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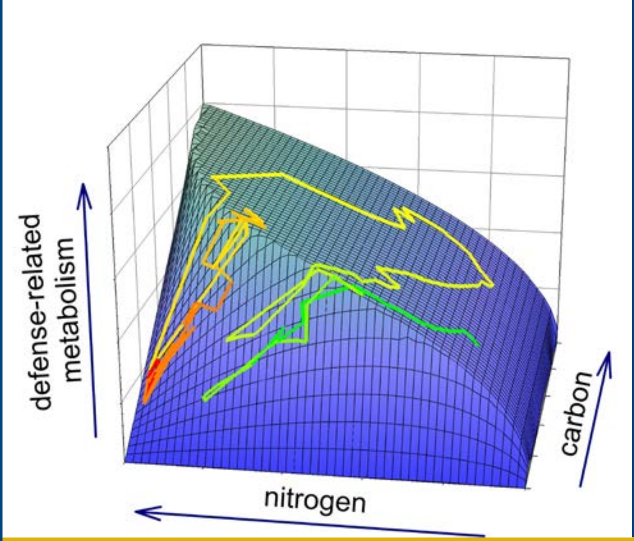
The Alternatives Growth and Defense: Resource Allocation at Multiple Scales in Plants

Rainer Matyssek, Ulrich Lüttge, and
Heinz Rennenberg (Eds.)



Deutsche Akademie der Naturforscher Leopoldina –
Nationale Akademie der Wissenschaften, Halle (Saale) 2013

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The Alternatives Growth and Defense: Resource Allocation at Multiple Scales in Plants

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With 91 Figures and 20 Tables

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Cover:
Simulated allocation rates to the defense-related plant metabolism, depending on carbon and nitrogen availability, which both fluctuate during the vegetation period. The colored line represents the time course of a scenario with juvenile beech trees starting in May (green) and ending in September (red); also see GAYLER and PRIESACK, this volume; adapted from GAYLER, S., PRIESACK, E., FLEISCHMANN, F., HELLER, W., RÖTZER, T., SEIFERT, T., and MATYSSEK, R.: Modeling the defensive potential of plants. In: MATYSSEK, R., SCHNYDER, H., OSSWALD, W., ERNST, D., MUNCH, J. C., and PRETZSCH, H. (Eds.): Growth and Defence in Plants: Resource Allocation at Multiple Scale. Ecological Studies (Springer) *220*, 375–400 (2013).

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Prologue

Ulrich Lüttge ML (Darmstadt)

Writing a prologue to this number of *Nova Acta Leopoldina* Neue Folge gives me the welcome opportunity to put into perspective four meetings and symposia of Leopoldina in the organization of which I participated within one decade. The first one addressed biology in general the other three were focusing on plant biology:

- 2002 – Nonlinear Dynamics and the Spatiotemporal Principles of Biology (Nova Acta Leopoldina NF Vol. *88*, No. 332, 2003),
- 2004 – From Plant Taxonomy to Evolutionary Biology (Nova Acta Leopoldina NF Vol. *92*, No. 342, 2005),
- 2007 – Imaging and Integrating Heterogeneity of Plant Functions: Functional Biodiversity from Cells to the Biosphere (Nova Acta Leopoldina NF Vol. *96*, No. 357, 2009),
- 2011 – Growth and Defense in Plants: Resource Allocation at Multiple Scales (Nova Acta Leopoldina NF this volume).

The basic philosophy of all of them was to consider spatiotemporal dynamics at a large range of scalar levels in space and time with strictly interdisciplinary approaches. For the latter, almost used like a dogma, I like the metaphor of the magic tripod, i.e. where the ancient Greek goddess Pythia sat uttering the oracles in Delphi or on which the medieval witches cooked their broths. In our science the three legs of the magic tripod are

experiment and observation,
theory,
modeling.

At the time of the first of the four Leopoldina-events the era of the "-omics" sciences was in full vigorous development. It was a real outcry for the theory leg of the tripod to help mastering the mountains of data heaped up by the "-omics" researchers. The symposium was organized such that in each of the different sessions both empiricists and theorists were speaking. The introductory comment of the volume asks for establishing the time scale to arrive at dynamics from the static data information (Thellier 2003). It was realized that non-linear dynamics with important roles of deterministic chaos and regulatory functions of noise were providing robustness to the biological systems. Theory of networks and supra networks implicitly touched scalar levels and indicated the path to a new holistic understanding rather than just data-collection based systems biology as summarized in the synopsis:

"[…] it is realized that it is not enough to concentrate on the molecular structure of genes and their regulating power in cell performance alone, but that it is the whole spatiotemporal nonlinear dynamics of interacting cell agglomerations which is responsible for the behavior and conduct of living organisms. Here the whole richness, variety and complexity of nonlinear systems come into play, as well as the fine-tuning of noise-enhanced signaling and the precise conditions for synchronization within a system" (Beck and Lüttge 2003).

The second of the Leopoldina-events was dedicated to the memory of Nees von Esenbeck (1776–1858, President of the Leopoldina 1818–1858) and held at his birthplace in Reichelsheim in the Odenwald, where he was born in the castle Reichenberg. Nees von Esenbeck was a very broadly learned and scientifically productive scholar and among many other things an outstanding botanist who had described thousands of new plant species. Perhaps such systematics and taxonomies were the early 19th century version of heaping up data-mountains. However, researchers like Nees von Esenbeck were already thriving for a "natural system" of organisms. The Leopoldina-meeting then picked taxonomy up to follow to dynamics of diversification and phylogeny of plants and populations with the participants covering the vast scope of disciplines given by evolutionary biology, phylogeography, molecular genetics, physiological ecology, plant physiology and biochemistry.

The third event was firmly installed on all three legs of the tripod. Interdisciplinary also was the demonstration of how the most modern technologies including optical remote-sensing were acquired for imaging to make visible the spatiotemporal dynamics of cellular communication processes leading to growth of organs, whole plants and stands. Thus the scalar levels for illustrating functional biodiversity ranged from molecules to ecosystems (e.g. Fig. 3 in Lüttge and Hütt 2009) and thence to biosphere. The experimental observations came out as adornments. It was realized that theoretical approaches to derive functional principles from the dynamic patterns will be scale-invariant, as we wrote "[…] the development of systems biology based on molecular information has been compared with the historical development of astronomy […] The way from designing instrumentation (Galileo) and accumulation of data (Brahe) towards an understanding of natural laws (Newton) went *via* interpretation of patterns in the data (Kepler). If this analogy holds, biology at present is at a most exciting point of this path: the move from the patterns towards the natural laws and fundamental principles" (Lüttge and Hütt 2009). Here it was pronounced again that we live with the inspiring challenge to advance to real systems biology where information *via* knowledge merges to understanding.

Thus, the fourth of the Leopoldina-events emerged as a natural continuation of the previous three ones further pushing the view of systems biology on the basis of the magic tripod. Moving from the nightmare of non-penetrated -omics information and the quest of holistic systems biology (see first event) to scale invariant theoretical understanding of the imaged patterns of systems (see third event) the fourth event in its four sessions spanned the arch over objects, processes, scales and systems. Scales ranged again from molecules to the biosphere now also imaged as the supra organism or holobiont Gaia, the latter in strict terms of nature-science. In the holistic view of systems in biology the role of theory is now far more than management of information. There is first simulation by statistical analysis and probabilistic modeling. This allows deriving tests and corresponding quality criteria. There is second, numeric modeling using mathematical operators for comprehending dynamics. Progress in biology will only be possible combining both approaches (zu Castell 2013). As a concise and as such in a way limited albeit overarching example the growth differentiation balance (GDB) theory was the concrete core of the symposium as outlined in the introduction (Matyssek et al. 2012). One major new insight was that with the trade-off between growth and defense the GDP has far reaching power explaining performance of plant systems and yet so often is fooled by the astonishing plasticity of plants. Thus, from the thorough evaluation of GDB outreaching general ideas emerged with stress memory of plants, answers by plasticity, potentials for repair functions with hopeful outlooks on self-organization and self-sustainment in the plant life of a threatened biosphere or holobiont Gaia under fearful global environmental threatening.

The four events confirm that with corporative unrestrictedness more than any other institution an academy like Leopoldina can accommodate a unique culture of interdisciplinarity, *sensu* the magic tripod, in research. The German Academy of Sciences Leopoldina in its foundation in the year 1652 has written in the demand of *naturae curiosorum*. It is institutionally occupied with policy advice, public debate, and editing statements and recommendations. The symposium once again showed that with curiosity and scientifically based opinion Leopoldina embraces both *naturae curiosorum* and concerns of existential requirements of mankind.

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The Alternatives Growth and Defense: Resource Allocation at Multiple Scales in Plants – Introduction to the Symposium

Rainer Matyssek[1] (Freising), Ulrich Lüttge ML[2] (Darmstadt),
and Heinz Rennenberg ML[3] (Freiburg)

1. Scope of the Symposium

The international Leopoldina Symposium on "The Alternatives Growth and Defense: Resource Allocation at Multiple Scales in Plants", held during July 4–6, 2011, in Freising (Germany), was focused on the "conflict" of plants in coping with and balancing between diverse ecophysiological demands during the process of resource allocation. Such balancing was exemplified through the presumed trade-off between growth and stress defense along with the associated cost/benefit relationships (cf. Herms and Mattson 1992, Matyssek et al. 2013a). Given the case of such a trade-off, growth is conceived as the pre-requisite for staying competitive in resource acquisition (Schwinning 1996, Grams and Lüttge 2010), and defense for retaining resources once incorporated (Matyssek et al. 2002, 2005). Hence, growth and defense are fundamental plant functions, as they determine individual plant fitness. Resolving the regulatory challenge between these two functions requires plants to optimize their resource fluxes in response to internal needs and external availabilities (Gayler et al. 2013). The mechanistic understanding of this regulation marks a current frontier in plant science (cf. Bazzaz and Grace 1997), challenging the comprehension of the plant's persistence in the field at the transition between molecular, ecophysiological and ecosystem-level processes (Matyssek et al. 2013a).

The outlined subject was intended to be examined, as a starting point of the symposium, for the extent to which related evidence and theory in plant science are currently available and backed by mechanistic and ecologically relevant foundations. At the same time, the symposium marked the concluding keystone of a 12-year, interdisciplinary research program, supported by the German Research Foundation (DFG) through the funding instrument of a "Sonderforschungsbereich" (SFB), i.e. a collaborative research centre, entitled as "SFB 607: *Growth and Parasite Defense in Plants – Competition for Resources in Economic Plants from Agronomy and Forestry*". Active from 1998 through 2010, SFB 607 was borne by up to 20 research teams with expertise in different areas of plant biology (i.e. molecular genetics, biochemistry, eco-physiology), agricultural and forest sciences, physics as well as biomathe-

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matics and modeling theory. The teams were affiliated with the three major research institutions in the Munich area, namely *Technische Universität München* (with R. Matyssek as coordinator of the SFB), *Helmholtz-Zentrum München*, and *Ludwig-Maximilans-Universität München*. The integrated outcome from SFB 607 is provided by a recent volume of the book series "Ecological Studies" (Matyssek et al. 2013b).

With 12 local speakers and 13 invited external keynoters, the symposium was to project rationale, evidence and conclusions from SFB 607 to envisaged follow-up research, i.e. widening the horizon of gained knowledge towards new scientific frontiers. In doing so, process cross-linking between functional and structural plant and ecosystem-scale organization hierarchies was explored as a pre-requisite for comprehending spatio-temporal response patterns towards an integrative mechanistic "plant system" understanding (Sandermann and Matyssek 2004, Lüttge and Hütt 2009).

2. Theoretical Grounds

Conceptual starting point of SFB 607 was the "Growth Differentiation Balance Theory" (GDB), which basically claims, originating from early definitions by Loomis (1953) and Lorio (1988), a stimulation of the growth-related metabolism in parallel to increasing resource supply, and hence, increasing gross primary productivity (Herms and Mattson 1992, Matyssek et al. 2005, 2013a). This kind of stimulation is thought to occur at the expense of the defense-related metabolism (being aware of functional overlaps between primary and secondary metabolites, Schwachtje and Baldwin 2008). At reduced resource supply, the growth-defense relationship is claimed to be reversed (with high defense capacity at low growth activity), unless both growth and defense are constrained at severe resource limitation. Hence, a trade-off is reflected by this theory, being consistent with some kind of dilemma a plant has to resolve, and associated with so-called "opportunity costs", which incur once the plant's "decision" is made (Stitt and Schulze 1994, Matyssek et al. 2013a). Such costs, mirrored in the internal resource balance, quantitatively denote chances forgone by the plant upon deciding in favor of the one rather than the other alternative (Rötzer et al. 2013). GDB is thought to be the currently most advanced theory on the subject, with high potential for further development on mechanistic grounds (Koricheva et al. 1998, Matyssek et al. 2005, 2013a). Are plants actually caught on such grounds, however, in a "dilemma" while coping with conflicting resource demands? To which extent may "functional plasticity" allow plants to "escape" or, at least, mitigate such a dilemma?

3. Recent Gain in Knowledge Setting the Stage

Clarification required spatio-temporal process scaling, as reflected by the sub-title of the symposium, acknowledging the intense resource interchange of plants with their abiotic and biotic environment during the internal regulation of process resource allocation. As crucial interfering agents emerged competitors, pathogens, consumers and organisms of the mycorrhizosphere (Gayler et al. 2013, Rötzer et al. 2013, Grams et al. 2013, Osswald et al. 2013, Agerer et al. 2013). The biotic interactions turned out as determinants in the control of the plant's resource allocation, as they drive the conflict between growth and defense (Gayler et al. 2013). It became evident, as one major conclusion towards mechanistic understanding, that plants, their parasites and associated mycorrhizospheric organisms form – under the prevailing competitive site scenario – a functional unity in the allocation process (Matyssek et al. 2013c). Such evidence required the extension of the conventional view on spatio-temporal scaling from cell and organ levels to those of individuals and stands by the additional scaling dimensions of ontogeny (from juvenile to adult plants) and growth scenarios (from controlled chamber to prevalent field conditions; Matyssek et al. 2005). Nevertheless, the challenge was to functionally link, plant-internally, metabolic control (signaling, genomics, transcriptomics, proteomics) with metabolic activity (biochemical and physiological processes). This task required the synthesis of molecular and organismic biology in view of the gap in understanding to be closed between micro-scale causality (as mostly demonstrated under controlled conditions) *versus* macro-scale spatio-temporal complexity (as typically encountered at stand and ecosystem levels and beyond, Glinski and Weckwerth 2006, Ernst et al. 2013). Given this challenge, the basis for a new holistic concept of systems biology was elaborated, reaching to beyond the molecular scale (cf. Lüttge and Hütt 2009). It became apparent, in addition, that the complexity of the challenge is to be met only by a combination of empirical assessment and modeling theory (Matyssek et al. 2013b). On such grounds, what is to be expected then about the out-reach of trade-offs in resource allocation *sensu* GDB across spatio-temporal scales?

4. How far do Allocation Trade-offs Reach across Scales?

In linking metabolic control with activity, consistent regulatory patterns became apparent at the gene level (of the shikimate pathway) under oxidative stress (Ernst et al. 2013). These were related to increased transcript and protein levels, and in addition, to up-regulated metabolic end products related to defense. Genetic control, in addition, yielded an overall down-regulation of primary metabolism *sensu* GDB, although direct transcript-protein overlap was not substantiated. Once initiated through gene regulation, processes beyond the molecular level attain their own “momentum” so that decoupling from the gene basis is pretended (Matyssek et al. 2013a, c). Nevertheless, transcriptome analysis revealed similar gene grouping in herbaceous and woody plants at the level of metabolic control, with regulation being more pronounced in juvenile than adult trees (Ernst el at. 2013). Regarding the biochemical and physiological process level, trade-offs did indeed occur between growth and defense-related metabolism, although the emergence of such conflicts strongly depended on the metabolite classes and their hierarchical significance within the plant-internal metabolic organization (Koricheva et al. 1998, Osswald et al. 2013). The overall picture on allocation trade-offs *sensu* GDB, however, was ambiguous, being traceable to several reasons: Plants have the capacity to escape trade-offs through accelerating growth under stress (as long as permitted by resource availability), enabling for compensation of organ loss (Maurer and Matyssek 1997). Organ “value” in a plant is given through organ abundance (Zangerl and Bazzaz 1992), which if becoming low may foster defense, and hence, trade-off. The latter may be pronounced also, if defense is energetically expensive (however, see Häberle et al. 2009). It must be cautioned, though, that “full costs” of defense, also accounting for storage, transport and recycling of metabolites, including respiration (Schwachtje and Baldwin 2008, Bolton 2009, Lehmeier et al. 2013, and Schnyder et al. 2013), are hardly assessable (Lerdau and Gershenzon 1997). In addition, defense can induce new sinks for carbon which then drive, i.e. up-regulate photosynthesis, in such a way mitigating or circumventing trade-offs (Osswald et al. 2013).

Evidently plants do have means, i.e. "opportunities", to de-escalate trade-offs rather than being strictly caught within "opportunity costs" (see above) associated with trade-offs. Notwithstanding, irrespective of the degree of being relevant, resource trade-offs are parts of spatio-temporal scales, and hence, of a hierarchical structure of cause-effect networks ("knots" and "edges") within and across scales. Therefore, even though substantiated perhaps within one "knot" or along one "edge", trade-offs do not necessarily extend throughout entire networks (Lüttge 2013) – neither, this must be presumed on theoretical grounds.

5. Experiment and Modeling – Intrinsically Tied to Knowledge Gain

Given the plant's regulatory "opportunities", mathematical modeling bears potential of elucidation, if capable of mirroring dynamics in resource allocation along the source-sink gradients of growth and defense under phenological and ontogenetic influences, and most importantly, of accounting for internal availabilities of carbohydrates and nutrients (Priesack et al. 2013, Gayler et al. 2013, and Rötzer et al. 2013). Cautioning that models represent integrated lines of hypotheses themselves, and that modeling, once being validated, must be able to mechanistically explain empirical evidence, agreement with the latter can be taken as indirect confirmation of underlying presumptions. Eventually, scenario analysis through model simulations can foster theory development. At this stage the "magic tripod" is established, integrating empiric research, modeling and theory development (Lüttge 2013).

On such grounds, resource trade-offs were shown to be driven distinctly through seasonal phenology and even result in conflicting outcomes, as depending on relative availabilities of carbon *versus* nutrients (Gayler et al. 2013). Dominated were such relationships by biotic interactions, remarkably, by intensities of competition. The latter can determine incurred "opportunity costs" (see above) which indeed result from allocation conflicts between growth and defense. Such costs appear to become ecologically relevant, in particular, in long-lived trees (Rötzer et al. 2013).

Aided are such explorations by novel approaches in statistical modeling (zu Castell et al. 2013), in particular, if the focus is on the degree of generality or universality of empirical findings. One novel means here is based on the theory of "*unsupervised learning*" (Vapnik 1995). Aim is at identifying such variables, which most distinctly respond to same driving factors under different scenarios. The complementarity of modeling theory and empirical approaches yielded consistency patterns in allocation response to stress, irrespective of herbaceous or woody life form, ontogenetic stage and growth conditions (zu Castell et al. 2013). Nevertheless, the significance of genotype, ontogeny and site ecology for plant performance must not be overlooked.

Remarkable was the wide range of „functional plasticity", emerging as *the* intrinsic feature of plant response to stress, and hence, becoming the key to understanding plant performance mechanistically (Matyssek et al. 2013b, c). Knowing the range of potential responsiveness opens access to mechanistic understanding. As a consequence, „systemic" empirical and theoretical approaches beyond the gene level are required (Lüttge and Hütt 2009) – establishing a new quality of „systems biology", which is to integrate molecular and organismic rationales in relation to defined ecological scenarios (Sandermann and Matyssek 2004).

The foundation has been laid for meeting such a requirement, as reflected by the contributions to the symposium, with the outcome from SFB 607 providing major input.

6. Extending the Horizon towards New Frontiers

The conclusion about the need for an extended "systems biology" affirms the conceptual view of a functional unity represented by plant-associated mycorrhizospheric organisms, competitors (including both intra-specific and inter-specific competition), parasites and the plant itself, as striving to mechanistically understand the "system plant" (with its internal and external substrate and energy interchanges, intrinsic information signaling and response plasticity within ecologically relevant contexts). Directing the view, therefore, towards research questions as arising from the recent, above outlined knowledge gain, one may pose the hypothesis that "working points" of evolution are not individual species but "multi-species holobionts", represented by the hologenome of the involved species (cf. Zilber-Rosenberg and Rosenberg 2008). Hence, the functional unity or entity of relevance in evolution may actually be the holobiont. Defined in a strict sense (and as starting point of consideration) it originally focused on associations with microorganisms. In this definition holobiont is considered as a host organism (plant or animal) in interaction with all associated microorganisms (bacteria and fungi, van Dam and Heil 2011, Vannette and Hunter 2011). Such a holobiont is an open system in terms of its resource and energy fluxes, typically would have resulted from its inherent co-evolution, and thus, would make the individual organism appear as an arbitrary construct, in view of spatio-temporal performance and evolution (see Matyssek 2013, this volume, Lüttge 2013, this volume). Subject of examination might then be as to whether the evolutionary strength of the holobiont is founded in the high genetic micro-organismic variability along with fast reproduction cycles in compensation for the host's slow adaptability to a changing environment. Also "fitness" ought to be re-considered then, extending the conventional scope of definition on reproduction capacity and offspring survivability by the valuation of long-term niching success at the ecosystem level and co-evolutionary potential.

In the symposium the definition of "holobiont" was extended from its original strict sense, i.e. host with its microorganisms, to fit an advanced integrative view of systems biology (for development of the argument in detail see Matyssek and Lüttge 2013, this volume). Interlinking the widened concept holobiont, sensu "system plant", with the ecosystem level might then as a consequence provoke the thought of viewing competition and facilitation not as the interaction between plant individuals but between holobionts. Even ecosystems might be viewed as holobiont-like systems, i.e. as the functional integration of the involved holobionts. Such thoughts might be projected further until reaching the level of global ecology and its functional, i.e. mechanistic understanding (e.g. by exploring the Gaia hypothesis within a framework explicitly based on nature sciences, Lovelock 1979, Matyssek and Lüttge 2013, this volume). Regarding all such spatio-temporal views on holobionts and holobiont-like systems, the guiding question may be posed as to whether similar, perhaps consistent, patterns prevail regarding resource, energy and information fluxes and capacities in functional self-organization. Do holobiont-like principles prevail, in such respects, regardless of the spatio-temporal scale of biological organization? And if so, which is the emergent evolutionary value of holobiont-like systems, marking the co-evolutionary advantage relative to the conventional view on the evolution of single species (with their individuals rather than organismic associations conceived as the focal points of selection)?

7. Structure of the Symposium

In introducing into questions as developed above, the symposium advanced towards a new and deeply penetrating understanding of "systems biology", newly defined beyond the molecular scale and within an ecological context. To this end, the presented evidence focused on multi-organismic genotype/species networks in resource flux and information signaling, integrating science theory, experimentation, bio-mathematical concepts and modeling approaches (representing the core of the "magic tripod" of research, Lüttge 2013).

The outlined subject was evaluated by four sessions:

I The Objects: Hosts, Pathogens and Symbionts
II The Processes: Competition versus Facilitation
III The Scales: Spatio-Temporal Pattern Formation
IV The Systems: Holobionts and Hierarchy Theory

The advancement fosters capacities for consolidating cause/effect-based assessments of natural response variability on the mechanistic grounds of multi-organismic systems across spatio-temporal scales. Publications which have originated from the presentations at the symposium make up, in the following, the contents of this volume of Nova Acta Leopoldina NF. In addition, summary papers on each of the four sessions comprehend key conclusions along the lines of the symposium's rationale (Rennenberg and Simon 2012, Matyssek 2013, Priesack 2013, Lüttge 2013, each this volume).

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Memory Processes in the Control of Plant Growth and Metabolism

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With 7 Figures and 5 Tables

Abstract

Plants are sensitive to many different stimuli, to which they react by an almost immediate, transient elevation of cytosolic free calcium. This Ca^{2+} signal has various consequences, including an altered expression of certain genes, and the final response may be a movement or, more generally, a metabolic or morphogenetic modification. Plants can "memorize" the information corresponding to a stimulus that they have perceived. In some cases, after exposure to a first stimulus, plants modify the subsequent transduction of the same or other stimuli; this may therefore be termed the "habituation" form of memory. Another form of plant memory has been studied using mainly three experimental systems ("inhibition of hypocotyls elongation", "breaking the symmetry of bud growth" and "induction of epidermal meristems"); this may be termed STO/RCL because it involves storage of information and recall of that information at a later time. In all three systems, information may be transferred from the stimulated to the reactive area at a rate that is always close to 100 $\mu m \cdot s^{-1}$; an electric "slow wave" is apparently involved in this transfer of information. Information storage may be caused by stimuli such as manipulation, drought, wind, cold shock and even low-intensity electromagnetic radiation. After a period of consolidation, which is especially short for mechanical stimuli (~ 2 min), the storage of information is usually robust and commonly lasts for periods of up to several days or even weeks. Enabling the plants to recall stored information is often easily reversible, with the consequence that it has been possible to repeatedly recall stored information. STO/RCL is related to the internal rhythms of the plants. Moreover, STO/RCL is sensitive to the mineral conditions and, in particular, to calcium and potassium concentrations. It seems likely that Ca^{2+} waves and Ca^{2+} condensation/decondensation are involved in the control of the STO and RCL functions, respectively. The synthesis and/or post-translational modification of specific proteins (e.g. *via* transient protein phosphorylation) are associated with the activation of only the STO function or of only the RCL function or of both these functions. Under natural conditions, the STO/RCL form of memory can help plants adapt to the many stimuli to which they are subjected by optimizing the allocation of their resources to their main requirements such as for growth and defense. A mechanistic model of plant memory is proposed.

Zusammenfassung

Pflanzen sind gegen viele verschiedene Stimuli empfindlich. Sie reagieren darauf beinahe unmittelbar mit einer vorübergehenden Erhöhung der Konzentration an freiem Calcium im Zytosol. Dieses Ca^{2+}-Signal hat verschiedene Konsequenzen, u. a. eine veränderte Expression bestimmter Gene. Die Signalkette endet mit einer Bewegung oder ganz allgemein mit einer metabolischen oder morphogenetischen Modifikation. Pflanzen können sich an Information „erinnern", die einem aufgenommenen Stimulus entspricht. In manchen Fällen modifizieren Pflanzen, nachdem sie einem ersten Stimulus ausgesetzt waren, die Verarbeitung anschließend erhaltener Stimuli derselben oder auch einer anderen Natur. Dies kann man die „Habituationsform" des Gedächtnisses nennen. Eine andere Form des pflanzlichen Gedächtnisses wurde vornehmlich mit drei experimentellen Systemen untersucht: (*a*) Hemmung der Hypokotyl-Streckung, (*b*) Symmetriebrechung beim Austreiben von Knospen, (*c*) Induktion epidermaler Meristeme. Dies kann man STO/RCL-Gedächtnis nennen, denn es umfasst Speicherung (engl. *storage*, STO) der Information und späteres Wiederaufrufen (engl. *recall*, RCL) derselben Information. In allen drei Systemen kann die Information vom stimulierten Bereich zum reagierenden Bereich mit einer Geschwindigkeit weitergeleitet werden, die immer nahe bei 100 $\mu m\ s^{-1}$ liegt. Eine „langsame elektrische Welle" ist wahrscheinlich an dieser Informationsübermittelung beteiligt. Die Speicherung von Information kann durch Stimuli ausgelöst werden, wie Trockenheit, Wind, Kälteschock und sogar elektromagnetische Strahlung niedriger Intensität. Die Informationsspeicherung ist normalerweise robust und kann in der Regel für eine Zeitdauer von bis zu mehreren Tagen oder sogar Wochen anhalten. Es ist oft leicht reversibel, die Pflanzen in einen Zustand zu versetzen, wo sie gespeicherte Information wieder aufrufen können. Die Konsequenz davon ist, dass gespeicherte Information wiederholt abgerufen werden kann. STO/RCL steht mit endogenen Rhythmen der Pflanzen in Beziehung. STO/RCL hängt auch mit der Mineralstoffversorgung der Pflanzen zusammen, besonders den K^+- und Ca^{2+}-Konzentrationen. Es ist wahrscheinlich, dass Ca^{2+}-Wellen und die Ca^{2+}-Kondensation/De-Kondensation an elektrisch geladenen Oberflächen bei der Regulation der STO- bzw. RCL-Funktionen eine Rolle spielen. Die Synthese oder post-translationale Modifikation spezifischer Proteine (z. B. durch reversible Protein-Phosphorylierung) führt zur Aktivierung entweder allein der STO-Funktion oder allein der RCL-Funktion oder gleichzeitig beider Funktionen. Unter natürlichen Bedingungen kann die STO/RCL-Form des Gedächtnisses den Pflanzen helfen, sich mit den vielen Stimuli, denen sie ausgesetzt sind, zurecht zu finden, indem sie die Allokation ihrer Ressourcen für ihre wichtigsten Bedürfnisse nämlich Wachstum und Verteidigung optimieren. Ein mechanistisches Modell des pflanzlichen Gedächtnisses wird vorgestellt.

1. Do Plants Perceive and Remember?

A plant cannot move from one place to another to optimize environmental conditions. In the holobionts to which they belong, plants thus have to adapt their functions to their local environment. This means that they have to adapt to many stimuli (and their fluctuations) over a long period and not just to one stimulus at one time. If they get it wrong, they fail to prosper and may die. How then should they process the information available to them, i.e. integrate many different variables, so as to choose the best strategy? Clearly, they should make this choice only after having perceived and memorized environmental stimuli for a sufficiently long time. Do they?

2. Perception of Stimuli by Plants and Plant Memory

Most people draw a stark distinction between organisms endowed with sensitivity (i.e. animals and humans) and plants, which are considered inert and insensitive. In France, somebody whose brain has been irreversibly damaged is said to have become “*un véritable légume*” (a real vegetable). Plants are, however, sensitive to many different stimuli, e.g. wounds inflicted by herbivorous animals, attack by pests, and stimuli generated by wind, rain, touching, drought, cold, etc.

There is ample evidence that plants usually react to environmental stimuli by an almost immediate, transient elevation of cytosolic free calcium (Knight et al. 1991, 1992, 1995, Bush 1995, Knight and Knight 1995, Klüsener et al. 1995, Polisensky and Braam 1996, Cessna et al. 1998, Knight 2000, Plieth 2001, and Verdus et al. 2007). Initially, this Ca^{2+} signal has various cellular consequences, including an altered expression of certain genes. Finally, plants respond to stimuli by modification of their metabolism, by morphogenetic changes and by movements that may occasionally be rapid (for instance when an insect alights on a leaf of a Venus flytrap, this stimulus causes the leaf to close up thus leading to the insect being trapped and ultimately digested).

Sometimes, the response is made by the stimulated organ itself. For instance, rubbing a growing internode of a *Bryonia* plant increases the activity of certain peroxidases in this internode and inhibits internode elongation (Boyer et al. 1979). In other cases, the response takes place far from the stimulated area. For instance, cutting or pressing 10 mm above the root extremity of a wheat seedling led to a significant decrease in the growth rate of the root tip less than 80 s later (Gautheret 1935), which means that growth-inhibition information migrated at a rate of at least 100 $\mu m \cdot s^{-1}$ from the stimulated to the reactive area. Subsequently, other examples in plants of responses occurring at a distance from the stimulated area have been described (see e.g. Davies and Schuster 1981) and, again, the rate of information migration as evaluated from the authors' data is in the range of one to a few hundred $\mu m \cdot s^{-1}$.

In 1982, we showed that plants can also "memorize" the information corresponding to a stimulus that they have perceived (Thellier et al. 1982). Since then, many authors have confirmed the existence of plant memory. For reviews of plant memory, see Thellier et al. (2000), Trewavas (2003) and Ripoll et al. (2009). The examples studied so far fall into two categories (Trewavas 2003). In the first form of memory, after exposure to the first stimulus, plants modify the pathway transducing new stimuli. This form of memory may be termed habituation because it attributes to plants the "learning behavior" attributed to lower animals (Quinn et al. 1974, Folkers and Spatz 1981, Aceves-Pina et al. 1983). The second form of plant memory, which has been intensely studied in our group, is closer to the usual concept of memory as used in disciplines as different as computer science and psychology. It involves storage of information and recall of that information at a later time (Thellier et al. 2000).

3. Evidence for the "Habituation" Form of Plant Memory

There are cases in which, after exposure to the first stimulus, the initial response of plants is to modify the transient elevation of cytosolic free calcium that is caused by subsequent stimuli. In *Nicotiana plumbaginifolia* seedlings, a single wind stimulus causes cytosolic Ca^{2+} to rapidly increase, but repeated wind stimuli make the plant cells refractory to further Ca^{2+} signaling for ~ 1 min (Knight et al 1992). In *Arabidopsis thaliana*, the elevated level of cytosolic Ca^{2+} due to hyperosmosis is further increased by hyperosmotic-stress pre-treatment, whilst this level is reduced by an oxidative-stress pre-treatment (Knight et al. 1998). Again in *Arabidopsis thaliana*, cold pre-treatments attenuate the increase of cytosolic Ca^{2+} due to cold shock (Plieth et al. 1999).

In other cases, it is the final response of the plant to a stimulus that is modified after one or several previous stimuli. With maize coleoptiles, the effect of a gravitropic stimulus is only opposed by a compensatory counter-stimulus when the time between these stimuli is less than 90 min (Nick and Schäfer 1988). The history of phosphate levels in the media affects the way the uptake system adapts when *Anabaena variabilis* cells are transferred from a phosphate-poor to a phosphate-rich medium (Falkner and Falkner 2003, Aubriot et al. 2011). The closure of a leaf of the Venus flytrap can be initiated by a single electrical stimulus above a threshold value; but a series of subthreshold stimuli also cause closure when their sum equals the threshold value (Volkov et al. 2008).

4. Studies of the "Storage/Recall" Form of Memory

Three different systems have been studied in our group, "inhibition of hypocotyl elongation", "breaking the symmetry of bud growth" and "production of epidermic meristems". The group of Nicole Boyer (Bourgeade et al. 1989) has discovered a fourth system, "increased peroxidase activities".

4.1 Inhibition of Hypocotyl Elongation

In this investigation, we have used seedlings of *Bidens pilosa* L (Desbiez et al. 1983, 1987a). Under natural conditions, the seedling hypocotyls start elongating shortly after the beginning of seed germination (D_0) and reach full elongation after ~ 2 weeks.

4.1.1 The STO and RCL Functions

In the first series of experiments (Fig. 1), the seedlings, which were initially grown in a classical nutrient medium, were either left in this nutrient medium (batch b) or transferred into deionised water on D_5 (batch a) or D_8 (batch c). At these ages, the transfer had no important effect on hypocotyl elongation, but it did affect the response to an abiotic stimulus (a few needle pricks) given on D_6. With seedlings bathing in water (batch a) when the abiotic stimulus (vertical arrow) was given, hypocotyl elongation was inhibited immediately by ~ 25 %. With seedlings bathing in the nutrient medium when the abiotic stimulus was given (batch b), hypocotyl elongation was not appreciably inhibited; if, however, 2 days later water was substituted for the nutrient medium (batch c), hypocotyl elongation was immediately inhibited by ~ 25 %. This means that (*i*) the abiotic stimulus stores an information of "hypocotyl-elongation-inhibition" within the seedlings (STO function), (*ii*) this information remains latent as long as the stimulated plants bathe in the nutrient medium (stimulus-response period, here 2 days), (*iii*) transferring the seedlings to water leads to recall of the stored information and inhibition of hypocotyl elongation (RCL function) and (*iv*) whether the plants are transferred to water before or after the stimulation does not affect the final result (~ 25 % hypocotyl-elongation inhibition): the RCL and STO functions thus act independently from one another. Bathing the seedlings in 20 µM solutions of LiCl had the same effect as bathing them in the nutrient medium whilst NaCl had the same effect as water. The memory process (especially the RCL functioning) is therefore sensitive to the mineral conditions. The "hypocotyl-elongation-inhibition" information initiated by the stimulus was transported from the stimulated cotyledons to the hypocotyl at ~ 100 $\mu m \cdot s^{-1}$.

Similar results were obtained with plants other than *Bidens* (e.g. flax, var Ariane) and by replacing the traumatic pricking-stimulus by the non-traumatic deposition of droplets of solutions of dextran or fructose (possibly with added KCl) on the seedling cotyledons. Table 1 describes such an experiment, using flax seedlings previously transferred to deionised water. The effect of pricking was similar to that observed with *Bidens* seedlings. In the absence of KCl, the effect of the droplets of sugar solutions was either not visible or poorly visible but in the presence of 6 % KCl in the sugar solutions, this effect was clearly apparent (though less pronounced than that of pricking).

4.1.2 Main Properties of the System

In one series of experiments with *Bidens* seedlings, gene expression was studied in relation to the activation of the STO and RCL functions (Henry-Vian et al. 1995). The levels of proteins of 20, 35, 37, 38 and 38' (two different proteins with similar mass values), 41, 48 and 49 kDa changed when *Bidens* seedlings were stimulated by (*i*) pricking (storage of information of hypocotyl-elongation-inhibition, i.e. STO activation) or (*ii*) transfer to deionised water (enabling the recall of stored information, i.e. RCL activation) or (*iii*) both pricking and transfer. Specific

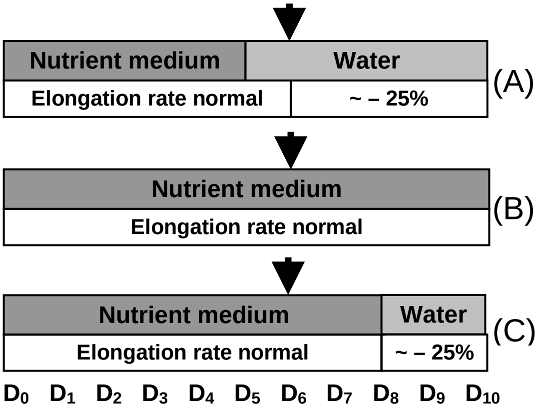

Fig. 1 Inhibition of hypocotyl elongation. *Bidens* seedlings were studied from germination (D_0) to the 10th day (D_{10}). They were initially germinated and grown in a classical nutrient medium (*dark grey*). They were either left in this nutrient medium (batch *B*) or transferred to deionized water (*light grey*) on either D_5 (batch *A*) or D_8 (batch *C*). In all cases, the seedlings were subjected to a pricking stimulus on D_6 (vertical arrow). As long as the plants were neither stimulated nor transferred to water (up to D_5 in batch *A* and D_6 in batches *B* and *C*), the elongation rate of the hypocotyl was normal. When non-stimulated seedlings were bathing in water (from D_5 to D_6 in batch *A* and from D_6 to D_8 in batch *C*) or when stimulated seedlings were bathing in the nutrient medium (from D_6 to D_{10} in batch *B* and from D_6 to D_8 in batch *C*), the elongation rate was also normal. When the seedlings were both stimulated and bathing in water (from D_6 to D_{10} in batch *A* and from D_8 to D_{10} in batch *C*), the elongation rate was decreased by ~25 %.

Tab. 1 Inhibition of the hypocotyl elongation of flax seedlings previously transferred to deionised water and subjected to various types of stimuli*

Stimulus		Hypocotyl elongation (% vs. non-stimulated control) Mean value ± confidence interval
Cotyledon pricking		– 32 ± 9
Dextran (0.1 µg·mL^{-1})	No KCl added	– 4.6 ± 8.8
	+ KCl (6 %)	– 19.8 ± 5.8
Fructose (0.1 µg·mL^{-1})	No KCl added	– 14.9 ± 11.8
	+ KCl (6 %)	– 22.7 ± 6.2

* A total of 46,000 seedlings were studied in this experiment (M. O. Desbiez, unpublished data).

changes (Tab. 2) occurred at 37 and 41 kDa (STO activation alone) or at 20, 48 and 49 kDa (RCL activation alone). When both STO and RCL were activated, all the changes at 20, 37, 48 and 49 kDa occurred plus changes to two new proteins at 38 kDa that are probably involved in the final response; the lack of a change to the protein at 41 kDa suggests that this protein is produced only transiently after stimulation. The change at 35 kDa, which occurs under all circumstances, may be a non-specific response of the seedlings to any modification of their environmental conditions. In other experiments with *Bidens* seedlings, metabolic changes were studied following a stimulus. It was found that the level of ethylene precursors, the rate of ethylene release and some peroxidase activities increased in the hours after the stimulus.

Tab. 2 Gene expression in relation to the activation of the STO and RCL functions

Activated Functions	Changes to the proteins* (kDa) 20	35	37	38	38'	41	48	49
STO	no	↑	↑	no	no	+	no	no
RCL	↑	↑	no	no	no	no	↑	↑
STO + RCL	↑	↑	↑	+	↓	no	↑	↑

* Symbols: ↑ or ↓ = increase or decrease of a protein, + = appearance of a new protein, no = no change; 38 and 38' correspond to two different 38-kDa proteins. Table drawn from data in Henry-Vian et al. (1995).

4.2 Breaking the Symmetry of Bud Growth

In the bud-symmetry experiments, we used *Bidens* seedlings slightly older (~ 3 weeks) than those in the experiments described in Section 4.1. At 3 weeks, these seedlings are bilaterally symmetrical. They comprise an axis (terminal bud [or "apex"], hypocotyl and root), the two opposite cotyledons and the buds at the axil of the cotyledons (i.e. the "cotyledonary buds").

4.2.1 The Asymmetry Index

The cotyledonary buds are normally quiescent. After removal of the apex (seedling "decapitation") both cotyledonary buds can grow. However, under limiting light and mineral levels, one bud starts to grow before the other (so breaking the bilateral symmetry of the seedling). When the seedling cotyledons are labeled "A" and "B" and their axillary buds "a" and "b", one may define (Desbiez et al. 1986) an asymmetry index, g, by

$$g = (n_b - n_a)/n_t \qquad [1]$$

where n_t is the total number of seedlings under study and n_b and n_a are the numbers of seedlings in which it is bud b or a that is the first to start to grow. When $g \approx 0$, this means that the set of seedlings is symmetrical, while $-1 \leq g < 0$ and $0 < g \leq 1$ mean that the set is asymmetrical in favor of bud a or b.

4.2.2 The STO and RCL Functions

In the experiments described below, the seedlings were subjected to pricking or other stimuli, either asymmetrical (e.g. 4A = 4 pricks given to cotyledon A) or symmetrical (e.g. 4A–4B = 4 pricks given simultaneously to each cotyledon A and B). When the seedlings were subjected to several successive stimuli, this was written for instance 4A(Δt h)4A–4B, meaning that an asymmetrical stimulus 4A was followed Δt hours later by a symmetrical stimulus 4A–4B. In the case of an asymmetrical pricking treatment, the buds at the axil of the pricked and non-pricked cotyledons were termed the proximal and distal buds, respectively.

Seedlings kept under homogeneous conditions and not subjected to any asymmetrical stimulus had g-values that were always close to zero, as might be expected. Unexpectedly, however, asymmetrically stimulated seedlings had g-values that were either close to zero or far from zero! A typical experiment (many others gave a similar result) is described in Table 3. Seedlings were subjected to an asymmetrical stimulus and were decapitated 2 days later, with a razor blade, either at the onset of daylight or at midday. The g-values were appreciably above zero in the first case (asymmetrical set of plants) while they were close to zero in the second case (symmetrical set of plants). That symmetry could be broken two days after the application of the pricking stimulus meant that symmetry-breaking information was stored in the stimulated seedlings. It is not possible that the stored information was deleted when seedlings were decapitated at midday because giving an appropriate thermal treatment (which was not asymmetrical) after the midday decapitation again gave g-values significantly greater than zero. Our interpretation (THELLIER et al. 2000) is that (*i*) stimulus 4A stores "symmetry-breaking" information (STO function) and (*ii*) the decapitation conditions and other treatments do not alter the stored information but render the seedlings reversibly able/unable to recall the stored information (RCL function).

Tab. 3 g-values under different experimental conditions*

Stimulus	Seedling decapitation	Further treatment	Mean g-value
4A	Morning	–	0.35
	Midday	–	0.08
	Midday	Thermal treatment	0.50

* *Bidens* seedlings at the age of 3 weeks were subjected to an asymmetrical stimulus 4A (pricking cotyledon **A** four times). The seedlings were decapitated 2 days later, with a razor blade, either at the onset of daylight (morning) or in the middle of the day. In one case, a thermal treatment (rapid cooling followed by slow re-warming) occurred immediately after seedling decapitation (modified from THELLIER et al. 2000).

4.2.3 Main Properties of the System

From more than 1 million seedlings studied by Marie-Odile DESBIEZ, it was found that "symmetry-breaking" information could be generated by many very different treatments. These included diverse asymmetrical abiotic stimuli (whether traumatic [pricking] or not [rubbing]), asymmetrically deposited droplets of a solution (1–100 $\mu g \cdot ml^{-1}$) of oligosaccharide fragments (given to us by Peter ALBERSHEIM) or asymmetrically deposited droplets of solutions (10 $\mu g \cdot ml^{-1}$) of D-glucose, D-galactose, D-fructose and myo-inositol. In this case of the droplets, symmetry was broken if the droplets contained 0.6 $\mu g \cdot ml^{-1}$ KCl or $CaCl_2$ (but not NaCl); symmetry was not broken by droplets that contained D-arabinose, mannitol and DL-leucine irrespective of whether KCl or CaCl2 were added (DESBIEZ et al. 1991a). Stored asymmetry was not changed by stimuli that were stronger and opposite to the original stimulus. Storage of symmetry-breaking information therefore appears irreversible. In the experiment in Table 3, the stimulus-response period was 2 days; but stimulus-response periods of up to 14 days were observed without any loss of information (DESBIEZ et al. 1984). With short stimulus-response periods (e.g. 2 days), recall of stored information was easily and reversibly enabled/disabled (i.e. the RCL function was easily switched "on" and "off") depending on the decapitation conditions and on the addition of pricking, thermal or other treatments. With long stimulus-response periods (Tab. 4), the RCL function was blocked "on" (i.e. the seedlings were always able to recall stored symmetry-breaking information). Whether the seedlings were stimulated (i.e. STO-activated) before or after being rendered able to recall stored information (i.e. RCL activated) did not change the final result (the g-value): again, the RCL and STO functions could be activated independently from one another. The memory process was dependent on the mineral conditions: information storage was only observed with mineral-starved seedlings and the effect of droplets of sugar solutions depended on the presence of Ca^{2+} and K^+ ions; information recall was strongly dependent on the composition and concentration of the bathing solution of the plants (Desbiez et al. 1987b).

Tab. 4 g-values under short and long values of the stimulus-response periods*

Stimulus-response Period (days)	g-value (mean ± confidence interval) for seedlings decapitated on	
	Morning	Midday
2	0.37 ± 0.03	0.01 ± 0.07
14	0.34 ± 0.04	0.40 ± 0.14

* The Table was computed from the results obtained with 78000 seedlings studied in different experiments. In these experiments *Bidens* seedlings were subjected to an asymmetrical pricking stimulus and then they were decapitated with a razor blade either in the morning or in the middle of the day, after a stimulus-response period of 2 or 14 days (M. O. Desbiez, unpublished data). The g-values with a stimulus-response period of 2 days are consistent with those in Table 3.

The "symmetry-breaking" information initiated by the stimulus was again transferred from the stimulated cotyledons to the buds at the rate of ~ 100 $\mu m \cdot s^{-1}$. *A priori*, information might be transmitted in a plant at that rate *via* signal molecules in the sap flow, waves of hydraulic pressure and waves of electric depolarization (Davies 1987, 1993, Malone 1992). Using extra-cellular electrodes, we have shown that, after pricking a cotyledon of a *Bidens* seedling, two different electric signals were propagated from the stimulated area: an action potential and a slow wave. The action potential was propagated at a rate much above that of information transfer; but the rate of the slow wave was of the order of 100 $\mu m \cdot s^{-1}$ and its amplitude was appreciably higher at the level of the proximal bud than at the level of the distal bud: i.e. the slow wave was a good candidate for being the vehicle for the transfer of symmetry-breaking information (Frachisse et al. 1985). However, given the sensitivity of both *Bidens* systems (hypocotyl elongation and bud asymmetry) to the deposition of droplets containing short or long-chain sugars, the transmission of information *via* the flow of such sugars in the sap cannot be excluded.

In the experiment described in Table 5, a small asymmetrical stimulus, 2A, was followed by one or several symmetrical stimuli, 2A–2B, with appropriate time lapses between them. Two days after being subjected to the first asymmetrical stimulus, the plants were decapitated in the morning with a razor blade. Symmetry-breaking information was always stored following this asymmetrical stimulus; but the plants were alternatively unable or able to recall the stored information, depending on the number of symmetrical stimuli and the moment when they were administered. Incidentally, this shows that the stored information can be recalled twice (Desbiez et al. 1991b). Moreover, when the seedlings were subjected to various pairs of stimuli (at least one of them being asymmetrical) there was a clear oscillation of the g-values as a function of the time lapse between the stimuli (Fig. 2). This oscillation of the g-values, plus their sensitivity to the time of the day when seedling decapitation is carried out (see Tab. 3 and the second in Section 4.2.2), suggest that the memory process may be related to internal rhythms of the plants.

Tab. 5 g-values for different combinations of stimuli*

Stimuli	g-value
Asym	0.06
Asym(1h)Sym	0.32
Asym(1h)Sym(3h)Sym	0.05
Asym(1h)Sym(3h)Sym(5h)Sym	0.34

* The seedlings were given an asymmetrical stimulus (Asym = 2 pricks on cotyledon A) followed by one or several symmetrical stimuli (Sym = 2 pricks simultaneously on cotyledons A and B). The time lapse between two successive stimuli is indicated in brackets. Modified from Desbiez et al. (1991b).

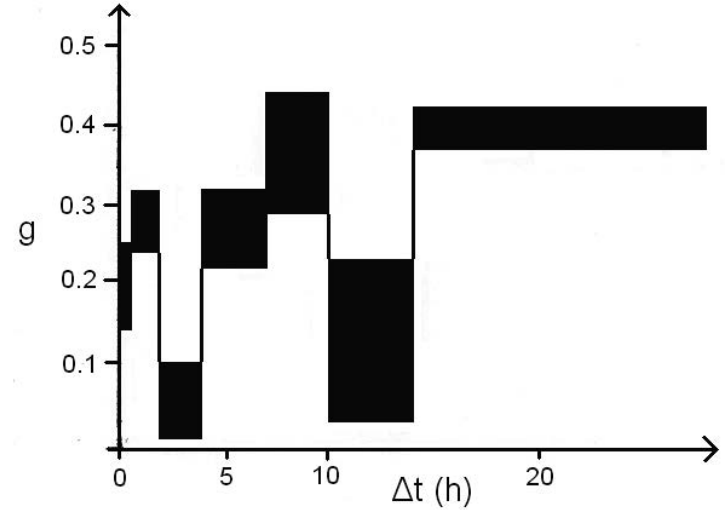

Fig. 2 Oscillations of the g-values of *Bidens* seedlings subjected to various pairs of stimuli (one of them, at least, asymmetrical) as a function of the interval of time, Δt, between them. The dark rectangles correspond to the mean g-value ± confidence interval (rectangle heights) during lapses of time, Δt (h), ranging from 0 to 0.5, 0.5 to 2, 2 to 4, 4 to 7, 7 to 10, 10 to 14 or larger than 14 (rectangle widths). The graph was computed with data obtained from 60,000 seedlings. Modified from Desbiez et al. (1991b).

When non-decapitated seedlings were subjected to an asymmetrical pricking stimulus, symmetry-breaking information was stored within the plants but, as could be expected, nothing externally visible occurred and the cotyledonary buds remained quiescent. The DNA content of the meristematic cells in these cotyledonary buds was measured 1 day after stimulation. In the non-stimulated controls the numbers of cells in the phases G1 and G2 of the cell cycle were approximately equal. In the stimulated seedlings, most meristematic cells were in the phase G1 (i.e. most cells that were previously in the phase G2 had divided following stimulation). Cyclins, or other substances or events involved in triggering the entry of G2 cells into mitosis, may thus be involved also in the storage of information in plants. Furthermore, nearly all cells underwent the "G2 → Mitosis" transition in the bud at the axil of the pricked cotyledon, whilst a non-negligible number of cells did not undergo this transition in the other bud. Hence, since the seedlings were not decapitated, an asymmetrical response to an asymmetrical stimulus can be observed at the cellular level without involvement of the RCL function (Desbiez et al. 1998).

4.3 Production of Epidermal Meristems

The experiments were carried out using flax seedlings (*Linum usitatissimum* L., var Ariane) shortly after germination.

4.3.1 The STO and RCL Functions

The main results are summarized in Figure 3. When flax seedlings were stimulated (e.g. by simply transferring them from their germination box to a classical growth solution [the so-called "manipulation stimulus"]) and then immediately subjected to transient (e.g. 2 days) Ca^{2+} depletion, epidermal meristems were produced in the hypocotyls during the three following weeks. Seedlings subjected to only the manipulation stimulus or to only the Ca^{2+} depletion produced far fewer (often practically no) meristems. When Ca^{2+} depletion was delayed relative to the stimulus, meristem production was correspondingly delayed; i.e. the signal initiated by the stimulus was memorized. Again, the stimulus leads to storage of "meristem-production" information (STO function). Ca^{2+} depletion does not alter the stored information but enables the seedlings to recall it to allow the production of meristems (RCL function). Stimulus-response periods (i.e. the time lapses between stimulus and Ca^{2+} depletion) of up to 8 days were observed without any loss of information. When the transient calcium depletion preceded the stimulation, the seedlings were already ready to recall stored information at the moment when they were stimulated and meristem production began immediately after the stimulation. Depleting Ca^{2+} before or after the stimulus did not change meristem production: once more, the STO and RCL functions act independently of one another (Verdus et al. 1997).

4.3.2 Main Properties of the System

For detail, see Verdus et al. (2012). Briefly, when seedlings were stimulated on D4, followed by 2-day Ca^{2+} depletion, they produced meristems as usual; then, if they were subjected to another Ca^{2+} depletion on D12 (when meristem-production had nearly reached a plateau), a second round of meristem production occurred (Fig. 4). The behavior of stimulated seedlings that were subjected to a combination of 2-day Ca^{2+} depletion and Ca^{2+} excess (three times the normal Ca^{2+} concentration) is depicted in Figure 5. When stimulated and Ca^{2+}-depleted seed-

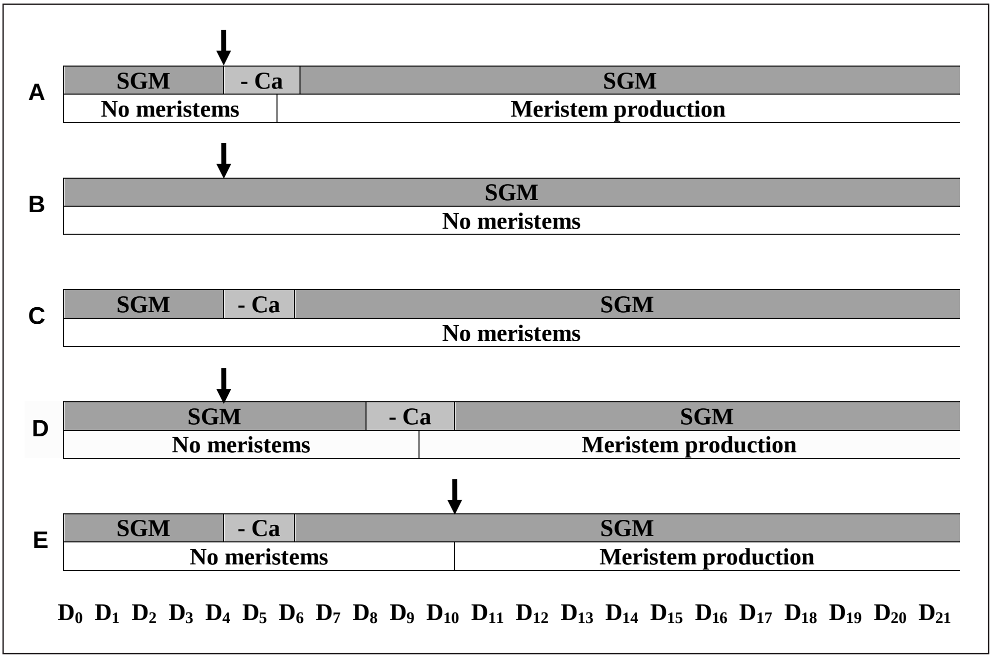

Fig. 3 Induction of meristem production. Seed germination started on D_0 (day zero). The seedlings were initially grown in a standard growth medium (SGM) and, apart from the controls, were subjected to a manipulation stimulus (*vertical arrow*) and to a 2-day calcium depletion (– Ca). The meristems produced were counted during ~ 3 weeks following germination (e.g. until D_{21}). (*A*) When the seedlings were stimulated on D_4 and immediately subjected to calcium depletion, meristem production started around the end of the period of calcium depletion. (*B*) and (*C*) When the seedlings were only stimulated or only subjected to calcium depletion, practically no meristems were produced. (*D*) When calcium depletion was delayed relative to plant stimulation, the production of meristems was correspondingly delayed. (*E*) When the seedlings were subjected to calcium depletion before being stimulated, meristem production started immediately after the stimulation. Figure drawn from data in Verdus et al. (1997, 2011).

lings were subjected to 2-day calcium excess on D8 to D10 (a time when they were actively producing meristems), the meristem production was rapidly and severely inhibited (with a 4-day Ca^{2+} excess, the inhibition of meristem production would have been total [not shown]). When seedlings, previously stimulated and subjected to Ca^{2+} depletion followed by Ca^{2+} excess, were subjected to another 2-day Ca^{2+} depletion, a second round of meristem production occurred. Therefore, after storage of “meristem-production” information (activation of the STO function following a stimulus), Ca^{2+} depletion and excess can reversibly switch the RCL function “on” and “off”. The experiments in Figures 4 and 5 also show that stored information can be recalled and take effect at least twice.

Meristem-production information is stored after stimuli caused by manipulation, drought, wind, cold shock and even low-intensity electromagnetic radiation at 0.90 GHz (from a mobile telephone) or at 105 GHz (from a Gunn oscillator) (Tafforeau et al. 2002b and 2004). Alain Vian, Françoise Paladian and their collaborators, working in the University of Clermont-Ferrand in France, have confirmed that plants are sensitive to electromagnetic radiation (Roux et al. 2006, Vian et al. 2006). Plant sensitivity to electromagnetic radiation in the approximate range 1–100 GHz is hard to understand. Mobile phones are far too recent for

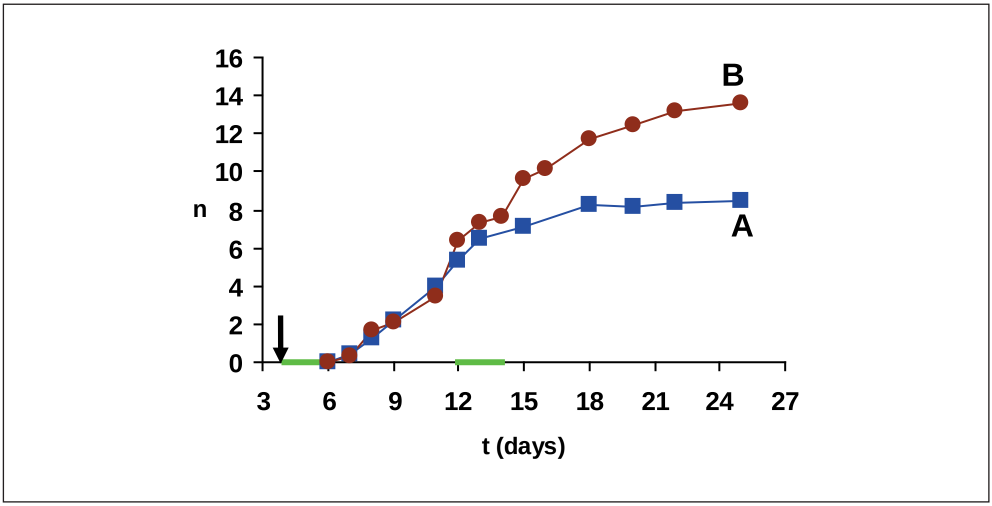

Fig. 4 Mean number, n, of meristems produced per seedling as a function of the time, t (days), when subjecting the seedlings to repeated 2-day Ca^{2+} depletions. The seedlings were all stimulated on D_4 (vertical arrow), and then they were subjected to transient Ca^{2+} depletion (thick green horizontal dash) either (*A*) only once (from D_4 to D_6) or (*B*) twice (from D_4 to D_6 and D_{12} to D_{14}). A second round of meristem production occurred after the second period of calcium depletion. Modified from Verdus et al. (2012).

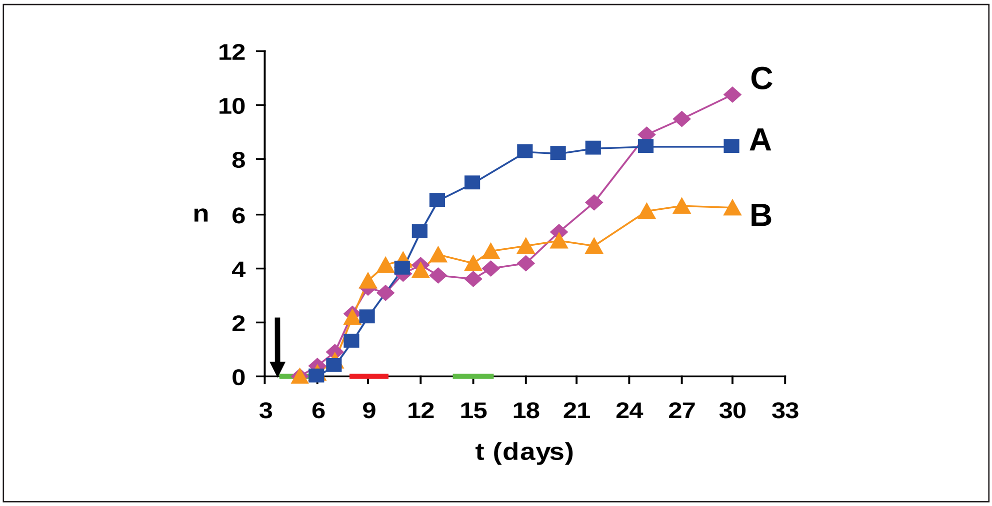

Fig. 5 Mean number, n, of meristems produced per seedling as a function of the time, t (days), when subjecting the seedlings to combinations of 2-day Ca^{2+} depletion and Ca^{2+} excess. The seedlings were all stimulated on D_4 (*vertical arrow*) and then they were subjected to transient Ca^{2+} depletion (thick green horizontal dash) and/or transient Ca^{2+} excess (thick red horizontal dash). (*A*) Control: stimulated seedlings subjected to a single Ca^{2+} depletion from D_4 to D_6 (the same control as curve *a* in Fig. 4). (*B*) Stimulated seedlings subjected to Ca^{2+} depletion from D_4 to D_6 and to Ca^{2+} excess from D_8 to D_{10}. (*C*) Stimulated seedlings subjected to Ca^{2+} depletion from D_4 to D_6, to Ca^{2+} excess from D_8 to D_{10} and again to Ca^{2+} depletion from D_{14} to D_{16}. The period of Ca^{2+} excess severely inhibited meristem production, but after the second Ca^{2+} depletion a second round of meristem production occurred. Modified from Verdus et al. (2012).

plants to have had the time to adapt to the radiation emitted by them. It is unclear whether there is a natural stimulus or process that might lead to plants being able to detect or use radiation at such frequencies and, at present, we cannot explain this sensitivity. We can only note that plants do perceive electromagnetic radiation at these frequencies and that nothing, in the experiments performed by us and by the group in Clermont-Ferrand, has indicated that this radiation has any adverse effect on plants.

Repeated stimuli increase meristem production. Storage of meristem-production information was inhibited by addition to the nutrient media of pharmacological agents that affect Ca^{2+} availability and transport (Verdus et al. 2007), consistent with the results of others (see the second in Section 2) that the perception of a stimulus is associated with cell Ca^{2+} uptake. Use of the same pharmacological agents revealed a period of vulnerability during information storage that was less than 2 min for mechanical stimuli and over 5 min for other abiotic stimuli (Verdus et al. 2007); in higher animals, memory for facts and events is not acquired in a stabilized form but undergoes a process of consolidation over time (Dudai 2004, Lesburguères et al. 2011); the same thus seems to be true in plants; moreover, in plants, the storage of mechanical stimuli would be consolidated particularly fast. We have seen above (Figs. 4 and 5) that recall was enabled by transient Ca^{2+} depletion and disabled by transient Ca^{2+} excess; no other treatments were found to affect RCL. Ca^{2+} depletion effective in RCL required Ca^{2+} levels of less than 0.23 mM, while the normal Ca^{2+} concentration of the growth medium is 2.33 mM (Verdus et al 2012). In the April-June period, the *total number* of meristems increases greatly; but the *ratio* of the number of meristems produced in stimulated, Ca^{2+}-depleted seedlings to that in non-stimulated or non-Ca^{2+}-depleted controls stays around 10.

The proteome of the flax seedlings was studied by 2D gel electrophoresis (Tafforeau et al. 2002a, 2006). Seedling stimulation (leading to information storage), Ca^{2+} depletion (enabling the recall of stored information) or both were responsible for proteome modifications: some spots moved, others disappeared and new spots appeared. Among these changes, some were transient whilst others were long lasting and some were specific to one particular type of stimulus whilst others were common to several different stimuli. The $^{31}P/^{12}C$ ratio in the protein spot "Touch 1" was measured by SIMS at different times after the stimulus and was correlated with the position of that spot relative to neighboring spots at each time-point, indicating that a transient displacement of "Touch 1", which was observed following subjecting the seedlings to manipulation stimulus, was caused by the transient phosphorylation of that protein (Tafforeau 2002, Tafforeau et al. 2002a, 2006). For a detailed study of gene expression in tomato plants subjected to electromagnetic radiation, see Vian et al. (2006).

4.4 Increased Peroxidase Activities

In tissue cultures prepared from stimulated *Bryonia* internodes (see the third in Section 2), the increases in the different peroxidase activities were maintained for several months, depending on the type of peroxidase, before progressively returning to the level before stimulation (Bourgeade et al. 1989). In these experiments, it is as if the information to increase peroxidase activity was stored in plants spontaneously able to recall that information (RCL function blocked "on"). When a peroxidase activity returned to the level before stimulation, it is not known if this was because the plants lost the stored information or because they became unable to recall that information (RCL function blocked "off").

5. Reflection and Perspectives

To interpret the above data, we first reflect on the nature of the constraints on plants. In the light of these reflections, we then propose a descriptive model of plant memory; this model is based on the interaction between two genes (or groups of genes) and the physico-chemical properties of the Ca^{2+} ion, in particular, Ca^{2+} waves and Ca^{2+} condensation. Finally, we discuss the ecological significance of plant memory and draw a brief comparison between plant and animal memory.

5.1 What is Vital for a Plant

It is vital for a plant not to produce a myriad of different responses to as many different stimuli. It is vital for a plant to produce a phenotype that is coherent with itself and with the environment. To achieve such coherence, a plant has to share its (usually limited) resources between different functions, especially defense (against biotic and abiotic aggressions) and growth (HERMS and MATTSON 1992, GAYLER et al. 2006, 2008, GAYLER 2010). It is therefore vital for a plant to have mechanisms that can generate coherence and control resource allocation.

5.2 Calcium Waves

Ca^{2+} waves (TREWAVAS 1999) are involved in the transient elevation of cytosolic Ca^{2+} that occurs on perception of a stimulus by a plant (see the second in Section 2). In the cell cytosol, the Ca^{2+} concentration is normally very low, but the cell possesses Ca^{2+} stores (wall, vacuole, etc.) that are connected with the cytosol *via* IP3-dependent channels (IP3 = inositol trisphosphate). These channels are normally closed. After a stimulus, the channels open according to an autocatalytic process involving IP3. The Ca^{2+} released at the mouth of an open channel tends to open the neighboring channels. A wave of Ca^{2+} enters the cytosol. This transient elevation of cytosolic Ca^{2+} has the consequences indicated above (see the second in Section 2) and, more precisely, leads to responses such as the opening of ionic channels and Ca^{2+}-dependent gene expression (KNIGHT et al. 1998, TREWAVAS 1999). The opening of Cl^- and Na^+ channels may contribute to the propagation of electric signals involved in the induction of responses distant from the stimulated area. The kinetics and magnitude of the transient elevation of cytosolic Ca^{2+} are different for different stimuli and are thought to orient the system towards different possible responses (DOLMETSCH et al. 1997, MCAINSH and HETHERINGTON 1998, KNIGHT et al. 1998, SANDERS 2002, NGO and MCAINSH 2003). Since information storage occurs in the minutes following stimulus perception (VERDUS et al. 2007), we may reasonably assume that it is somehow related to the Ca^{2+} wave. Beside all these effects, the Ca^{2+} wave activates ATPases that pump Ca^{2+} back to the stores, and the channels close.

5.3 Counter-ion Condensation

Consider (Fig. 6) a 1D-structure bearing negative charges and bathing in a solution containing co-ions (here negative ions) and counter-ions (here positive ions) that are either divalent or univalent. When the density of anionic sites, ξ_0, on the 1D-structure is low, the co- and counter-ions diffuse freely in a sort of ionic atmosphere. If some event causes ξ_0 to increase to a value, ξ, which is above a critical value, ξ_c, counter-ions “condense” on the 1D-structure until ξ decreases back to the critical value, ξ_c. Divalent ions condense before univalent ions. Condensed ions are free to move along the charged 1D-structure, but, even at infinite dilution, they remain in the close vicinity of this structure (MANNING 1969). This threshold-dependent phenomenon does not obey the mass-action law and may result in abrupt modifications to the relative concentrations of condensed and free Ca^{2+}.

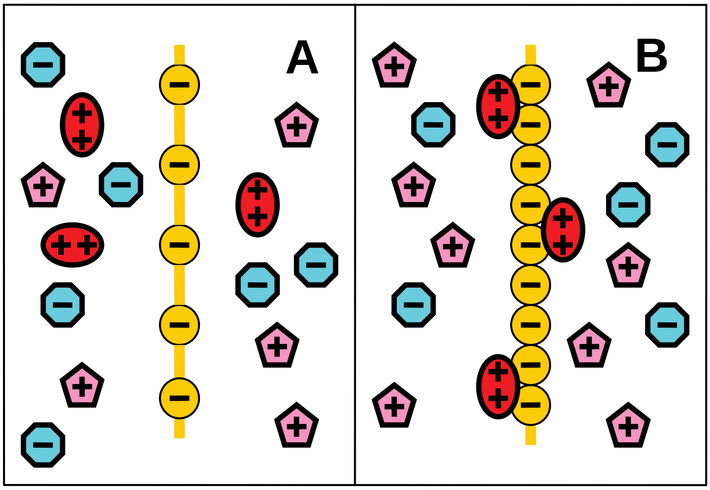

Fig. 6 The process of counter-ion condensation. A 1D-structure bearing negative charges is bathing in a solution containing negative and positive inorganic ions (co-ions and counter-ions, respectively). (*a*) When the charge density of the 1D-structure is low, the inorganic ions diffuse freely in the solution. (*b*) When the charge density of the 1D-structure is above a critical value, counter-ions "condense" on this structure until its charge density becomes equal to the critical value. The divalent counter-ions condense before the univalent. Symbols: circles = charges on the 1D-structure, octogons = free inorganic co-ions, pentagons = free univalent counter-ions, ellipses = free divalent counter-ions.

Cells contain networks of ionized, negatively charged 1D-structures (e.g. nucleic acids, cytoskeleton, 1D-assemblies of proteins) on which counter-ions may condense (or from which they may decondense) (RIPOLL et al. 2004). In our working hypothesis, we assume that Ca^{2+} condensation/decondensation plays a decisive part in the recall of stored information by plants *via* the activation/inactivation of Ca^{2+}-dependent processes (including kinases and phosphatases). When Ca^{2+} is condensed, the Ca^{2+}-dependent processes that are physically associated with the charged 1D-structures are activated, while those in the bulk are inactivated; reciprocally, when Ca^{2+} is not condensed, the processes associated with the 1D-structures are inactivated while those in the bulk are activated. A modification of the ξ-value (making it larger or smaller than ξ_c) may thus act as a switch in the overall functioning of the system.

5.4 *Modeling Plant Memory*

A mechanistic model of plant memory is shown in Figure 7. The functioning of the "habituation" form of memory can be explained if the Ca^{2+} wave exerts a negative (and probably indirect) effect on its own production following the perception of an appropriate stimulus. The functioning of the STO/RCL form of memory is explained below.

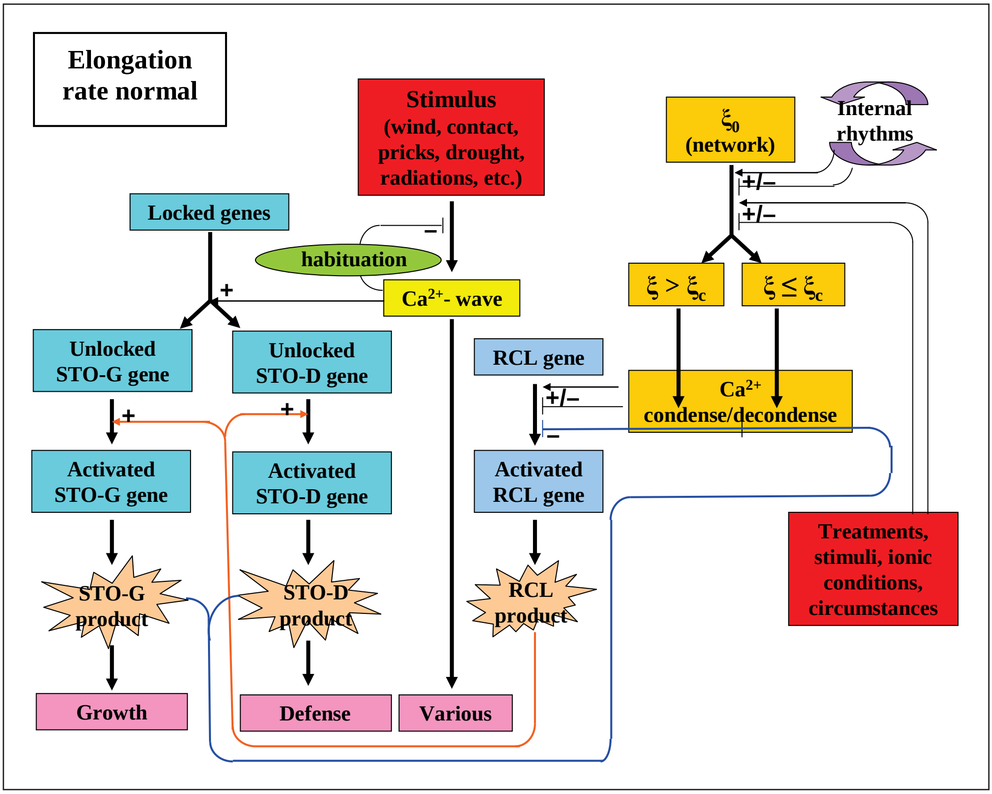

Fig. 7 A mechanistic model of plant memory. In this model, the functioning of the memory system is based on an interaction between two genes: the "STO gene" and the "RCL gene". Initially, the STO gene is locked off. A variety of stimuli can unlock it, for instance by local DNA decompaction and probably *via* a series of steps. This change is not easily reversible, which permits robust storage of the information that the plant has perceived by the stimulus. To generate its product, the STO gene requires the presence of a suitable activator. The RCL gene is potentially functional (unlocked) but it requires activation (or derepression) to generate its product. The expression of the RCL gene is modulated positively or negatively by various biotic and abiotic stimuli, interactions with internal rhythms and the ionic conditions. The RCL product is an activator for the STO gene and the STO product is a repressor for the RCL gene. The STO and RCL products are progressively degraded; therefore they are not present in the system when they are not produced. The presence of the STO product allows the ultimate, morphogenetic or metabolic effects to take place, for instance in the relative allocation of resources to growth and defense. Symbols: G = growth, D = defense, thick arrow = implication, thin lines = positive or negative regulations; for charge densities ξ, ξ_0 and ξ_c, see text (Section 5.3).

5.4.1 Information Storage

Two salient characteristics of information storage are taken into account in our model. The first is that an irreversible or poorly reversible alteration of the genetic material is likely to be responsible for the storage of information, given its robustness. The second is that such storage of information helps resources to be allocated optimally after the perception of many different stimuli by the plant.

In differentiated cells, a number of genes are locked off (e.g. by histones) in a compacted form of DNA. When a plant perceives a stimulus (wind, contact, prick, etc.), the characteristic features of the Ca^{2+} wave are known to depend on the nature of the initial stimulus and to induce various cellular processes involved, for example, in growth or defense. In a first approach of the question, let us assume that (*i*) the Ca^{2+} wave unlocks only one of two genes (or of two groups of genes) involved in the control of growth (G-gene) or defense (D-gene) and that (*ii*) the expression of these genes requires the binding of a suitable activator (see below, Section 5.4.2). When the activator is present, the unlocked, activated (G or D) gene is transcribed and translated to yield a product that leads to growth or defense, respectively. When the activator is absent, the (G or D) gene remains unlocked thereby storing the information not only that there has been a stimulus but also that the nature of the appropriate response to this stimulus is G or D; however, this gene is not transcribed and translated until the activator becomes available. We thus term these G or D genes STO-G and STO-D genes.

5.4.2 Information Recall

Let ξ_0 be the density of ionisable sites on the network of cellular 1D-structures. Various factors (e.g. internal rhythms, ionic concentrations, external conditions or treatments) possibly favoring the induction of reactions such as protonation/deprotonation or methylation/demethylation, may cause the effective charge density, ξ, to become different from ξ_0. According to whether $\xi > \xi_c$ or $\xi \leq \xi_c$, Ca^{2+} is condensed or free. Let us assume that there exists a gene (or group of genes) of which (*i*) the activation is favored by Ca^{2+} condensation and the inactivation by Ca^{2+} decondensation, or inversely, and (*ii*) the product is an activator of the STO genes. We term this gene an RCL gene: when it is activated, the unlocked STO-G or STO-D gene is expressed, ultimately promoting growth or defense. If ξ is not too different from ξ_c, it is easy to increase or decrease it reversibly above or below ξ_c; hence it is easy to render the plants reversibly able/unable to recall stored information. If ξ is very different from ξ_c, then the RCL function seems to be blocked “on” or “off”.

Let us also assume that (*i*) the RCL, STO-G and STO-D products undergo degradation or dilution in such a way that they are not present provided they are not produced and (*ii*) the STO-G or STO-D product has a negative, relatively slow effect on the activation of the RCL gene (*via* an effect on Ca^{2+} condensation/decondensation). Considering for instance our third system (Section 4.3), this means that, firstly, meristem production is limited with time because the RCL, STO-G and STO-D products progressively disappear and, secondly, meristem production can be induced repeatedly (i.e. stored information repeatedly recalled) by manipulating Ca^{2+} condensation/decondensation in such a way as to activate, inactivate, activate again, etc. the RCL gene.

5.5 Suggestions for Further Work

Our mechanistic model of plant memory is still far from complete. It requires: (*i*) identifying the STO and RCL genes (*ii*) evidencing that the Ca^{2+} waves really act on the STO function and Ca^{2+} condensation/decondensation on the RCL function, (showing that the Ca^{2+} wave encodes the final phenotype (e.g. growth or defense) rather than (*iii*) the various initial stimuli, (*iv*) checking if growth and defense are really the only two possible final responses or if more diverse final responses may occur, (*v*) learning whether it is Ca^{2+} condensation or Ca^{2+} decondensation that has a positive effect on the activation of the RCL gene and (*vi*) elucidating the pathways between the initial stimulus and the Ca^{2+} wave, between the Ca^{2+} wave and STO gene activation, between the STO products and the ultimate metabolic or morphogenetic effects, between the treatments, circumstances or internal rhythms and Ca^{2+} condensation/decondensation, between Ca^{2+} condensation/decondensation and RCL gene activation, etc.

Finally, in order to make experimentally testable predictions, there is a need for a mathematical modeling of the functioning of plant memory. In such modeling, our mechanistic model could serve as the basis for new mathematical approaches along the lines of those previously undertaken (Kergosien et al. 1979, Desbiez et al. 1994, Demongeot et al. 2000, 2006, Thellier et al. 2004) in collaboration with Yannick Kergosien (catastrophe theory), Donald Mikulecky (diffusion-reaction systems), Jacques Demongeot (differential continuous formulation) and René Thomas (logical, discrete formulation). Moreover, the dynamical model PLATHO (Gayler et al. 2006) could be used for the numerical simulation of plant growth and the production of defense molecules under various environmental conditions.

5.6 Ecological Significance of Plant Memory

In our approach of plant memory as described above, the experiments have been carried out on developing, aerial parts of often very young herbaceous plants grown under controlled laboratory conditions. Therefore, it is not known if the STO/RCL form of memory also exists in trees, in adult herb tissues or in roots. Moreover, the responses that we have observed correspond to plants subjected to a single stimulus, or to a restricted number of well-defined stimuli, which is a situation totally different from that of plants subjected to a variety of changing stimuli and stresses under natural conditions. With these limitations in mind, we may, however, begin to discuss the ecological significance of the plant memory mechanisms.

Since internal rhythms interfere with the STO and RCL functions (see Fig. 2 and the third in Section 4.2.3), this might mean that an internal clock is involved in plant memory. In particular, seasonal ionic (especially Ca^{2+}) rhythms could help control the recall of stored metabolic or morphogenetic information, *via* the sensitivity of the RCL function to the ionic (especially the Ca^{2+}) conditions (see Section 4.1 and the first in Sections 4.2.3, 4.3.1 and 5.4.2).

In Nature, in which conditions often change, it is unlikely that plants allocate all their resources to either growth or defense. On the contrary, it is likely that plants allocate their resources to these functions in a balanced, complex and integrated way depending on the pattern of environmental stimuli, stresses and aggressions. There is a clear role of the STO/RCL mechanism in attaining this balanced allocation. Firstly, the mechanism could affect responses other than those directly involved in growth or defense; for instance, a response such as the opening of Cl^- and Na^+ channels may contribute to the propagation of electric signals, possibly including the signals involved in the transfer of information from stimulated to reactive areas. Secondly, the STO/RCL mechanism could integrate several possible growth responses or several possible defense responses. Evidence for varied responses include (*i*) control over particular aspects of growth in the STO/RCL form of memory (see Section 4) rather than growth in general, namely hypocotyl or stem elongation (see Section 4.1 and third in Section 2), precedence between cotyledonary buds (see Section 4.2) and meristem production (see Section 4.3) and (*ii*) the expression of specific stress-induced genes in the habituation of *Arabidopsis thaliana* plants to hyperosmotic stimuli (Knight et al 1998), rather than the promotion of defense in general.

More generally, in a sense the ecological significance of plant memory reflects the importance of plants in the ecosystem. Terrestrial ecosystems usually depend on which plant species survive and proliferate; this, we would argue, depends on their possession of memory insofar as the habituation and STO/RCL forms of memory allow plants to adapt to both environmental stresses and opportunities. An obvious example would be if the STO/RCL memory were to allow plants to remember the nature and frequency of attacks by particular predators, such as insect pests, so that by integrating this information over time they were able to develop a more effective defense. A somewhat different example would be if the STO/RCL memory were to allow a seasonal memory such that the plant could remember in the spring which species had been growing next to them the previous autumn and adapt accordingly (e.g. anticipating that the neighboring plant would have a thick foliage in summer that would put it in the shade).

5.7 Plant Memory versus Animal Memory

There are a number of striking resemblances between plant and animal memory. In particular, the transfer of information is associated with the propagation of an electric depolarization whilst the storage of this information is associated with a period of consolidation before storage becomes robust and this stored information can be repeatedly recalled. The mechanisms involved, however, are clearly different since plants have no neurons let alone the equivalent to the specialized brain tissues (hippocampus, neocortical areas) that are central to the memory of higher animals (Wang and Morris 2010). The relative simplicity of plant tissues compared with the brain of higher animals may make it easier to elucidate the basic biophysical processes needed to memorize a piece of information.

The study of plant memory could thus contribute to our understanding of memory in general.

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Section I
The Objects: Hosts, Pathogens and Symbionts

Nova Acta Leopoldina NF *114*, Nr. 391, 45–54 (2013)

Resistance of Spruce to *Heterobasidion*: A Realistic Proposition?

Stephen Woodward (Aberdeen, UK)

With 7 Figures

Abstract

Heterobasidion spp. cause the largest timber losses of all pathogens in European forestry. Various techniques can be used to reduce losses, although in long-managed forests, options are very limited. The most effective way of controlling any plant disease is through the use of resistant hosts, but whether such an approach would be useful against long-lived and persistent root diseases of trees is debatable. Work in the UK over the last 25 years has focused on understanding chemical and physical defense responses of Sitka spruce to *Heterobasidion annosum*. Inoculation of clonal materials has demonstrated that variation in susceptibility to the pathogen is present in Sitka spruce. The tree, therefore, deploys a range of defenses in order to reduce the chance of pathogen ingress.

In this paper, the constitutive defenses in bark tissues of *Picea* species are contrasted with the induced defenses that occur following mechanical damage and/or pathogen attack. The potential roles of terpenoid and phenolic compounds in defense are discussed, in terms of utility as markers for relative host susceptibility to *H. annosum*. Concurrent with the deployment of chemical defenses, a cascade of events is initiated leading initially to production of a ligno-suberized boundary zone (LSZ) in cells extant at the time of wounding and/or pathogen attack. The LSZ enables de-differentiation of bark cortex and secondary phloem to produce a trauma-induced phellogen (wound phellogen) tissue; this generative tissue re-establishes the integrity of the periderm, producing wound phelloderm to the interior and wound phellem to the exterior. The application of metabolomics and genomics are increasing our understanding of the deployment of defenses in clones of Sitka spruce showing different levels of susceptibility to *Heterobasidion.*

Zusammenfassung

Von allen Pathogenen verursacht *Heterobasidion* spp. die größten Schäden in der europäischen Forstwirtschaft. Zur Reduktion dieser Schäden können verschiedene Maßnahmen getroffen werden, die aber überwiegend in Wirtschaftswäldern nur begrenzt einsetzbar sind. Der effektivste Weg, Pflanzenkrankheiten zu kontrollieren, ist der Einsatz resistenter Wirte. Ob dies jedoch auch gegen langlebige und persistente Wurzelpathogene bei Bäumen erfolgreich ist, wird derzeit kontrovers diskutiert. Untersuchungen in Großbritannien während der vergangenen 25 Jahre waren vor allem auf das Verständnis der chemischen und physikalischen Abwehrreaktionen der Sitka-Fichte gegen *Heterobasidion annosum* ausgerichtet. Durch die Inokulation von Klonen mit diesem Pathogen konnte eine hohe Variabilität der Empfindlichkeit der Sitka-Fichte nachgewiesen werden. Diese Art hat somit eine Reihe von Abwehrmechanismen entwickelt, um die Chance des Befalls mit dem Pathogen zu reduzieren.

Im vorliegenden Beitrag werden konstitutive Abwehrmechanismen der Rinde von *Picea*-Arten den induzierten Abwehrmechanismen, die nach einem Befall bzw. einer Schädigung durch Pathogene vorliegen, gegenübergestellt. Die potenzielle Bedeutung von Terpenoiden und Phenolen für die Abwehr von Pathogenen wird in Hinblick auf ihre Eignung als Marker für die Empfindlichkeit von Wirten gegenüber *H. annosum* diskutiert. Zusammen mit der Entwicklung chemischer Abwehrmechanismen wird durch einen Pathogenbefall eine Kaskade von Reaktionen initiiert, die zur Bildung einer Ligno-Suberin-Grenzschicht (LSZ) führt. Die LSZ führt zu einer Dedifferenzierung des Rindencortex und ermöglicht die Bildung eines Wundphellogens durch das sekundäre Phloem. Dieses generative Gewebe stellt die Integrität des Periderms wieder her, in dem Wundphelloderm nach innen und Wundphellem nach außen produziert wird. Durch den Einsatz von „Metabolomics“- and „Genomics“-Ansätzen wird unser Verständnis der Entwicklung von Abwehrmechanismen in Klonen der Sitka-Fichte mit unterschiedlicher Empfindlichkeit gegenüber *Heterobasidion* verbessert.

1. Introduction

The genus *Heterobasidion* comprises species of hymenomycete pathogens that cause heart rot and root decay in trees. The main problems associated with these species are the large losses in timber of managed and unmanaged gymnosperm forest trees (Woodward et al. 1998). Until the late 1970s, it was considered a single species in Europe, known as *Heterobasidion annosum* (the name *Fomes annosus* was used for many years, but by the rules of taxonomic nomenclature, *H. annosum* took precedence). Losses to *Heterobasidion* species are associated with decay in spruce, larch and fir (and many other gymnosperm trees) and killing in pines (Korhonen and Stenlid 1998, Asiegbu et al. 2005). In 1998, it was estimated that the economic impact of this pathogen in the European Union (EU) amounted to some €700 million per annum (Woodward et al. 1998); since that time, with the accession of further states into the EU, losses are likely to exceed €1 billion per annum.

It has long been known that the most effective way to control plant diseases is through the planting of resistant lines. Because of the long life cycles and habit, however, pathogens affecting and causing decay in the secondary roots of trees, however, present an unusual challenge to plant pathologists searching for less susceptible host genotypes.

Life cycle: *Heterobasidion* spp. enter plantations when basidiospores infect fresh stump surfaces during thinning and clear felling operations (Fig. 1, Redfern and Stenlid 1998). Following germination and establishment, the fungus grows into the dying root system, eventually colonizing adjacent standing trees at points of root-to-root contact (Stenlid and Redfern 1998, Asiegbu et al. 2005). The pathogen spreads from the root system of the newly colonized tree, eventually reaching the main stem and causing extensive decay. After substantial colonization has occurred, fruit bodies may be produced, from which the basidiospores are released.

2. Testing Spruce for Resistance to *Heterobasidion* Species

There have been many reports of potential resistance of Norway spruce to *Heterobasidion* over a number of years, as clonal materials of this species were available in quantity from the 1960s (Delatour et al. 1998). Sitka spruce, however, proved less amenable to vegetative propagation, and it was not until the late 1990s that clones were available with sufficient replication to warrant testing for relative resistance in properly designed experiments (Lee 1999, 2001). The most commonly used assay for resistance has been the extent of lesion development following artificial inoculation of young, potted plants of 3 to 5 years (Swedjemark et al. 1998, 1999, 2001, Bodles et al. 2006, 2007). More recently, however, more mature host materials, similar to the trees that would become infected in the forest, have been tested (Swedjemark et al. 1998, 1999, 2001, Deflorio et al. 2011, 2012).

These tests have demonstrated that different clones do respond differently in terms of extension growth of the pathogen following inoculation (Fig. 2). In recent work on resistance of Sitka spruce to *Heterobasidion annosum*, the most susceptible and most resistant clones, based on measurements of lesion lengths, have been compared in order to determine differences in host response to wounding and inoculation.

Quantitative PCR has confirmed that estimates of resistance based on lesion length are accurate in terms of the relative growth of the pathogen in different spruce clones (Hietala

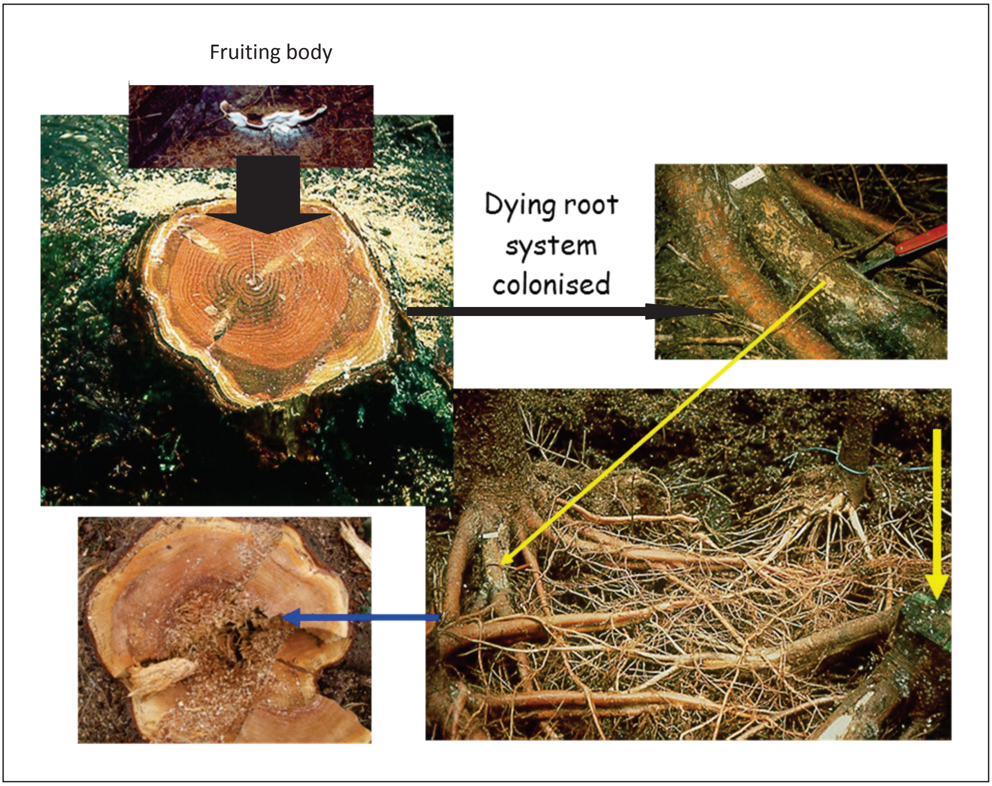

Fig. 1 Life cycle of *Heterobasidion*. Basidiospores released throughout the year colonize stumps of freshly felled trees. The pathogen grows down into the root system of the stumps and infection transfers to adjacent standing trees at points of root to root contact. Following growth through the root system of the standing tree, *H. annosum* causes severe decay in the heartwood, resulting in timber losses.

et al. 2003, Bodles et al. 2006), although the technique also proved that *H. annosum* grows far more rapidly and extensively in the sapwood of spruce than suggested by the extent of necrosis in the lesions (Fig. 3).

3. Host Defense Mechanisms

Defense of plants against pathogenic microorganisms can be divided into constitutive components, those present in the healthy host, and induced components, which result from the response of the plant to the detection of an invading pathogen. Understanding of these defenses in coniferous trees has caught up with the work on herbaceous angiosperms in the last ten years, with great insights into the operation of defense mechanisms in these ancient organisms.

Constitutive defenses: Trees, as long-lived organisms, require robust mechanisms to defend against pests and pathogens. Morphological defenses include the outer bark, the cuticle on foliage and young shoots, and the wood itself. The outer bark layers (periderm and rhytidome) and the cuticle contain large quantities of fats and fatty acids; in the rhytidome,

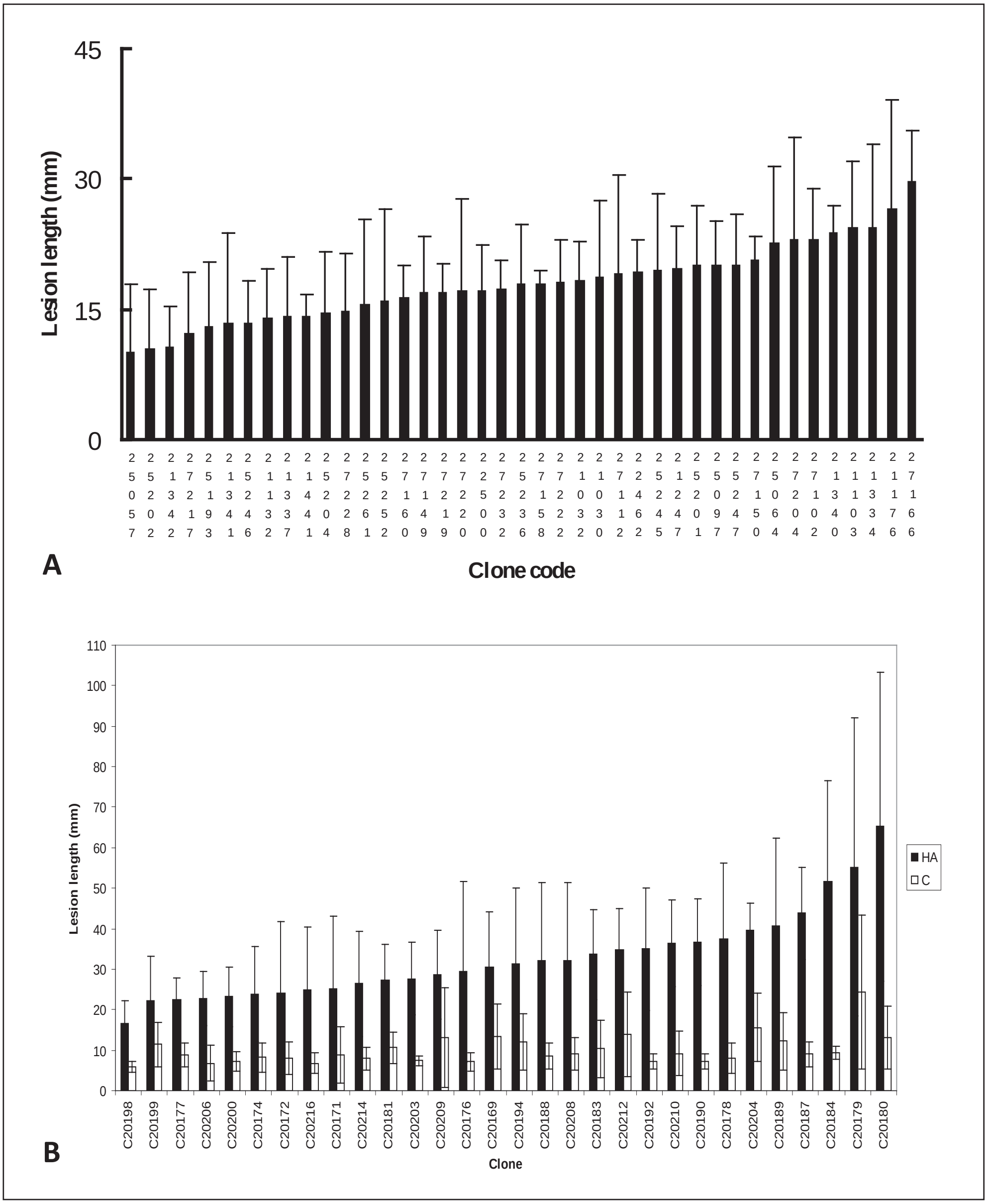

Fig. 2 Extent of lesions formed in Sitka spruce clones (*A*) 4 or (*B*) 20 years in age, following inoculation with *Heterobasidion annosum.*

the phellem includes layers of dead cells, the walls of which are impregnated with large quantities of suberin or lignin, both of which are highly recalcitrant to degradation by micro-organisms. Within the bark tissues, conifers produce abundant quantities of resins, mixtures of monoterpenes, with various di- and tri-terpenes and resin acids. In addition, the bark tis-

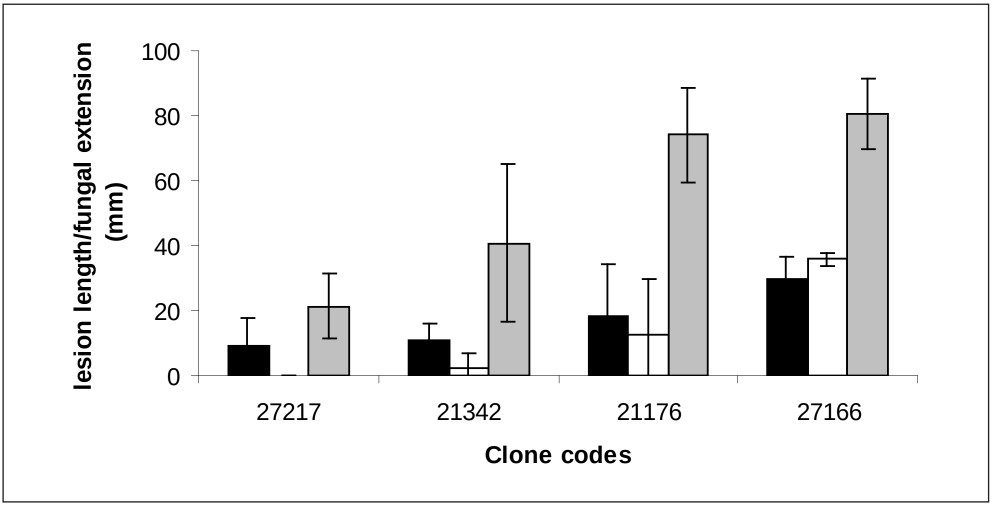

Fig. 3 Comparison between lesion lengths and fungal growth detected in bark and sapwood using RT-PCR, 42 days after inoculation of resistant (27 217, 21 342) or susceptible (21 176, 27 166) clones of Sitka spruce with *Heterobasidion annosum* (from Bodles et al. 2006). *Closed bar*: lesion extent based on visual measurement; *Open bar*: detection of *H. annosum* DNA in bark tissues; grey bar: detection of *H. annosum* DNA in sapwood.

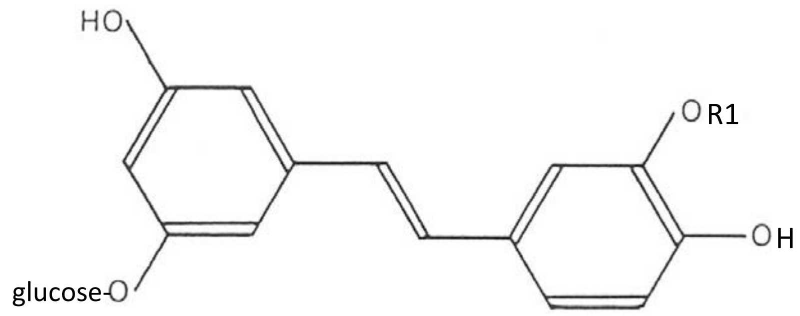

Fig. 4 Structures of the principal stilbene glucosides found in spruce bark. R1 = H, astringin; R1 = OH, isorhaponticin.

sues also contain large amounts of phenolic compounds; in spruce, the dominant phenolic compounds are stilbene glycosides, principally astringin and isorhapontin (Fig. 4) along with much smaller concentrations of piceid. In undamaged bark tissue, the stilbenes are stored in specialized polyphenolic parenchyma cells (Krekling et al. 2000, Fig. 5*A*, *B*), presumably to avoid causing damage to the plant itself.

Immediately on wounding, the resins and stilbenes provide a rapidly-deployed defensive response. The stilbenes are released from the polyphenolic parenchyma, presumably by the physical damage itself. It is likely that the stilbene aglycones, astringenin and isorhapontigenin, are released as soon as the tissue damage occurs by host-produced β-glucosidases.

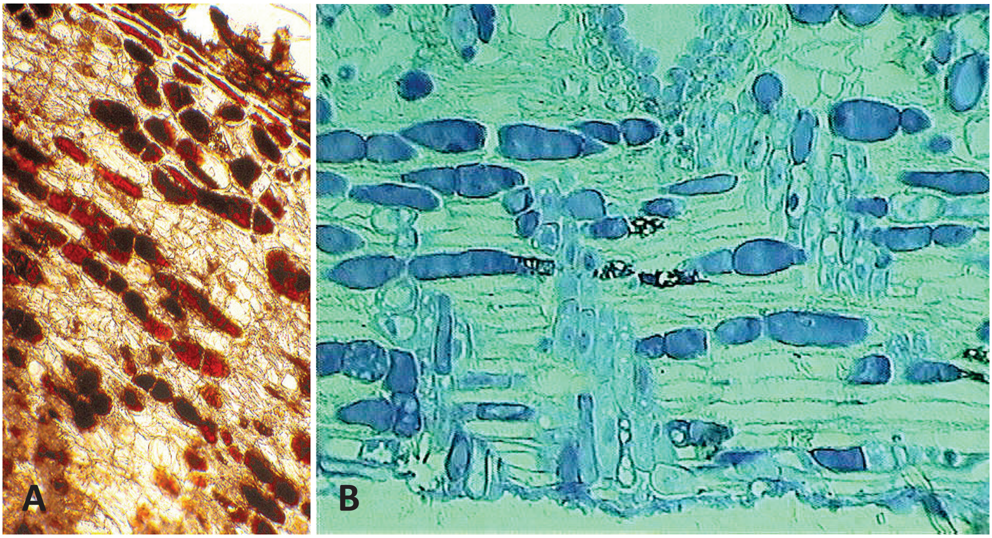

Fig. 5 Localization of stilbenes in healthy bark of Sitka spruce. The darkly staining swollen cells are the polyphenolic parenchyma, where the stilbene glycosides are stored. (*A*) 40 year old tree; (*B*) 3 year old tree. [Figure 5*A* courtesy of Dr. Claudia Toscano Underwood].

These aglycones are an order of magnitude more fungitoxic than the equivalent glycones (Woodward and Pearce 1988a). Moreover, the stilbene aglycones are thought to oxidize and polymerize, rapidly forming a phenolic barrier over the wounded tissues (Woodward and Jaspars, unpublished). At the same time, resins flow into the wounded area from the resin canals present in the bark; over a number of days, the monoterpenes volatilize and the resin hardens to form a rigid barrier to both pathogen ingress and to water loss from the damaged tissues (Gibbs 1968). Apart from this physical barrier presented by resin, the monoterpene components may also inhibit fungal growth directly (Cobb et al. 1968, Gibbs 1972, Henriks et al. 1980).

Induced defenses: Active host defenses against pathogens also include both chemical and physical components (e.g. Krekling et al. 2004, Nagy et al. 2004, Franceschi et al. 2005). Following wounding and/or infection of bark, cell death occurs close to the damaged tissues, with lignification and suberin deposition leading to formation of a discrete ligno-suberized boundary zone (LSZ) in cells extant at the time of wounding (Woodward and Pearce 1988b, Woodward 1992a, Solla et al. 2002, and Woodward et al. 2007). In Sitka spruce, the LSZ is first detectable within approx. 7 days of wounding (Woodward, unpublished), but continues to develop for up to 28 days. The wound periderm forms internal to the LSZ, from re-differentiated phelloderm and secondary phloem cells, eventually restoring the integrity of the damaged tissue (Woodward and Pearce 1988b; Fig. 6).

The molecular control of LSZ formation has received considerable interest, due to the possible role this tissue plays in resistance to canker and root-invading pathogens (Asiegbu et al. 1998, Spanos et al. 1999, Solla et al. 2002). The accumulation of phosphorus in the zone where the LSZ/wound periderm are forming (Fig. 7, Siebold et al. 2012) indicates the high energy input that is invested in raising these barriers. Work on the development of LSZ and

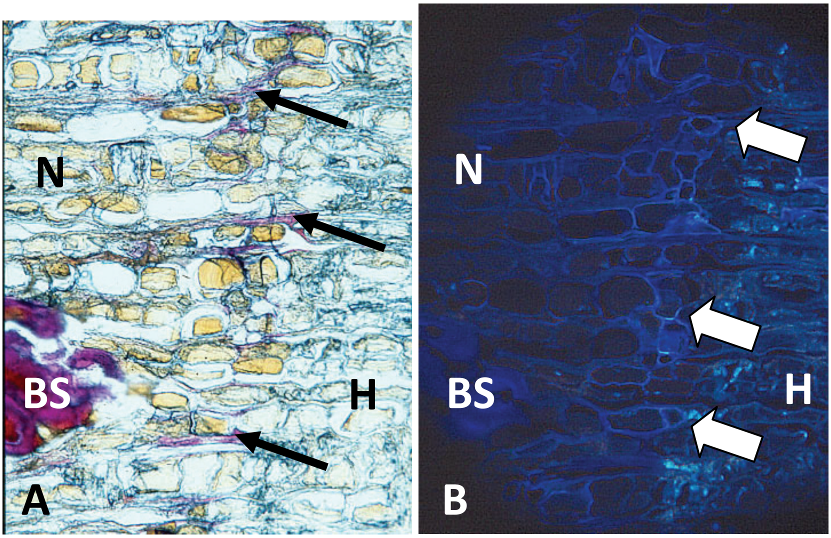

Fig. 6 Formation of the ligno-suberized boundary zone (LSZ; arrows) between the healthy (H) and necrotic (N) tissues in bark of mature Sitka spruce 16 days after wounding. The precise position of the LSZ can be determined by staining the section with phloroglucinol-HCl and viewing under incident (*A*) or UV light using 410 nm barrier filters (*B*). (*A*) Lignin in the boundary zone stains pink; (*B*) phloroglucinol-HCl quenches the autofluorescence of lignin but has no effect on the fluorescence of suberin. The large cell cluster staining positive for lignin is a brachysclereid (BS).

wound periderm (WP) in Sitka spruce suggested that the speed of formation of these barriers may be related to relative host resistance to root pathogens such as *Armillaria ostoyae* and *Heterobasidion annosum* (Solla et al. 2002), and that aggressive pathogens may disrupt the development of the barriers.

Resin production may also play a significant role in defense after wounding and infection. Additional traumatic resin canals (TRD) form (Christiansen et al. 1999, Franceschi et al. 2000, Nagy et al. 2000, Krekling et al. 2004) and the composition of the resin itself changes (Woodward et al. 2007). Although it is unlikely that the resin monoterpenes play a major role in defense against pathogens well-adapted to infection of spruce, these changes in resin profiles following *H. annosum* infection have been suggested as markers for the level of resistance to the pathogen (Woodward et al. 2007).

Recent work has demonstrated that several genes for the enzymes involved in the phenylpropanoid pathway and other defensive proteins are up-regulated rapidly after wounding in spruce and inoculation with *H. parviporum* or *H. annosum* (Fossdal et al. 2007, Deflorio et al. 2011). Three days after wounding and inoculation of Sitka spruce clones, genes encoding phenylalanine ammonia lyase (PAL), cinnamoyl-CoA reductase (CCR1), hydroxycinnamoyl-transferase (HCT1), cinnamyl alcohol dehydrogenase (CAD), peroxidase (PaPX3) and class IV chitinase (PaCHI4) were up-regulated in the bark tissues immediately surrounding the point of wounding and inoculation, but at 10 mm from the inoculation point, only CAD was up-regulated. In contrast, all genes except PaPX3 and PaCHI4, were down-regulated around

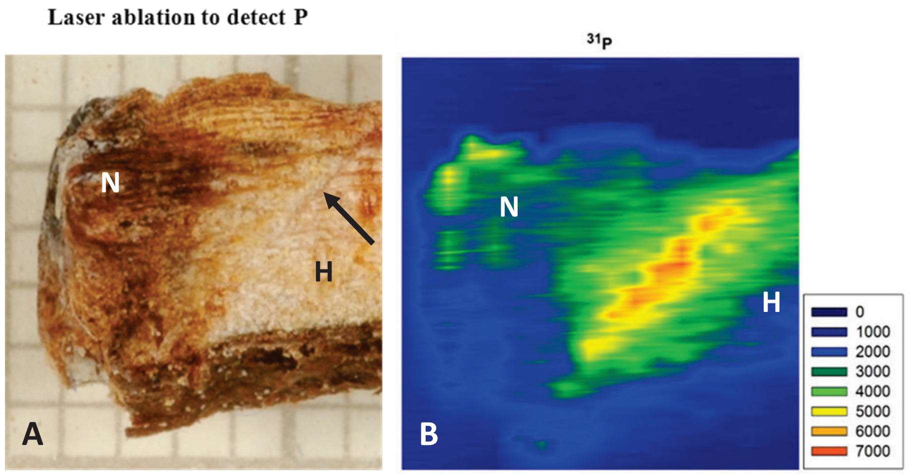

Fig. 7 Localization of the LSZ (arrow) and wound periderm in Sitka spruce bark 43 days after wounding using laser-ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). The wound was made to the left-hand side of the bark piece. (*A*) the LSZ is visible as a faint line crossing the tissue diagonally from top right to the left and separating the healthy (H) from the necrotic (N) tissue. (*B*) detection of high intensity of phosphorus in the LSZ-wound periderm by LA-ICP-MS suggests high energy use in the zone.

the point of wounding in sapwood. Bark was clearly more able than sapwood to respond to the treatments. However, there were few differences between putative resistant and susceptible clones of Sitka spruce.

4. Conclusions

Clearly, different clones of Sitka spruce show variations in susceptibility to extension growth by *H. annosum* in the bark and sapwood. Work over the last 25 years has given strong evidence that both chemical and morphological markers may be used to predict relative susceptibility of spruce clones at an early stage in the vegetative propagation cycle. Further work is required, however, before the full utility of these markers can be realized in routine applications. It is anticipated that the availability of the *Heterobasidion* genome (Olsen et al. 2012) will further increase the pace of research into this important pathogen, and the host responses to attack.

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Trade-offs in Host-Pathogen Interactions

Frank Fleischmann (Freising)

Abstract

Plant defense mechanisms against pathogens or parasites require plant internal carbon resources which might cause a trade-off between growth related and defense related metabolisms within the plant as predicted by the growth differentiation balance hypothesis (GDB) by Herms and Mattson (1992).

Within the SFB 607 we tested the significance of the GDB for defense against fungal pathogens of herbaceous and woody plants by changing the plant internal resource availability *via* variation of atmospheric CO_2 or O_3 and nitrogen fertilization.

Although we confirmed the existence of the predicted trade-off in most examined host-pathogen interactions, these trade-offs could not explain changes in plants susceptibility towards the corresponding pathogen in almost 50 % of the cases. Our results indicate, that the relevance of trade-offs between growth and defense related metabolism for the plants susceptibility strongly depends on the actual effective defense mechanism for the corresponding host-pathogen interaction, and on the scale of observation, as many defense mechanisms occur only locally surrounding the infection site.

Zusammenfassung

Die pflanzliche Abwehr gegen Pathogene und Schädlinge benötigt Kohlenstoffressourcen, was zu einem *Trade-off* zwischen Wachstums- und Abwehrprozessen in der Pflanze führen kann, wie dies die *Growth-Differentiation Balance*-Hypothese (GDB) von Herms und Mattson (1992) vorhersagt.

Im Rahmen des SFB 607 testeten wir die GDB in Bezug auf Abwehr gegenüber pilzlichen Pathogenen an krautigen und holzigen Pflanzen unter variabler pflanzeninterner Ressourcenverfügbarkeit. Dies wurde durch Variation des atmosphärischen CO_2 oder O_3 bzw. der Stickstoffdüngung erreicht.

Obwohl wir in den meisten Fällen den von der GDB vorhergesagten *Trade-off* bestätigen konnten, wurden dadurch nur in ca. 50 % der Fälle die beobachteten Veränderungen in der Anfälligkeit der Pflanzen gegenüber dem jeweiligen Pathogen erklärt. Unsere Ergebnisse weisen darauf hin, dass die Relevanz von *Trade-offs* zwischen Wachstums- und Abwehrprozessen für die pflanzliche Anfälligkeit stark von den tatsächlich wirksamen Abwehrmechanismen bei der jeweiligen Wirt-Pathogen-Interaktion abhängt und dass auch die Betrachtungsskala ausschlaggebend ist, da viele Abwehrreaktionen nur lokal um die Infektionsstelle auftreten.

1. Introduction

Plants are exposed to various biological stressors, like viruses, bacterial or fungal pathogens and herbivores, and higher plants developed a multitude of different mechanisms for their defense against these biological stressors. In general, defense mechanisms can be classified to be either constitutively expressed in healthy plants, or to be induced only upon pathogen attack. The former can be further divided into structural barriers and anti-microbial substances, such as phenolic compounds or tannins which directly inhibit the attacking pathogen or parasite, and into precursors of phytoanticipins (Van Etten et al. 1994), such as glycosides of stilbenes, saponines or glucosinolates. These precursors are inactive for plant defense in healthy plants, and will be enzymatically converted into toxic aglycons during pathogenesis. Induced defense mechanisms besides others include deposition of callose or lignin, the synthesis of pathogenesis-related proteins, of reactive oxygen species or of phytoalexins, which are "low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms" (Van Etten et al. 1994).

The synthesis of any defense mechanism requires carbon and/or nitrogen resources. This might cause a trade-off between plant growth and defense against pathogens or parasites, as both mechanisms compete for the same plant internal resources. Several hypotheses exist in literature about the nature of this trade-off as reviewed by Stamp (2003). According to her conclusion the expanded growth differentiation balance hypothesis (GDB) by Herms and Mattson (1992) is the most matured of these hypothesis, stating that any environmental factor that slows growth more than it slows photosynthesis can increase the resource availability for the defense related metabolism (Loomis 1932). The GDB focuses on so-called carbon based secondary compounds (CBSC) as defense mechanisms. This includes various classes of phenolic compounds as well as hydrolysable or condensed tannins. CBSCs belong to both, constitutive and induced defense mechanisms. The effectiveness of any defense mechanism strongly depends on the specific host pathogen interaction. As the GDB was originally developed for defense mechanisms against herbivores, its predictions mainly refer to constitutive defense' mechanisms. Within the SFB 607 (Matyssek et al. 2005) the predictions of the GDB were tested on host-pathogen interactions of fungal pathogens with herbaceous or woody host plants, where induced defense mechanisms in general dominate over constitutive ones.

2. Plant Defense under Changing Environmental Conditions

This manuscript summarizes the results obtained by several research groups of the SFB 607 regarding trade-offs between growth related and defense related metabolism of different host pathogen interactions between fungal pathogens and potato, apple or European beech as host plants, respectively. A more detailed review on this topic is given by Osswald et al. (2013). To test the GDB for the different host-pathogen interaction comparable environmental scenarios were applied to change the plant internal resource availability. Elevation of atmospheric concentrations of carbon dioxide or ozone were used to increase or decrease carbon availability, respectively. Different nitrogen fertilization regimes were applied to change the plant internal carbon-nitrogen balance. Soluble and cell wall bound phenolics were analyzed as surrogates for CBSCs in host plants. Besides this biochemical analysis, molecular analyses at transcript or protein level were performed to investigate induced defense mechanisms. Under growth promoting conditions a decrease in defense related metabolism was expected and, as a consequence, an increase in susceptibility against pathogens, and *vice versa*.

When studying the susceptible interaction of apple (cv. Golden Delicious) with the apple scab pathogen *Venturia inaequalis* all predictions of the GDB were confirmed (Leser and Treutter 2005). Increasing nitrogen fertilization promoted shoot growth and reduced the concentration of soluble phenolics in leaves. In parallel, susceptibility of leafs towards *V. inaequalis* increased with increasing N-fertilization. A similar response on nitrogen fertilization was observed in potato (cv. Indira) infested with the late blight pathogen *Phytophthora infestans* (Mittelstrass et al. 2006). However, susceptibility towards *Alternaria solani* – the causal agent of early blight of potato – decreased under the same growing conditions.

The latter observation was made for the majority of case studies performed on potato, apple and beech, where a trade-off between growth and defense related metabolism could be confirmed, but changes in plants susceptibility against fungal pathogens were not consistent with the observed trade-offs (Fleischmann et al. 2010, Leser and Treutter 2005, Mittelstrass 2004, Plessl et al. 2007). These results revealed that the analysis of CBSCs is not sufficient to explain changes in plants susceptibility towards fungal pathogens. In fact, different defense mechanisms are effective against late blight and early blight of potato, respectively (Fry 2007). The different responses of the hemi-biotrophic pathogen *P. infestans* and the necrotrophic *A. solani* on nitrogen fertilization might be explained by their different life strategies (Rotem 1994).

For the investigation of induced defense mechanisms transcript analyses were conducted for apple and potato using comparable macro-array techniques derived from pathogen enriched subtractive cDNA libraries. Using these techniques trade-offs between growth and defense related metabolism could also be confirmed at the molecular level (Ros et al. 2008, Zistler 2007). Induction of defense-related genes after pathogen attack led to a simultaneous repression of growth-related genes, mainly photosynthesis related genes. As these kinds of trade-offs occurred only after pathogen attack, they did not directly become manifested in growth parameters or biomass production. In general it was not possible to distinguish between reduced growth and loss of biomass by pathogen damage, respectively.

In contradiction to the GDB the induction of defense related gene expression was greater, the higher the susceptibility of host plants was. Again, similar observations were made for the potato – *P. infestans* (Ros et al. 2004, 2005) and the apple – *V. inaequalis* (Zistler 2007) interaction, when gene expression was calculated on leaf level. This discrepancy might be resolved when one assumes that induced defense responses primarily happen locally surrounding the infection site, while systemic responses will follow later on. Hence, when gene induction levels were re-calculated taking the actual infested leaf area into account, the induction of defense related genes in more resistant cultivars exceeded those in more susceptible cultivars by far (Osswald et al. in press). In consequence, trade-offs between growth related and defense related metabolism might be restricted to a few cell layers surrounding the infection site in highly resistant interactions. These trade-offs will hardly be traceable in total leaves not to mention in whole plant. Thus, the relevance of trade-offs strongly depends on the level of observation.

A completely different observation was made for European beech (*Fagus sylcatica*) infested with *Phytophthora plurivora*.[1] This highly aggressive root pathogen suppresses induced plant defense responses of beech very effectively as shown both on transcript and protein level (Schlink 2009, 2010). Under elevated CO_2 a strong increase in susceptibility was found in beech (Fleischmann et al. 2010), but hardly any trade-off between growth and constitutive CBSC levels was visible. The better pathogen growth might rather be the result of an higher carbohydrate content of roots under elevated CO_2, as sugar concentration can be a major force

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[1] *P. plurivora* was recently described as a new species of the genus *Phytophthora* after re-evaluation of isolates of *P. citricola* (Jung and Burgess 2009). Thus, original publications of the SFB 607 regarding this host-pathogen interactions used the pathogens old name *P. citricola*.

for the growth of *Phytophthora* (Henkel et al. 2012). Many of the beech plants managed to cope with a high infestation rate of *P. plurivora* for at least two vegetation periods. These plants were characterized by higher photosynthesis rates, by smaller root systems and by an increased specific root tip density (Fleischmann et al. 2010). Hence, these plants managed to tolerate the root infection by reducing the size of the organ targeted by the pathogen, and by increasing the carbon gain, which allows re-growth of destroyed root material without an apparent reduction of total biomass production. To compensate the reduced size of roots, their morphology changed to increase their efficiency. Such changes are not covered by the predictions of the GDB, and offer a new strategy for the adaptation of plants to pathogen attack.

3. Conclusions

The different investigations regarding host-pathogen interactions within the SFB 607 demonstrated the existence of trade-offs between growth related and defense related metabolism of plants in many cases, as it is predicted by the GDB. These observations were made not only on whole plant level, but also on the molecular level. However, these trade-offs were not able to explain changes in susceptibility in many cases. On the one hand, this discrepancy might be explained by the multitude of different defense mechanisms of plants, and the fact that different defense mechanisms might be effective in different host-pathogen interactions. For a reliable evaluation, the effective defense mechanism has to be examined. On the other hand, conclusions drawn e.g. on whole plant level are not necessarily transferable to organ or cellular level, and *vice versa*. Thus, the results of tests of the GDB also depend on the level of observation. Still, the GDB seems to be a useful hypothesis not only for plant defense against herbivores but also against fungal pathogens. However, not all plant responses to pathogen attack can be explained by the GDB, as showed by the beech -*P. plurivora* example, where plants adapted in ways not foreseen by the GDB.

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Nonlinear Dynamics and the Spatiotemporal Principles of Biology

Leopoldina-Symposium
gemeinsam veranstaltet von der Deutschen Akademie der Naturforscher Leopoldina
und dem Graduiertenkolleg 340 „Kommunikation in biologischen Systemen“
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Schwerpunkte der Veranstaltung bildeten nichtlineare Dynamik im Allgemeinen, stochastische Resonanz und Synchronisation. Alle diese Themen wurden mit engem Bezug zu aktuellen Anwendungen in der Biologie im Wechselspiel von Theorie und Empirie behandelt. Sobald man bei dem Versuch, ein mathematisches Modell eines biologischen Phänomens zu entwerfen, die dynamischen Variablen über lineare Flüsse miteinander verbindet, findet die Beschreibung im Rahmen der linearen Systemtheorie statt. Wenn dann nichtlineare Wechselwirkungen einbezogen werden, gelangt die Modellierung in den Bereich der nichtlinearen Dynamik. Die Beiträge behandeln als Kernthemen Oszillationen, zeitliche und räumliche Synchronisation und Rauschen. Die im Rahmen des Symposiums diskutierten Oszillationen in der Natur reichen von ultradianen zu circadianen Rhythmen, von der Dynamik einzelner Enzyme zu Zellen, Organismen und Populationen. Die Diskussion von Synchronisation reicht von springenden Brownschen Teilchen, Ionenkanälen, Mikroalgen, Wasserflöhen (Daphnien), Zellen in einem Organ, Neuronen, entwicklungsbiologischen Organisations- und Signalzentren, Fischen in einem Vortex bis hin zu Planktonpopulationen. Rauschen wird als Umwelteinfluss oder als interner Beitrag schneller Dynamiken beschrieben. Biologische Variabilität in nichtlinearen Systemen vermag, ähnlich wie Rauschen, raumzeitliche Muster zu induzieren. Mit dem Auftreten nichtlinearer Phänomene in der Biologie stellt sich die Frage eines evolutionär wirksamen Nutzens für das biologische System. So können Oszillationen etwa den evolutionären Vorteil einer Zeitreferenz und einer inneren Uhr mit sich bringen. Die Beiträge machen solche funktionellen Vorteile in der Synchronisation biologischer Prozesse und im auf Brownsche Motoren wirksamen Rauschen bzw. in der Kontrolle von Krankheiten aus. Die nichtlineare Struktur ihrer Dynamik verleiht lebenden Systemen Robustheit.
Alle Beiträge sind in englischer Sprache abgefasst.

Wissenschaftliche Verlagsgesellschaft Stuttgart

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The Powdery Mildew Fungus *Blumeria graminis* Reprograms Barley for Triggering Susceptibility

Ralph Hückelhoven, Caroline Hoefle, Reinhard Proels, and
Ruth Eichmann (Freising-Weihenstephan)

With 2 Figures

Abstract

Understanding the mechanism of host susceptibility to infectious diseases may pave the way for molecular breeding for disease resistance. Innate immunity to plant pathogenic microbes is robust and multilayered, hence allowing plants to survive in an environment with diverse and abundant parasites. However, successful pathogens have adapted to their host plants, which they are able to access by the use of host-specific virulence effectors. Pathogen effectors directly or indirectly manipulate the function of host targets, which are called susceptibility factors if they are required for full susceptibility. However, little is known about fungal effector molecules or host susceptibility factors in plant-fungus interactions. We identified several potential susceptibility factors of the cereal model barley. The corresponding proteins are involved in cell survival (endoplasmic reticulum stress regulator BAX INHIBITOR-1), signaling (small G-protein RACB) and carbohydrate metabolism (alcohol dehydrogenase). Targeted silencing of corresponding genes limits barley susceptibility to the powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. One of the proteins, the metazoan oncogene RAS-homolog small GTP-binding protein RACB, functions in polar growth processes of barley. This is co-opted by the powdery mildew fungus to establish a haustorium in an intact plant cell by inward growth of the extrahaustorial host membrane and by building a new apoplastic compartment.

Zusammenfassung

Das Verständnis der Mechanismen der Anfälligkeit von Pflanzen gegenüber Pflanzenkrankheiten ist ein möglicher Schlüssel zur molekularen Züchtung krankheitsresistenter Pflanzen. Die natürliche Immunität von Pflanzen gegenüber pathogenen Mikroorganismen ist robust und vielschichtig, so dass Pflanzen trotz ständigem Kontakt mit parasitären Organismen überleben. Erfolgreiche Pathogene haben sich aber soweit an ihre Wirtspflanzen angepasst, dass sie diese für sich mit Hilfe spezifischer Virulenzeffektoren zugänglich machen können. Diese Virulenzeffektoren manipulieren direkt oder indirekt die Funktion pflanzlicher Zielproteine, die in die Krankheitsentwicklung involviert sein können und dann Anfälligkeitsfaktoren genannt werden. In Pflanzen-Pilz-Interaktionen ist sehr wenig über Virulenzeffektoren und Anfälligkeitsfaktoren bekannt. Wir haben verschiedene Anfälligkeitsfaktoren im Getreidemodell Gerste identifiziert. Die einzelnen Proteine sind in das Überleben infizierter Zellen (Endoplasmatisches-Retikulum-Stress-Regulator BAX IHIBITOR-1), Signalübertragungsprozesse (kleines G-Protein RACB) oder den Kohlenhydratstoffwechsel (Alkohol-Dehydrogenase) involviert. Gezieltes Stilllegen der Expression der Anfälligkeitsfaktoren vermindert die Anfälligkeit gegenüber dem Echten Gerstenmehltaupilz *Blumeria graminis* f. sp. *hordei* (*Bgh*). Eines der Proteine, das kleine RAS-Onkogenhomolog-ähnliche GTP-bindende Protein RACB, hat eine Funktion in polaren Wachstumsprozessen. Diese Funktion wird vom Pathogen genutzt, um in den lebenden Zellen ein Haustorium zu etablieren, wobei die Zelle das Einwachsen des Pilzes durch die Biogenese einer extrahaustorialen Wirtsmembran und eines neuen apoplastischen Kompartimentes unterstützt.

Plants are resistant to the majority of plant-pathogenic microbes. This is explained by the existence of an innate immune system in plants, which is robust and possesses at least three layers of defensive barriers (Thordal-Christensen 2003). The first layer consists of preformed structural or biochemical barriers that the pathogen has to overcome for getting access to the resources of the plant. Preformed barriers are for instance the leaf cuticle, the cell wall and toxic phytoanticipins, which are released upon cell damage. Given a pathogen can cope with these barriers, e.g. by hydrolytic breakdown of host structures and by detoxification of defensive host chemicals, it is recognized by the plant as non-self. Non-self recognition takes place *via* conserved microbe-associated molecular patterns (MAMPs, also known as PAMPs, pathogen-associated molecular patterns) that are absent from plants. Alternatively, pathogens can be recognized *via* the damage caused to a host cell by the activity of the microbe leading to damage-associated molecular patterns (DAMPs) released from host structures during pathogenic invasion. The plant recognizes DAMPs as danger signals because they indicate non-self activity. Plants recognize both MAMPs and DAMPs *via* specific plasma membrane pattern-recognition receptors (Boller and Felix 2009). Upon recognition, signal transduction leads to activation of a host transcriptional response and cell wall-associated defense, which contributes to MAMP/DAMP-triggered immunity (MTI, Fig. 1) (Hückelhoven 2007, Boller and Felix 2009). If a pathogen can overcome MTI by the delivery of virulence effectors, the result is an effector-triggered susceptibility (ETS, Fig. 1). It is important to note that susceptibility is triggered with MTI acting in the background and that virulence effectors are often host species-specific and hence require pathogen adaptation to a certain host. The plant again can counteract ETS by effector-triggered immunity (ETI), in which host resistance proteins directly or indirectly interact with virulence effector molecules for triggering a strong immune response that often includes a local programmed cell death of infected cells, the hypersensitive reaction (Jones and Dangl 2006). However, the existence of resistance genes for ETI is usually host genotype-specific such that often only single host cultivars/accessions of a plant species are resistant to single races/isolates of an adapted pathogen.

Little is known about the plant targets of virulence effectors. Well-described examples of effector targets are components of MTI, e.g. receptor complexes or downstream signal transduction components. In plant-fungus interaction, however, only a few targets of virulence effectors are known (de Jonge et al. 2011). Besides direct targets of virulence effectors, a successful pathogen might require other components of the host plant to be a successful pathogen. It is conceivable that the pathogen might require the more support from its host plant the more it is specialized to it. In extreme cases, such as the powdery mildew fungi, the pathogens have become obligate parasitic biotrophs that cannot exist without support from living host cells (Eichmann and Hückelhoven 2008) (Fig. 2). In an evolutionary process, obligate host association apparently led to the loss of several genomic components usually found in other filamentous fungi (Spanu et al. 2010).

Host components can be considered susceptibility factors, if their genetic or pharmaceutical inhibition limits susceptibility. True function of a susceptibility factor is further supported if its over-expression or artificial activation enhances susceptibility in the authentic interaction (Hückelhoven 2005). Host susceptibility factors might be involved in general regulation of cell death/survival under stress, in negative regulation of defense, or in building structural or metabolic pre-requisites for pathogen nutrition.

The host cell actively contributes to pathogenesis if infection structures are accommodated in intact plant cells (Parniske 2000). In the interaction of plants with powdery mildew fungi, a haustorial complex is formed in infected host cells. The core of the complex is the fungal haustorium, a specialized hyphae that may serve nutrient uptake from the host and the delivery of effector proteins to the host. The haustorium is surrounded by an apoplastic

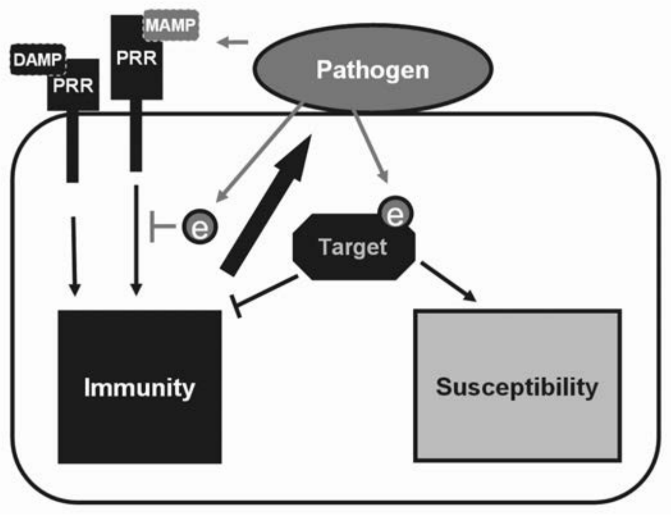

Fig. 1 MAMP/DAMP-triggered immunity and effector-triggered susceptibility in plants. Microbe- and damage-associated molecular pattern trigger a plant immune response, which is directly or indirectly suppressed by pathogen effectors in effector-triggered susceptibility. Effectors may also manipulate host targets for supporting susceptibility independent of suppressing host immunity. e, effector; DAMP, damage-associated molecular pattern; MAMP, microbe-associated molecular pattern; PPR, pattern-recognition receptor

extrahaustorial matrix, which is likely built with components from both the plant and the parasite. The extrahaustorial matrix is separated from the host cytoplasm *via* an extrahaustorial membrane, which is in continuum with the host membrane and may thus be of host origin (Hückelhoven and Panstruga 2011).

Several susceptibility factors have been described in the interaction of barley with the barley powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (*Bgh*) (Hückelhoven 2005, O'Connel and Panstruga 2006). The most prominent example of a susceptibility factor is the barley MLO (mildew locus o) protein, which is required for penetration of *Bgh* into leaf epidermal cells of barley. In homozygous *mlo*-mutants, which lack a functional MLO protein, fungal invasion is stopped before penetration of the host cell wall, which is associated with strong biochemical and transcriptional immune response as well as secondary cell death of underlying mesophyll cells (Büschges et al. 1997, von Roepenack et al. 1998, Hückelhoven et al. 1999, Piffanelli et al. 2002, Zierold et al. 2005). Immunity mediated by *mlo* is considered as complete and durable and thus is often applied in breeding of spring barley (Jørgensen 1994). The function of MLO is not yet understood at the mechanistic level but MLO might be a negative regulator of plant defense (Hückelhoven and Panstruga 2011).

Other susceptibility factors have been identified *via* transient biolistic transformation assays at the single cell level. One of them is BAX INHIBITOR-1 (BI-1), a conserved cell death regulator, which can, when over-expressed, substitute for MLO in a resistant *mlo* genetic background, which leads to re-constitution of susceptibility (Hückelhoven et al. 2003). Similar to MLO, BI-1 can be considered as a negative regulator of cell death and of cell

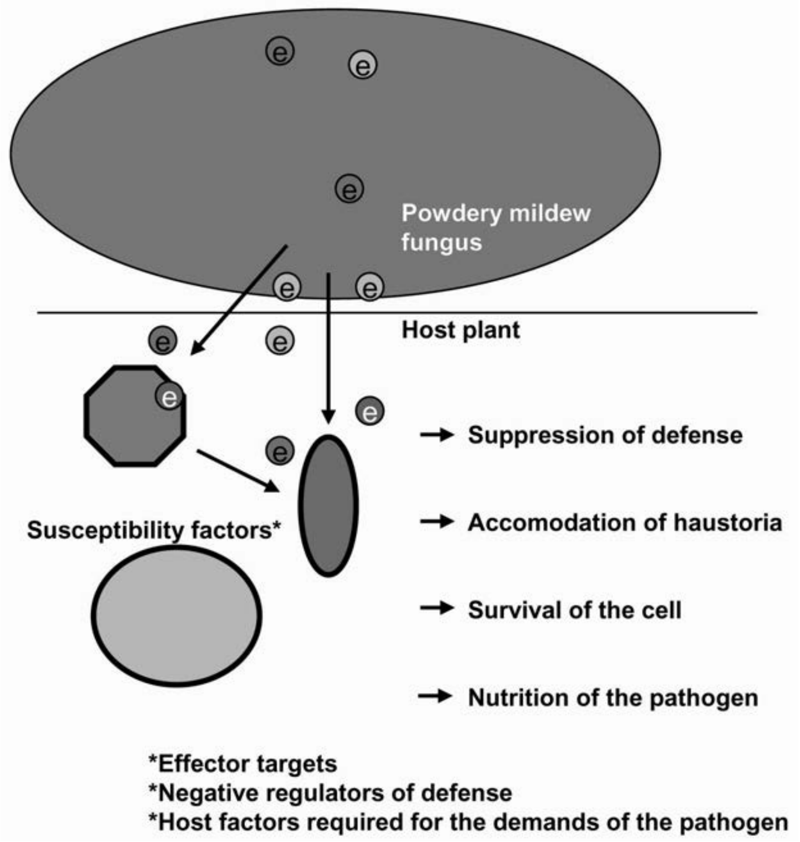

Fig. 2 Potential function of host susceptibility factors in the interaction with powdery mildew fungi. Effectors (e) released by the powdery mildew fungus may directly or indirectly (downstream) influence host susceptibility factors, which leads to manipulation of host cells for pathogenesis. Susceptibility factors may also support host cell functions from which the fungus profits without direct interference.

wall-associated defense (Eichmann et al. 2006). The precise mechanism of BI-1 function is not yet understood, but gene silencing experiments showed that normal expression of BI-1 is required for full susceptibility to penetration and symptom formation by *Bgh* (Eichmann et al. 2010).

A surprising finding was that also alcoholic fermentation appears to be involved in susceptibility to *Bgh*. The transcripts of barley alcohol dehydrogenases *ADH1* and *ADH2* accumulate to higher degrees in leaves of powdery mildew-infected barley when compared to non-inoculated controls, and ADH activity is increased (Pathuri et al. 2011, Proels et al. 2011). This is astonishing because one would not readily expect a hypoxia response in pathogen infection of fully air-exposed leaves. It is not yet clear whether local pathogen-induced changes of oxygen pressure are responsible for differential gene expression of ADH. Constitutive expression of *ADH1* in barley leaves suggests, however, that expression of *ADH* genes can correspond to other functions of ADH than response to oxygen deprivation. One explanation for expression of ADH in stress response of pathogen-infected tissue would be a high metabolic demand in combination with limited rates of respiration, which might occur under cellular stress. Interestingly, transient over- or under-expression of *ADH1* in barley supported or limited susceptibility to fungal invasion, respectively, and pharmacological inhibition of ADH limited fungal colony growth on barley. The latter effect could be complemented by the addition of glucose, which suggested that alcoholic fermentation normally might function in establishing a nutrient sink in the compatible interaction with the biotrophic pathogen (PATHURI et al. 2011, PROELS et al. 2011).

RACB, a small metazoan RAS oncogene homolog (RHO)-like GTP-binding protein, is another susceptibility factor of barley in interaction with *Bgh*. Recent findings suggest that the fungus may profit from the function of RACB in polar cell growth for ingrowth of the haustorial complex into the fully differentiated cells of the barley epidermis (HOEFLE et al. 2011). Originally, RACB was found in a targeted approach for identification of conserved regulators of defense and cell death in eukaryotes (HÜCKELHOVEN et al. 2001). It was then demonstrated that transient under-expression of the gene limited susceptibility to *Bgh*, whereas transient or stable expression of a constitutively activated version, CA RACB, enhanced susceptibility (SCHULTHEISS et al. 2002, 2003, 2005). The physiological role of RACB, however, might be found in cell and organ expansion. This is strongly supported by depolarization of root hair growth in CA RACB barley and the lack of root hairs in RACB under-expressors (PATHURI et al. 2008, HOEFLE et al. 2011). At the same time CA RACB plants develop more powdery mildew disease, whereas RACB under-expressors become less diseased. The latter is explained by less penetration success of the fungus in combination with the development of smaller haustoria when compared to wild type. RACB is thus a common element of epidermal root hair outgrowth and ingrowth of the haustorial complex. At the mechanistic level this might be explained by function of RACB in rapid anisotropic cell expansion and tip growth processes as reported for similar proteins in root hairs and pollen tubes. In these processes ROP proteins coordinate the actin and microtubule cytoskeleton as well as vesicle-based secretion (YALOVSKY et al. 2008, YANG 2008). A similar function appears conserved for RACB in barley, because RACB can modulate the polarity of filamentous actin during attack from *Bgh* (OPALSKI et al. 2005). Additionally, a molecular antagonist of RACB, MICROTUBLE-ASSOCIATED GTPASE ACTIVATING PROTEIN 1, MAGAP1, can shuttle between the microtubules and the plasma membrane depending on the activity status of RACB. MAGAP1 binds to RACB at the cell periphery where it likely switches off the G-protein by activating GTP hydrolysis. This may limit downstream signaling towards polar growth processes required for building the haustorial complex in the infected cell. Accordingly, under-expression and genetic modification of protein function supported a role of MAGAP1 in basal resistance to *Bgh* (HOEFLE et al. 2011).

The question emerges whether host susceptibility factors are direct or indirect targets of pathogen effector molecules. Interestingly, pathogenic *Escherichia coli* target BAX INHIBITOR-1 for blocking apoptosis in mammals (HEMRAJANI et al. 2010), such that BI-1 may be considered as conserved host susceptibility factor. However, no direct evidence is available that BI-1 is a direct target of plant pathogen effectors. The fact, however, that BI-1 can attenuate ETI-associated hypersensitive cell death reactions makes it an attractive target of virulence strategies (EICHMANN et al. 2006, KAWAI-YAMADA et al. 2009).

For RACB, preliminary data suggest that it might interact with peptides from *Bgh*. However, due to the fact that *Bgh* is not accessible for genetic manipulation, we have thus far accumulated only indirect evidences for a function of these peptides in influencing the interaction with barley (Mathias Nottensteiner, TUM, unpublished results). In mammals, RHO family proteins are, however, prominent targets of bacterial effector proteins. Effectors (cytotoxins) of diverse bacterial pathogens influence activity and stability of RHO proteins and direct cellular signaling towards bacterial invasion or suppression of immunity (Aktories and Barbieri 2005).

ADH may have a metabolic function in susceptibility to *Bgh*. In the oxygen deprivation response of *Arabidopsis*, ADH and ROP are linked *via* a regulatory network including the negative regulator ROP GTPase ACIVATING PROTEIN 4, ROPGAP4 (Baxter-Burrell et al. 2002). Interestingly, Arabidopsis *ropgap4* mutants are super-susceptible to powdery mildew (Huesmann et al. 2011). This indicates a possible link between ROP signaling, alcoholic fermentation and disease susceptibility. ADH could also be involved in signaling since genetic evidence suggested a function early in the establishment of pathogenesis (Pathuri et al. 2011). To our knowledge, a direct targeting of alcoholic fermentation in infectious diseases is not yet described.

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Reactions of the Ectomycorrhizosphere System to Plant Stress Imposed by Elevated Ozone

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Abstract

The focus of the present studies was on the composition and functioning of mycorrhizosphere soil and ectomycorrhizal communities of Norway spruce and European beech trees challenged by elevated ozone concentrations. As functional parameter, up to eight potential extracellular enzyme activities (acid phosphatase, N-acetylglucosaminidase, β-glucosidase, cellobiohydrolase, xylosidase, leucine aminopeptidase, glucuronidase, and laccase) of ectomycorrhizal roots and soil samples were measured in experiments that followed a scale concept of experimentation from small seedlings to adult trees under strongly controlled to field conditions. Ozone effects differed among tree species, tree ages, experimental settings and season. Despite this high variability, we found indications that nutrient cycles may be affected in the ectomycorrhizosphere of ozone challenged spruce and beech in different ways.

Zusammenfassung

Die vorgestellten Ergebnisse stammen aus verschiedenen Untersuchungen zum Einfluss erhöhter Ozonkonzentrationen auf die Zusammensetzung und Funktionen von Ektomykorrhizagemeinschaften und mikrobiellen Gemeinschaften in der Mykorrhizosphäre von Fichte und Buche. Experimentelle Skalen umfassten Sämlinge in kontrollierten Systemen bis hin zu adulten Bäumen im Bestand. Als funktioneller Parameter wurden potentielle Aktivitäten von bis zu acht verschiedenen Enzymen (saure Phosphatase, N-Aacetylglucosaminidase, β-Glucosidase, Cellobiohydrolase, Xylosidase, Leucinaminopeptidase, Glucuronidase und Laccase) an individuellen Ektomykorrhizen und in Bodenproben aus der Mykorrhizosphäre untersucht. Ozoneffekte waren beeinflusst und teilweise überlagert durch Pflanzenart, Baumalter, experimentelle Bedingungen und unterlagen jahreszeitlichen Veränderungen. Trotz dieser hohen Variabilität fanden sich Hinweise auf unterschiedliche Veränderungen von Nährstoffkreisläufen im Boden-Pflanze-System zwischen Fichte und Buche unter erhöhten Ozonkonzentrationen.

1. Introduction

Ectomycorrhizae are the dominant mycorrhizal symbiosis in boreal, subalpine and most temperate forest ecosystems. According to measurements of respired labeled carbon, ectomycorrhizal (ECM) trees invest 25–40 % of their newly assimilated carbon to support their ECM symbionts (Heinemeyer et al. 2007, Högberg et al. 2010). The fungal partners of ECM associations show a high diversity of several thousand species world-wide and can account for over 100 different taxa on a single tree (Bahram et al. 2011). However, the local ECM community composition is a result of complex interactions between soil properties, climate, and resources provided by the plant host(s) (Bruns 1995). Under elevated CO_2, an increase of ECM fungal species with high mycelial production was observed (Godbold and Berntson 1997) showing that the carbon flux to the fungal partners influences species composition.

Reversely, current elevated tropospheric ozone levels negatively affect carbon assimilation in sensitive plants resulting in reduced carbon supply to the roots (Matyssek et al. 2010) with consequences on the below-ground functioning (Andersen 2003). Reduced carbon supply to the belowground system was also experimentally manipulated by girdling of trees resulting in reduced ECM colonization (Kaiser et al. 2010, Högberg and Högberg 2002) or altered ECM species composition (Pena et al. 2010).

Tropospheric ozone (O_3) is a secondary air pollutant formed photochemically under UV radiation from diverse volatile organic compounds (VOCs) and nitrogen oxides (NO_x) as precursors and catalysts in the photochemical reaction (*The Royal Society* 2008). Actual (i.e. 2011) background ozone concentrations of over 40 ppb on average were calculated to cause 7 % of growth reductions in forest trees and the predicted future ozone levels bear the risk of strongly decreased growth in forest ecosystems thus diminishing or offsetting a key carbon sink for current CO_2 emissions (Wittig et al. 2009).

Ozone primarily affects leaf physiology in complex ways e.g. by interfering with stomatal control, photosynthesis, and phloem loading, by inducing antioxidant synthesis and repair processes altogether leading to reduced growth above and below ground of ozone sensitive plants (Matyssek and Sandermann 2003, Skärby et al. 1998, Broadmeadow 1998, Chappelka and Samuelson 1998, Matyssek and Innes 1999, Nunn et al. 2005, Nikolova et al. 2010). Ozone sensitivity differs among tree species with deciduous trees being generally more sensitive than conifers (cf. Wittig et al. 2009). Also tree age plays a role as young trees can differ in their ozone sensitivity from old trees (Nunn et al. 2005, Karnosky et al. 2007). In addition, other environmental factors such as elevated CO_2 or drought can interfere with ozone sensitivity (Wittig et al. 2009, Matyssek et al. 2010).

To account for these various factors, it has been suggested to use experiments at different scales of complexity and tree age (Karnosky et al. 2007). The joint research project SFB607 "Growth and Parasite Defense – Competition for Resources in Economic Plants from Agronomy and Forestry" used a multidisciplinary approach with common experiments to enable studies at different experimental scales and to integrate results from above ground and below ground plant reactions to ozone stress. The whole research concept has been described in detail by Matyssek et al. (2005).

2. Outline of Experimental Systems in SFB607

Two model tree species were selected namely Norway spruce (*Picea abies* (L.) Gaertn.) and European beech (*Fagus sylvatica* L.) both being of high economic and ecological importance in Central Europe. In all experiments of SFB607, the focus was on chronic effects of continuously elevated ozone concentrations. Therefore, ozone fumigation was doubled compared to the ambient concentration but restricted to an upper limit of 150 ppb to avoid acute ozone damage of foliage. Soil in all experiments was natural forest soil. The common experiments followed a scaling concept with experiments at three different levels of complexity.

Level 1: In the phytotron studies, young 1–3 year old plants (beech and or spruce) were grown in containers and studied over 1–2 growing season in climate controlled chambers called "phytotrons" that can be run under completely controlled climate scenarios (Luedemann et al. 2009, Pritsch et al. 2005, Luedemann et al. 2005, Kozovits et al. 2005a, b). In particular, phytotron studies allowed experimental multiple stress scenarios and stable isotope labeling experiments that could not be performed under field conditions.

Level 2: The lysimeter experiment aimed at bridging the ontogenetic level between the very young trees in the phytotrons and the adult trees in a stand (Pritsch et al. 2008, Schloter et al. 2005). The cylindrical lysimeters had a surface area of 1m², a soil depth of 2 m and were planted with European beech. Detailed descriptions were given by Schloter et al. (2005) and Winkler et al. (2009). The final lysimeter harvest after 4 years of ozone exposure comprised a complete retrieval of the soil columns of each lysimeter down to 2 m depth.

Level 3: The stand level was represented by a mature stand of Norway spruce and European beech with 60–70 years old trees at "Kranzberger Forst" with a world-wide unique free-air fumigation system (Werner and Fabian 2002, Nunn et al. 2002). Ozone fumigation of a group of 5 beech and 5 spruce trees with double ambient concentration (< 150 ppb) started in 2001 and ended in 2008.

3. Ozone Effects on the Plant-Soil System

Ozone does not act directly on the root-soil-system (Blum and Tingey 1977) as it quickly reacts with dry surfaces (Subke et al. 2009) and its diffusion is hampered in moist soils probably due to limited pore space (Toet et al. 2009). Thus, the predominant effect of ozone on the plant-soil system is indirect due to altered carbon supply by the vegetation exposed to and reacting towards elevated ozone resulting in reduced root growth, as well as changes in the quality and quantity of root exudates (Andersen 2003). The complexity of interactions in the plant-soil-system, therefore, is an enormous challenge when studying below-ground ozone effects because causalities may be masked by interactions. It has been hypothesized that ecosystem relevance of below ground effects may exceed above ground ozone effects as mineral nutrient and carbon cycling may become impaired (Andersen 2003).

To get insights into below-ground changes caused by ozone, the present studies addressed the ectomycorrhizosphere of trees subjected to elevated ozone as the interface between plant and soil. The studied parameters represented different levels of complexity from individual ECM tips to aggregated parameters such as mycorrhizosphere soil community composition and functioning, to account for a complex system influenced by plant stress.

4. Studies in the Ectomycorrhizosphere System

The composition of ECM communities was studied using morphological-anatomical characterization (Agerer 1991) and identification based on DNA sequence analysis of the ribosomal internal spacer region (ITS) (White et al. 1990). The ECM community composition was assessed by counting the number of root tips colonized by each morphotype. In addition, the functional diversity of ECM communities was assessed in some experiments using the method of enzyme activity profiling (Pritsch et al. 2004, Courty et al. 2005, Jones et al. 2011, Pritsch et al. 2011). This method is based on subsequent measurements of extracellular enzyme activities on individual ECM tips and has become one of the few methods that allow assessing a number of functional traits on individual ECM tips collected from the field

(Koide et al. 2007). From their phylogenetic background of multiple evolution from saprotrophic groups (Hibbett and Matheny 2009), ECM fungi share several enzymes with their saprotrophic relatives. The chosen set of eight enzyme activities (acid phosphatase, N-acetylglucosaminidase, β-glucosidase, cellobiohydrolase, xylosidase, leucine aminopeptidase, glucuronidase, and laccase) is potentially involved in the enzymatic mobilization of nutrients such as nitrogen and phosphorus from organic compounds contained in plant residues and litter components and as such not readily available for their plant host (Pritsch and Garbaye 2011). The same set or a subset of enzyme activities was measured in mycorrhizosphere soil samples collected from soil attached to mycorrhizal roots in order to detect potential changes of nutrient cycling enzymes in this soil compartment influenced by root exudates and secretions. Following the hypotheses of Andersen (2003), we postulated that due to altered carbon allocation belowground and subsequent changes in the availability and chemical composition of soil and plant carbon sources, ECM communities will be influenced in their composition and functioning.

5. Results and Discussion

5.1 ECM Community Diversity and Composition

ECM communities of young and adult spruce showed rather similar overall responses towards ozone. Overall, diversity of spruce ectomycorrhizae i.e. the number of taxa was not found to be changed by elevated ozone while the composition of the communities was considerably altered. The results on diversity of ectomycorrhizae of beech differed from spruce. In young containerized beech seedlings grown in the sun, respectively, the shade crown in a mature forest stand, ozone significantly decreased the ECM diversity (Železnik et al. 2007). Contrastingly, ECM diversity was not different in juvenile beech grown for six years in lysimeters under field conditions (Pritsch et al. 2009). In mature beech at the field site "Kranzberger Forst" the overall ECM diversity of adult beech was not strongly affected by ozone, although the significantly higher presence of *Cenococcum geophilum* on ozone treated trees indicated a shift in the community composition (Grebenc and Kraigher 2007, Haberer et al. 2007). Similar results were found after girdling of beech trees thus interrupting the flow of assimilates from the leaves to the roots. Girdling reduced ECM diversity of beech but only of the rare species while the remaining 90 % showed a similar community structure i.e. the same evenness of ECM communities compared to non-girdled control trees (Pena et al. 2010).

Unpublished results from container grown seedlings and adult trees showed a tendency that the community composition i.e. the distribution of ECM morphotypes became more homogeneous under elevated ozone. These shifts may be related to different carbon demands of different exploration types which have been categorized according to their extramatrical mycelia and the space in their soil habitat they can potentially occupy (Agerer 2001). In brief, contact types have almost smooth mantle surfaces, short-distance types show rather limited growth (few mm) into the soil, and types with rhizomorphs can be grouped into medium distance types (including 3 subtypes: fringe, mat, smooth) with rather far reaching rhizomorphs and an exploration distance of several cm, and finally long-distance exploration types characterized by differentiated rhizomorphs that reach up to several dm into the surrounding soil (Agerer 2001). For each exploration type, an estimate of the potential and actual mycelial space occupation has been provided that could be useful to assign biomasses and potential space occupation by ECM communities in the field (Weigt et al. 2011). However, as pointed out by Weigt et al. (2011), the colonized root length of each exploration type would be needed for exact calculations. As these data are quite rare and difficult to obtain, the number of root tips colonized by a distinct morphotype may be at least serve as an approximation for the percent colonization by the different exploration types. Using our data from counting the number of colonized root tips by distinct morphotypes on spruce in the mature stand "Kranzberger Forst", we found a shift from morphotypes with large mycelia to types with less extensive mycelia under elevated ozone treatment but only at the end of the vegetation period (Jana Ernst, unpublished results). That major differences are only apparent late in the growing season may be related to the seasonality of carbon allocation to the belowground system which can be up to 500 % more in the late than in the early growing season as shown by results from a Scots pine (*Pinus sylvestris* L.) stand (Högberg et al. 2007). Contrasting to our results of a reduced proportion of long-distance exploration types, results from girdled beech trees showed a reduction of species diversity but no apparent effect of girdling on the exploration types (Pena et al. 2010). Again, these contrasting results may be a consequence of tree species specific behavior in carbon allocation belowground with beech showing a more dynamic fine root system than spruce (Nikolova et al. 2010) which may compensate for carbon loss from actual photosynthesis by reserve mobilization as discussed by Pena et al. (2010). Another explanation is the long-term matter of chronically reduced carbon allocation belowground under elevated ozone with other possible mechanisms of adaptation of ECM fungal communities compared to instant disruption of actual carbon allocation below-ground after girdling with short-term drastic consequences for the ECM fungal composition and functioning.

5.2 Functional Changes of ECM Communities

Enzyme activity measurements on distinct mycorrhizal roots were performed on juvenile beech in the lysimeter experiment and on spruce in the mature stand. First of all, we found distinct enzyme activity patterns of different ECM species or morphotypes with a high functional redundancy of most enzyme activities measured (Courty et al. 2005). In the lysimeter experiment, enzyme activities of ECM communities of juvenile beech had not significantly changed at the end of the fourth vegetation period under elevated ozone fumigation (Pritsch et al. 2009). Contrary to that, ECM communities of adult spruce at Kranzberger Forst showed a shift at the end of the seventh vegetation period under elevated ozone concentrations with unchanged or decreased potential activities of most hydrolytic enzymes but the oxidative enzyme laccase was stimulated (Jana Ernst, unpublished results). Stimulated laccase activity may indicate the degradation of more recalcitrant substrates such as protein-phenol complexes or a stress response towards toxic compounds i.e. phenolic substances (Bending and Read 1996, Courty et al. 2007, Burke and Cairney 2002). Overall, these results suggest at least temporary changes of nutrient cycling enzyme activities at the level of ECM community functioning. It is not yet clear if these changes reflect poorer substrate quality of the litter or are an indication for stress reactions.

Differences in the belowground reaction of spruce and beech towards ozone stress were shown by different carbon allocation patterns belowground (Nikolova et al. 2010). These overall differences between the two tree species may also explain the different responsiveness of their ECM community composition and functioning. However, data supporting this interpretation need to be further elaborated.

5.3 Changes in the Mycorrhizosphere Soil System

Enzyme activity measurements and analyses of microbial community composition in mycorrhizosphere soil which were performed to allow a more systemic view on the biological activity at the soil-plant interface showed no consistent patterns of ozone reactions. Enzyme activity measurements in mycorrhizosphere soil samples revealed temporal fluctuations and an overall high variation dependent upon different plant species (spruce *versus* beech), tree ages (seedlings, juvenile, respectively adult trees), and experimental settings including plant competition, abiotic or biotic stress.

In the lysimeter study with juvenile beech, differences in soil enzyme activities were found in the initial stage of the experiment (SCHLOTER et al. 2005) but not in subsequent years (PRITSCH et al. 2008, 2009). Contrary to functional traits, phospholipid biomarkers revealed shifts in the composition of microbial communities at the end of the four-year ozone fumigation period (PRITSCH et al. 2009) suggesting that the same set of functions was performed by different members of the community (ESPERSCHÜTZ et al. 2009, PRITSCH et al. 2009). Lipid biomarkers also indicated a shift in the composition of plant residues (PRITSCH et al. 2009) indicating more stress related compounds in plant litter. However, other soil organic matter fractions should also be studied to support this finding which was only based on lipid markers.

Similarly variable findings came from the Aspen-FACE-experiment in Rhinelander Wisconsin (DICKSON et al. 2000). With respect to soil enzyme activities, LARSON et al. (2002) detected no differences due to the elevated ozone treatment after the second full growing season following planting, while CHUNG et al. (2006) two years later detected a significant decrease of β 1,4 glucosidase activity under elevated ozone. The evaluation of long-term data-sets and measurements in the tenth year of ozone fumigation showed that cellobiohydrolase activity was significantly decreased in the forest floor organic horizon of the elevated ozone plots, however, the changes were not found in the A horizon (EDWARDS and ZAK 2011). Interpretation of long-term data series on soil enzyme activities in the Aspen-FACE-Experiment suggested elevated O_3 to have variable stimulatory and repressive effects depending on the soil horizon and time point examined (EDWARDS and ZAK 2011).

Thus, enzyme activity measurements in the lysimeter experiment of SFB607 and the Rhinelander FACE experiment with different plants and soil types showed remarkably similar results of small and inconsistent changes which nevertheless indicate the risk of potentially changed food webs in the soil system of ozone challenged plants.

6. Conclusions

All in all the results suggest relatively small changes in soil microbial including mycorrhizal functions during the experimental timelines of 2–7 years. However, reduction in root growth and the changes in carbon allocation to ECM symbionts may be a long-term matter to be concerned of. As ECM mycelia also serve as carbon and nitrogen source and thus drive nutrient cycles in forest ecosystems, fungal mycelia and their functioning deserve further studies. In addition, the interaction between mycorrhizal fungi and decomposer communities may be of relevance when carbon allocation belowground is disturbed as shown with girdling experiments that revealed a decrease of fruiting bodies of ECM fungi but not of saprotrophic fungi following the disruption of assimilate transport to the roots (Högberg et al. 2001). Future experiments should aim at addressing mycelia for example by using compartment studies excluding or including hyphae and roots systems to allow undisturbed growth and thus to open the possibility to get experimental access to field-grown mycelia. In addition, soil enzyme activity studies would profit from simultaneous studies of the natural enzyme substrates or indicators of substrate quality to gain more insights into biochemical changes of the whole plant-soil system.

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Cuscuta reflexa Infestation: Molecular Components of a Parasitic Plant – Host-Plant Interaction

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With 4 Figures

Abstracts

Parasitic weeds such as *Cuscuta reflexa* are obligate holoparasites with low host specificity. In mild climate agricultural areas, the parasites cause substantial damage to important crops. In order to design plant protection strategies, but also because this plant – plant interaction has interesting facets from a scientific point of view, the molecular processes of *Cuscuta* infection were studied in detail. The characterization of some exemplary genes, the encoded proteins and their function in the host – parasite interaction are described. The acquired knowledge about the molecular events was transferred into a strategy to repel *Cuscuta reflexa* from host plants.

Zusammenfassung

Parasitäre Pflanzen, wie *Cuscuta reflexa*, sind obligate Holoparasiten mit geringer Wirtsspezifität. In landwirtschaftlich genutzten Gebieten mit mildem Klima können die Pflanzen an wichtigen Nutzpflanzen Schäden anrichten. Um Strategien zur Bekämpfung von *Cuscuta* zu entwickeln, aber auch weil die Biologie dieser Pflanzen-Pflanzen-Interaktion interessante wissenschaftliche Aspekte hat, wurden entsprechende molekulare Komponenten dieser Interaktion studiert. Die Charakterisierung exemplarischer Gene, der darin kodierten Proteine und deren Funktion in der Wirt-Parasit-Interaktion ist hier dargestellt. Ausgehend von dem erarbeiteten Wissen über die molekularen Vorgänge, konnten Strategien entwickelt werden, die zu einer Resistenz gegenüber *Cuscuta reflexa* führen.

1. Introduction

Parasitic weeds comprise more than 4100 species, classified into 19 families. The so-called holoparasites lack chlorophyll and are consequently entirely dependent on their host plant for the supply of water, nutrients and fixed carbon. Another group, the hemiparasites, is capable of photosynthesis and is consequently not completely dependent on nutrients from a host plant; they can be either obligate or facultative parasites. In general, parasitic plants have common characteristics, such as a reduced root system. In many cases, interaction with the host plant is accomplished by contact organs, called haustoria, which connect to either or both of the phloem and xylem (Fig. 1). Dependent on the host contact region, the plants are divided into either root or stem parasites.

Parasitic plants such as *Cuscuta reflexa* or the *Orobanchaceae* are obligate holoparasites with low host-specificity. They are endemic in regions with a relatively mild climate. In farming areas, infestation with parasitic plants can cause substantial damage to many crops such as, for instance, sugar beet, alfalfa, pepper, cucumber, tomato, potato or allium (Dawson

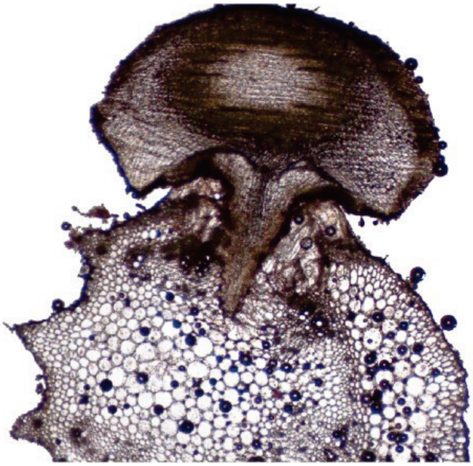

Fig. 1 Cross section of *Cuscuta* (upper) and tomato (lower) at the contact site.

1994). However, *Cuscuta* spp., together with other neophyte plants, was also observed recently in less mild climates (close to Mannheim and Heidelberg in Germany, for example (see http://www.guenther-blaich.de/pflgs.php?par=kune&lan=e [Lambdon et al. 2008]). Currently, an effective control of *Cuscuta*-spreading is based on preventive strategies, including control of seed contamination and application of herbicides prior to seed emergence. For an overview, refer to https://www.uni-hohenheim.de/www380/380b/science/supraregional/control.htm. In the case of an established infestation, the use of herbicides for weakening parasitic plants appears to be effective and not harmful to the host plant if it is herbicide-resistant (Nadler-Hassar and Rubin 2003, Nadler-Hassar et al. 2009). Due to the complexity of the parasite-host interaction, there are no crops created by conventional breeding techniques which possess resistance to parasitic plant infestation. Molecular biology and genomic research on parasites is needed in order to develop new, more effective and reliable control strategies (Davis et al. 2009, Stewart et al. 2009).

2. Biology of the Host – Parasite Interaction

Besides the commercial and agronomic relevance to control parasitic plants, the parasite – host interaction is also a fascinating biological system. One of the most noteworthy examples is the *Cuscuta pentagona* – tomato interaction, which demonstrates the influence of volatiles in sensing of the host by the parasitic plant. Moreover, volatile compounds found in *Impatiens* spp., tomato or wheat, induced very specific reactions: those from tomato and wheat elicit directed growth towards the host by *Cuscuta*, whereas one compound from wheat is repellent to the parasite. These specific reactions indicate that *Cuscuta* seedlings can not only detect the position of potential hosts, but also differentiate between host species (Runyon et al. 2006).

In order to obtain more information about the biology of the parasite – host interaction and corresponding molecular processes, we used several approaches to identify genes that are upregulated in host or parasitic plants. Suppression subtractive hybridization was applied to identify genes that are induced in the host tomato plant during early phases of the interaction with *Cuscuta reflexa*. In addition, we constructed a *Cuscuta* cDNA-library with mRNAs from the early stages of haustoria development, and used differential hybridization on macroarray slides to screen for haustoria-specific genes. In a further set of experiments, cDNA Amplified Fragment Length Polymorphism (AFLP) was used to identify *Cuscuta* genes that are expressed after host attachment. In summary, we observed something akin to a dialogue on a molecular basis between the host and *Cuscuta*.

3. The Host – Parasite Dialogue

One of the early steps in this 'conversation', initiated by the parasitic plant, is the synthesis of enzymes to soften host cell wall material. *Cuscuta* cDNA which codes for pectinases and proteinases were found to accumulate in this initial phase. A haustoria-specific cysteine proteinase was further characterized, and named *cuscutain*, consistent with similar proteins from other plants, such as, for example, papain (from papaya [Bleischwitz et al. 2010]). The sequence comprises the region of the enzyme containing the catalytic center, an intrinsic inhibitory peptide and a propeptide responsible for the extracellular transport (Fig. 2). The entire protein, as well as protein regions, were heterologously expressed in *Escherichia coli*, purified and biochemically characterized.

The course of events mediated by cuscutain during the initial stage of infestation is assumed to be as follows. The entire protein is expressed briefly after physical contact with the host in an enzymatically inactive form. After export from the parasitic cell, the protein is processed in such a way that the export sequence and the inhibitory peptide are cleaved off. The proteinase, as well as other degrading enzymes, then have access to the surface of

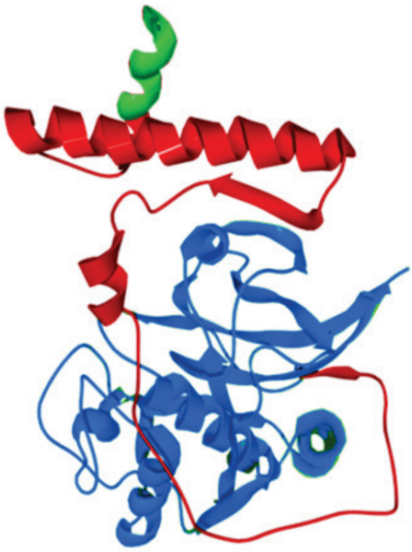

Fig. 2 Model of the cysteine proteinase, cuscutain. The regions of the prepeptide (*green*), the inhibitory propeptide (*red*) and the core enzyme (*blue*) are indicated by different colors.

the host and start to soften host cell wall material in order to facilitate its penetration by parasitic hyphae.

Shortly after the initial physical contact, an elongation of subepidermal cells in the host tissue was observed (Ihl et al. 1988, Sahm et al. 1995). The effect was accompanied by an increase of both local auxin concentrations and synthesis of the corresponding proteins (Löffler et al. 1999, Werner et al. 2001). Concurrently, *Cuscuta* emits signals which induced Ca^{2+} spikes and Ca^{2+}-induced gene expression in the host plant (Albert et al. 2010a, b). Ca^{2+} signals were monitored as bioluminescence in aequorin-expressing tomato plants after the onset of *Cuscuta reflexa* infestation. Signals at the attachment sites were observed from 30 hours to 48 hours after infection, which suggests that the Ca^{2+} signals were not caused by wounding but by constituents specific to *Cuscuta*. In an assay using leaf disks of aequorin-expressing tomato, which had been treated with different *Cuscuta reflexa* plant extracts, it was shown that the substance that induced Ca^{2+} release in the host plant is delivered by the parasite's haustoria. The heat-lability of this substance suggests that it is of a proteinaceous or volatile nature.

Subsequently, to the release of the second messenger substance, we observed an increase of specific gene activity in the host. One of the identified genes encodes a tomato xyloglucan endotransglycosylase/hydrolase (XTH), an enzyme involved in cell wall elongation and restructuring. In addition, it was found that XTH mRNA accumulation can be induced by external application of auxin (Albert et al. 2004). Another identified host mRNA encoded a so-called arabinogalactan protein (AGP). In tomato it accumulated precisely at the site of *Cuscuta* attack. It was therefore named 'attachment AGP' or 'attAGP'. attAGP is a plasma membrane-bound protein localized to the cell wall. Virus-induced gene silencing targeted at attAGP indicated a correlation between attAGP expression level and the attachment force of the parasite to host tomatoes. Decrease of attAGP expression level reduced the force of *C. reflexa* attachment and its ability to adhere to the host surface. It is concluded that *C. reflexa* infection induces a signal in the host leading to expression of tomato attAGP, which promotes the parasite's adherence (Albert et al. 2006).

As outlined at the beginning of this report, infection of crops with parasitic plants can cause severe problems in agriculture. As a proof of concept, we were interested if knowledge of the molecular processes could be used to prevent or heal a parasitic plant infestation. For

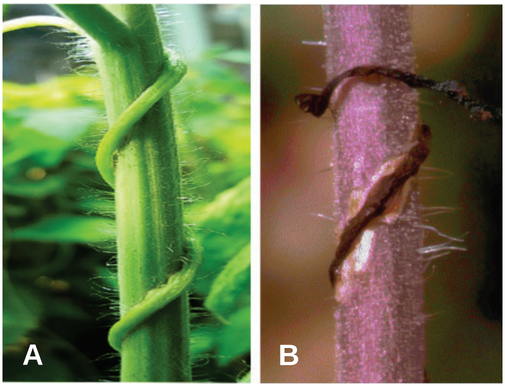

Fig. 3 *Cuscuta reflexa* on tobacco before (*A*) and after (*B*) treatment with an inhibitor-propeptide solution of cuscutain.

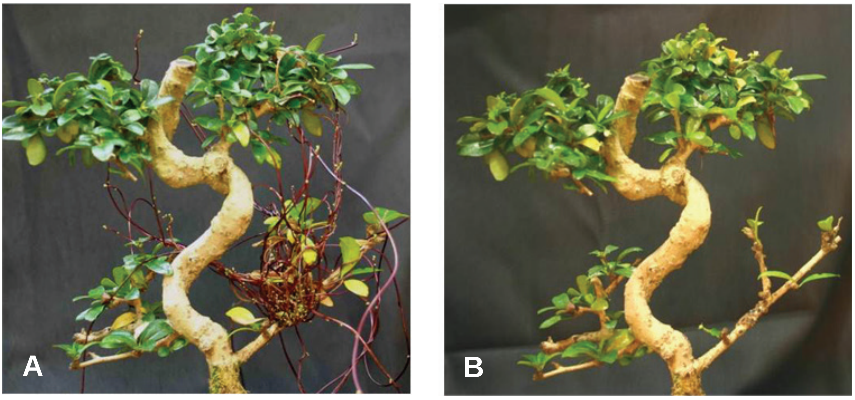

Fig. 4 *Cuscuta asiatica* on bonsai elm trees before (*A*) and after (*B*) treatment with an inhibitor-propeptide solution of cuscutain.

example, if cuscutain activity is essential for a successful infection, inhibition of the enzyme could be one way of reducing the effectiveness of *Cuscuta* infestation. To test this assumption, tobacco plants infected with *Cuscuta* were sprayed with an inhibitor propeptide solution. The parasites on these treated plants appeared to be thinner and less vital when compared to controls, and the number of prehaustoria and haustoria was decreased, whether attached or not to the host. *Cuscuta* plants that resided on treated plants dried out after about two weeks without further spraying (Fig. 3) (Bleischwitz et al. 2010). The polypeptide solution was also effective on woody plants, as demonstrated by its use on bonsai elm trees (Fig. 4). It is interesting to note that our current results also show a positive effect of the inhibitor solution on different species of parasitic plants such as *Phelipanche* (*Orobanchaceae*), although it is a root parasite. This suggests common molecular mechanisms, which possibly rely on common morphological structures, such as haustoria (Rehker et al. 2012).

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Effects of Abiotic and Biotic Stress on Gene Transcription in European Beech: From Saplings to Mature Trees

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With 2 Figures

Abstract

Abiotic and biotic environmental stress factors influence the growth of plants and its defense system. To investigate the chronic effects of ozone on forest trees and the interactions of ozone with endophytic fungi, transcriptional changes in the leaves of European beech were analyzed after ozone and fungal treatments. Beech saplings were cultivated under controlled conditions in a greenhouse and under partially controlled conditions in a local open-air lysimeter system; additionally, adult trees at the Kranzberger Forst free-air fumigation site were studied. Ozone-induced transcripts of genes for signal transduction, disease/defense and secondary metabolism were evaluated under these experimental conditions, and ontogenetic effects were analyzed by comparing young saplings with adult trees. The adult trees showed less pronounced transcriptional changes upon ozone treatment, as compared to saplings. Furthermore, gene expression was more strongly affected by ozone treatment and leaf morphology than by endophyte infestation (*Apiognomonia errabunda*).

Zusammenfassung

Abiotische, als auch biotische Stressoren beeinflussen pflanzliches Wachstum und Abwehrverhalten. Zur Untersuchung chronischer Ozoneffekte, sowie der Interaktionen von Ozon mit endophytischen Pilzen, wurden transkriptionelle Änderungen in Blättern der Europäischen Buche nach Ozon- und Pilzbehandlung untersucht. Wenige Jahre alte Buchen wurden unter kontrollierten Gewächshausbedingungen und unter teilweise kontrollierten Freilandbedingungen auf einem lokalen Lysimeter untersucht. Adulte Bäume wurden an der Freiluftozonbehandlungsstelle im Kranzberger Forst analysiert. Ozon-induzierte Transkripte von Genen der Signaltransduktion, Erkrankung/Verteidigung und des Sekundärmetabolismus wurden in allen Experimenten nachgewiesen. Ontogenetische Effekte wurden durch den Vergleich von wenige Jahre alten Buchen mit adulten Bäumen erfasst. Im Gegensatz zu jungen Buchen zeigten adulte Bäume nur geringe transkriptionelle Änderungen. Des Weiteren war die Genexpression stärker durch die Blattmorphologie und Ozonbehandlung als durch die pilzliche Infektion mit *Apiognomonia errabunda* beeinflusst.

1. Introduction

In recent decades, the effects of tropospheric ozone, a phytotoxic air pollutant, have been broadly studied at the biochemical and physiological levels (Matyssek and Sandermann 2003, Karnosky et al. 2005, Matyssek et al. 2008, Renaut et al. 2009). Furthermore, the mechanistic responses of plants at the transcriptional level have been elucidated (Langebartels et al. 2002, Jaspers et al. 2005, Heath 2008, Matyssek et al. 2008). It has also been shown that transcript patterns after ozone fumigation or after pathogen attack are comparable; ozone has, therefore, been described as an abiotic elicitor of plant defense reactions (Sandermann et al. 1998, Heath 2008).

Acute ozone exposure (between 0.2 µl l^{-1} and 0.3 µl l^{-1} for several hours) results in transcriptional changes of ozone-responsive genes, and microarray analyses have led to the characterization of such genes and there links to various functional groups and metabolic pathways, including primary and secondary metabolism, cell growth and structure, protein synthesis, signal transduction, disease and defense (Tamaoki et al. 2003, Tosti et al. 2006, Matyssek et al. 2008, Puckette et al. 2008). Chronic ozone exposure experiments (up to twice the ambient level for several days, weeks or years) have been performed under both controlled chamber conditions (Langebartels et al. 1997, Miyzaki et al. 2004, D'Haese et al. 2006, Bohler et al. 2010) and free-air conditions (Karnosky et al. 2007, Winkler et al. 2009). It was shown in *Arabidopsis thaliana* and *Thellungiella halophila,* but also in trees, such as trembling aspen, European beech and paper birch, that several genes are similarly affected by chronic and acute ozone exposure, although to a lesser extent by chronic ozone fumigation (Gupta et al. 2005, Olbrich et al. 2005, Miyazaki et al. 2004, Li et al. 2006, Olbrich et al. 2009, Kontunen-Sopella et al. 2010). In addition, great differences in transcript abundances between growth-chamber and field-condition experiments have been observed (Miyazaki et al. 2004).

2. Ozone Exposure of Beech Saplings under Controlled Greenhouse Conditions

European beech saplings at four years of age were fumigated in the greenhouse with 0.15 – 0.19 µl l^{-1} ozone for 63 d, and the effects on stress-related expression of genes of the shikimate pathway and ethylene biosynthesis were observed.

The shikimate pathway, an intermediary pathway between primary metabolism and secondary metabolism, consists of seven enzymatic reaction steps, and the pathway has been shown to be regulated at the transcriptional level during plant development and by environmental stresses, such as wounding, pathogen attack and elicitor treatment (Görlach et al. 1995). All genes of the shikimate pathway were up-regulated in the leaves of beech saplings upon ozone treatment (Fig. 1, Betz et al. 2009b, c), a response that has also been described for tobacco (Janzik et al. 2005). Furthermore, increases of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase 3 and 3-dehydroquinate dehydratase/shikimate dehydrogenase at the protein level were in agreement with the increased transcript levels of these enzymes (Fig. 1, Betz et al. 2009b). This result is also in accordance with higher enzymatic activities of shikimate dehydrogenase in ozone-treated leaves of poplar (Cabané et al. 2004). Chorismate synthase (CS), which is up-regulated at the transcriptional level in the leaves of beech upon ozone fumigation (Fig. 1, Betz et al. 2009b), produces chorismate, which is also a central metabolite in the biosynthesis of salicylic acid (SA) and gentisic acid (GA) (Fig. 1). Upon ozone treatment, increased levels of conjugated SA and GA were observed in parallel to an increased level of *CS* transcripts (Fig. 1, Betz et al. 2009b), similar to the levels of conjugated SA in ozone-treated tobacco leaves (Janzik et al. 2005). Therefore, the accumulation of both metabolites is consistent with the increased transcript levels of shikimate pathway genes.

Another well-known response of plants to ozone is the induction of ethylene biosynthesis (Kangasjärvi et al. 1997). Ethylene is synthesised via 1-aminocyclopropane-1-carboxylic acid (ACC) by two enzymes, (*i*) ACC synthase (ACS) and (*ii*) ACC oxidase (ACO). In ozone-fumigated beech leaves, increased transcript levels for *ACS* and *ACO* were evident, and a slight accumulation of ACC and an increased emission of ethylene were also found (Fig. 2, Betz et al. 2009a). These results are in accordance with studies in herbaceous plants

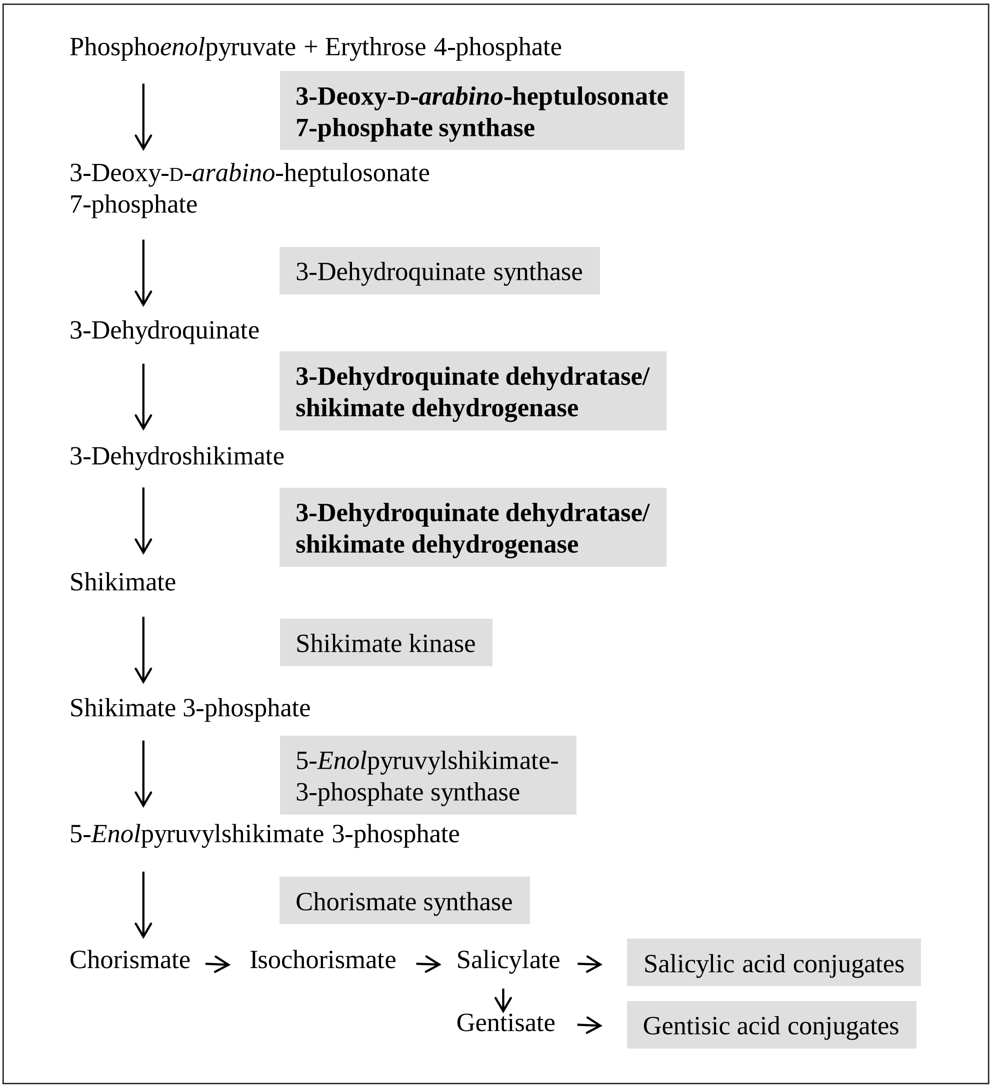

Fig. 1 The steps of the shikimate pathway that are up-regulated by ozone and the related accumulation of conjugated salicylic and gentisic acids in European beech (related transcripts and metabolites are highlighted, proteins are in bold) (Betz et al. 2009b).

(Kangasjärvi et al. 1997) and a previous study of beech trees (Nunn et al. 2005), and indicate a link between increased transcript levels and the accumulation of the corresponding metabolites.

In conclusion, the data demonstrate (*i*) a clear transcriptional ozone response of beech saplings, (*ii*) a similar transcriptional response of herbaceous plants and juvenile woody plants upon ozone exposure, and (*iii*) a correlation between changes in transcript accumulation and changes in protein and metabolite levels.

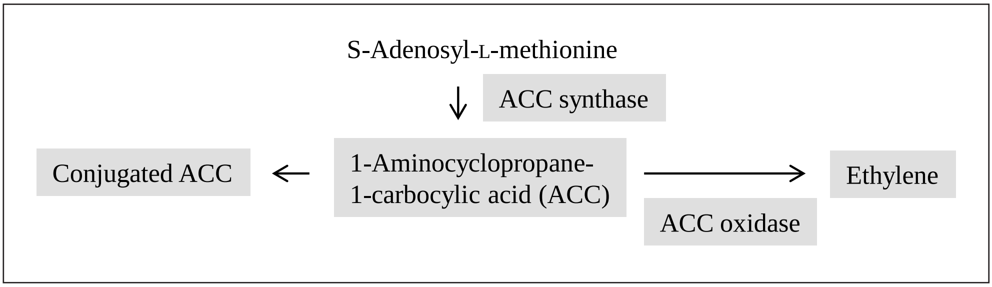

Fig. 2 The steps of ethylene biosynthesis that are up-regulated by ozone and the related accumulation of metabolites (related transcripts and metabolites are highlighted) (Betz et al. 2009a).

3. Ozone Exposure of Beech Saplings at an Outdoor Free-Air Fumigation Site

In 2002, three-year-old beech saplings were planted at the lysimeter area of the Helmholtz Zentrum München[1] and were then grown for four years under ambient and twice ambient ozone levels (Winkler et al. 2009). The leaf material was sampled over two vegetation periods (Olbrich et al. 2009) in 2005 and 2006, and transcript analyses were performed using a custom-made microarray, consisting of 1246 expressed sequence tags (ESTs) (Olbrich et al. 2005). Transcripts belonging to disease/defense, secondary metabolism and signal transduction mainly appeared to be up-regulated, whereas transcripts belonging to photosynthesis, chloroplast cell structure and the Calvin cycle were predominantly down-regulated (Olbrich et al. 2009). Although the expression profiles for 2005 and 2006 were not identical, a common trend of transcriptional changes was evident. These changes were more pronounced in 2006, which may be explained by a higher stomatal conductance in 2006, when compared to 2005, and as a result an enhanced ozone-flux (Olbrich et al. 2009).

Proteomic analyses showed that approximately 9 % of the total protein spots resolved by 2-D gel electrophoresis were differentially regulated upon ozone fumigation (Kerner et al. 2011). In a comparison of the transcriptomic and proteomic data, only three proteins showed a direct overlap with the corresponding transcript (Kerner 2011). This result was not surprising and has been reported previously in the literature, emphasising the fact that post-translational processing and gene silencing must be taken into account in these analyses (Perco et al. 2010, Sánchez-Pons et al. 2011). However, comparing the data by the functional classification of protein groups, a higher correlation was observed for the transcripts and proteins that are related to photosynthesis, the Calvin cycle, and disease and defense (Olbrich et al. 2009, Kerner 2011).

In conclusion, the data demonstrate (*i*) the importance of field studies and its comparison with controlled-chamber studies and (*ii*) a moderate correlation between the transcript and protein data from comprehensive transcriptomic and proteomic analyses.

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[1] http://www.helmholtz-muenchen.de/en/lysimeter/home/index.html.

4. Ozone Exposure and Infestation with *Apiognomonia errabunda* of Adult Beech Trees at "Kranzberger Forst"

The "Kranzberger Forst" is located near Freising (Germany, Bavaria); at this site, 65-year-old beech trees were fumigated since 2000 with twice ambient ozone by a free-air fumigation system (Karnosky et al. 2007). Transcriptomic analyses of RNA isolated from both sun and shade leaves were conducted in 2005 and 2006, and changes in gene expression were observed for approximately 0.2 % of all of the ESTs analyzed (Olbrich et al. 2010a). Furthermore, transcriptional differences between sun and shade leaves and between the two vegetation periods were evident (Olbrich et al. 2010a), indicating that, in addition to ozone, other external factors (e.g., weather conditions) affected gene expression. In addition, the few transcriptional changes in the adult trees, as compared to the beech saplings, indicate that ontogenetic effects also have to be taken into account (Olbrich et al. 2009, 2010a).

A controlled infection study with leaves using *A. errabunda* was also carried out at "Kranzberger Forst". Background populations of *A. errabunda* were found in the shade leaves under ambient ozone, and artificial fungal inoculation increased the infestation level considerably (Olbrich et al. 2010b). In contrast, ozone treatments of twice the ambient level resulted in reduced fungal infestation and fewer leaf necroses in the shade crown. In the sun crown, fungal infestation was approximately 50 times lower compared to the shade crown, indicating the strong influence of the light conditions (Olbrich et al. 2010b). Moreover, under hot and dry weather conditions, *A. errabunda* was barely detectable in the leaves, thus overriding the ozone effect (Bahnweg et al. 2005). At the level of gene expression, only a few transcripts were found to be altered upon *A. errabunda* infestation. Comparing all of the *A. errabunda*-, ozone-, and leaf type-affected ESTs, it was determined that gene expression was more strongly affected by the leaf morphology than by the ozone level and was scarcely affected by fungal infestation (Olbrich et al. 2010b).

In conclusion, the data demonstrate (*i*) a different transcription pattern in sun *versus* shade leaves and between different growing years, (*ii*) increased transcriptional changes in beech saplings compared to adult trees upon ozone fumigation, (*iii*) sublethal ozone exposure mediated strengthening of beech leaves against fungal attack and (*iv*) that gene expression was more strongly affected by leaf morphology and ozone treatment than by *A. errabunda* infestation.

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From Plant Taxonomy to Evolutionary Biology – Von der Taxonomie zur Evolution der Pflanzen

Leopoldina-Meeting
vom 10. bis 12. Juni 2004 in Reichelsheim (Odenwald)

Nova Acta Leopoldina N. F., Bd. *92*, Nr. 342
Herausgegeben von Peter K. Endress (Zürich), Ulrich Lüttge (Darmstadt) und Benno Parthier (Halle/Saale)
(2005, 239 Seiten, 38 Abbildungen, 10 Tabellen, 25,95 Euro, ISBN 3-8047-2252-0)

Das Leopoldina-Meeting war dem langjährigen Präsidenten der Leopoldina Christian Gottfried Nees von Esenbeck (1776–1858) gewidmet, der auch als Pflanzentaxonom von Bedeutung ist. Der Band wird mit der Begrüßung durch den Präsidenten der Leopoldina, Volker ter Meulen, eingeleitet. Ein historischer Beitrag von Michael Kaasch und Benno Parthier, Alt-Präsident der Leopoldina, hebt die große Bedeutung von Nees von Esenbeck für die Entwicklung der Leopoldina im 19. Jahrhundert im Rahmen national-akademischer Reformbestrebungen hervor und führt den Leser bis zu aktuellen Problemen, die vor den Akademien in der Gegenwart stehen.
Der fachwissenschaftliche Teil gibt 11 Vorträge über aktuelle Probleme der botanischen Evolutionsforschung wieder. H. Peter Linder (Zürich) und Nigel P. Barker (Grahamstown) spannen den Bogen von Nees von Esenbeck zur Gegenwart am Beispiel des Kenntnisstandes über eine Unterfamilie der Gräser (Danthonioideae). Einen weiteren Schwerpunkt setzen zwei Vorträge, die Phylogenie, Evolution und Diversität von Großgruppen von Pflanzen in den Mittelpunkt stellen (Volker Knoop, Bonn, sowie Wilhelm Barthlott und Mitarbeiter, Bonn). Der dritte Schwerpunkt befasst sich mit der Diversität und Evolution auf Art- und Gattungsniveau sowie geographisch-ökologischen Faktoren der phylogenetischen Differenzierung. Beiträge hierzu liefern Herbert Hurka und Mitarbeiter (Osnabrück) sowie Guido Grimm und Koautoren (Tübingen). Tod F. Stuessy und Mitautoren (Wien) stellen die Ausbreitungs- und Diversifizierungsgeschichte bei verschiedenen Gattungen vor. Joachim W. Kadereit und Hans-Peter Comes (Mainz) befassen sich mit der räumlichen und zeitlichen Diversifizierung von Hochgebirgs-Clades der Blütenpflanzen in den Alpen. Ulrich Lüttge (Darmstadt) behandelt die evolutiven Aspekte der Physiologie und Morphologie bei Blütenpflanzen und gibt eine Übersicht über das Vorkommen und die Evolution des Crassulaceen-Säurestoffwechsels. Manfred Kluge (Darmstadt) diskutiert an der Gattung *Kalanchoë* Aspekte des Crassulaceen-Säurestoffwechsels. Im Spannungsfeld von Morphologie und Evolution sind die Beiträge von Günter Theissen (Jena) über die Blütenentwicklung bei Angiospermen sowie Peter K. Endress (Zürich) über die Möglichkeiten und Aufgaben für die evolutive Morphologie der Blütenpflanzen angesiedelt. Die botanischen Beiträge sind in englischer Sprache verfasst.

Wissenschaftliche Verlagsgesellschaft Stuttgart

Nova Acta Leopoldina NF *114*, Nr. 391, 93 – 96 (2013)

Synthesis of Section I: Growth and Defense in Plants – the Players

Heinz Rennenberg and Judy Simon (Freiburg)

Defense by plants is usually seen as an investment of resources by the plant host to counteract an attack by a pathogen and/or by herbivores. In more general terms, defense can be defined as an investment of resources by the plant target to counteract an environmental stress, either biotic or abiotic, and can impact above-ground and below-ground parts of the plant target. In this sense, defense is not restricted to more or less rare events, but constitutes part of the plants' everyday life. Considering the broad range of environmental stresses, it appears unlikely that all these constraints can be counteracted by one common mechanisms of defense. Still common strategies of defense have frequently been suggested. These strategies include the activation of the plants' anti-oxidative system, since many of these environmental stresses pose oxidative stress on plants (Noctor 2006), and the accumulation of defense-related transcripts and transcripts belonging to secondary metabolism (Ernst et al. 2013, this volume). In a set of studies by Fleischmann (2013, this volume) accumulation of phenolic metabolites differed depending on particular hosts and pathogens indicating that a much broader range of secondary metabolites has to be considered as defense compounds.

The general idea that at a given net primary productivity (NPP) plants in their specific environment allocate part of their NPP to growth and part of their NPP to defense, has to be extended by considering several aspects. First, the need for defense may generate new sinks that may enhance NPP. As a consequence, defense may not necessarily reduce the resource availability for growth (Horst et al. 2010, Fleischmann 2013, this volume). Second, simultaneous exposure to different constrains may not necessarily result in an enhanced investment of resources for defense, but may even result in a trade-off in the use of resources between different types of defenses. For example, in *Arabidopsis* a pathogen attack can reduce the investment of the plant in UV protection pigments in favor of pathogen defense compound production (Schenke et al. 2011). Third, perennial plants that are able to accumulate a significant amount of resources as storage reserves, can uncouple resource investment from resource acquisition (Rennenberg et al. 2009, Rennenberg and Schmidt 2010). Under these conditions, the investment of stored resources may be different compared to the use of recently gained resources, and a trade-off between the investment of resources in growth plus development and defense may no longer be required.

The players involved in pathogen attack of plants are not restricted to parasitic fungi (Ernst et al. 2013, this volume, Fleischmann 2013, this volume, Hückelhoven et al. 2013, this volume, Woodward 2013, this volume) or parasitic weeds (Kaldenhoff 2013, this volume), but include herbivorous animals, in particular insects, and pathogenic bacteria and viruses as well. In addition, solute sucking insects (Inbar and Gerling, 2008), rhizospheric bacteria competing for growth-limiting resources (e.g., nitrifying bacteria using N compounds to gain energy, Rennenberg et al. 2009), and well adapted plant species competing for growth-limiting nutrients with less well adapted species (e.g., early successional understorey species and tree natural regeneration in forests upon drought, Gessler et al. 2007) should also be considered in this context. Therefore, types of stress should not only distinguish between the site (above- *versus* below-ground), but also between the mode of action, i.e. cell destruction and consumption, solute consumption, herbivory, and nutrient depletion.

Already the observation that the site of stress response is not necessarily identical to the site of defense (e.g. stomatal closure upon drought, Ernst et al. 2010, effects of atmospheric ozone on rhizospheric processes: Haberer et al. 2007) indicates the significance of the exchange of information in stress response and defense strategies. It is most interesting that the exchange of information is not restricted to signaling processes inside the plant host, but can also include reprogramming of the metabolism and the induction of developmental processes in a bidirectional exchange of information between the plant host and the parasite (Kaldenhoff, this issue). In addition, the presence of constitutively preformed defense measures that may be present as scavengers of oxidants (Noctor 2006) and UV irradiation (Jenkins 2009), or may be liberated upon cell destruction by an attack (Halkier and Gershenzon 2006) are of high significance for plants to defeat environmental stresses. As a result of this exchange of information or/and the action of preformed defense measures, a biotic or abiotic stress may be defeated, may take over / become injurious, or may be stopped by morphological or/and chemical barrier formation (Walters 2011). The latter may result in mutualistic coexistence or symbiosis. Formation of ectomycorrhizae between the roots of trees and fungi in temperate forests are considered a typical example for such a symbiosis (Prtisch, this issue).

In this context, the questions have to be addressed, who controls resource allocation and, hence, fungal growth in such symbioses and which processes are involved in this control (Kogel et al. 2006). In recent years it became evident (*i*) that the slowly adaptable partner (i.e. the tree) controls the growth of the highly adaptable partner (i.e. the fungus) (Matyssek et al. 2013, this volume, Dannenmann et al. 2009, Rennenberg et al. 2009) and (*ii*) that this control constitutes a tritrophic interaction between the tree, ectomycorrhizal fungi, and rhizospheric bacteria (Rennenberg et al. 2009) that may be considered an interaction within a holobiont system (Matyssek et al. 2013, this volume). Under unfavorable conditions, the control of fungal growth in ectomycorrhizal symbioses can be achieved, apparently, not only at the level of infestation, but also at the level of biodiversity. At reduced carbon allocation to the roots as a consequence, e.g., of drought or girdling, “costly” mycorrhizal fungi with large extra-radicular mycelia may be replaced by “less costly” fungi with small extra-matrical mycelia (Pena et al. 2010, Weigt et al. 2011). In particular it is important to consider the different impact of restricted and extended space occupation by the extra-matrical mycelium on the exploitation of the soil and the uptake of nutrients (Agerer 2013, this volume). In this area of research, it is a big challenge to elucidate, (*i*) which mechanisms prevent a microbial infestation from becoming pathogenic, (*ii*) which processes mediate the dynamic response to environmental stresses, and (*iii*) the role of memory processes (Thellier et al. 2013, this volume) in symbiotic interactions. We can expect diverse strategies and, hence, diverse answers to these questions for different types of symbioses.

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Section II
The Processes: Competition *versus* Facilitation

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Carbohydrate Allocation to Growth and Defense-Related Metabolism – A Modeling Approach at the Whole-Plant Level

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With 5 Figures

Abstract

Experimental factors such as nutrient and water supply, concentration of atmospheric carbon dioxide or irradiance can impact on the allocation of carbohydrates and nitrogen to the different and often competing processes in plants. In particular, the balance between growth-related primary metabolism and defense-related secondary metabolism is the object of several plant-defense theories which provide conceptual ideas how the level of defense-related carbon-based secondary compounds varies with changing environmental conditions. Under these hypotheses, the Growth-Differentiation-Balance theory was identified to be the most mature in theory development about plant defense. During the last decades, many experiments were carried out to test the adequacy of this theory for different plant species and experimental scenarios. However, in many cases these tests did not reveal consistent reaction patterns of carbon-based secondary compounds concentrations on experimental treatments.

The Growth-Differentiation-Balance theory predicts for nutrients and water a non-linear relationship between resource availability and allocation to secondary metabolism with highest allocation rates in case of intermediate resource availability. Consequently, evaluating the Growth-Differentiation-Balance theory needs to quantify the availability of the required resource in a given experiment on a scale from low to high resource availability. Further difficulties in testing the Growth-Differentiation-Balance theory arise from the fluctuating source and sink strength dynamics of plant internal resource pools during different growth stages and between different experiments, which cannot be fully addressed by a conceptual theory. As an approach to overcome such difficulties, we developed the numeric plant growth simulation model PLATHO, which simulates allocation to carbon-based secondary compounds.

We used this model to examine the extent to which patterns of carbon-based secondary compounds concentrations may relate to specific growth situations. To clarify the circumstances under which plants do vary allocation to defense-related metabolism as predicted by the Growth-Differentiation-Balance theory and to identify scenarios in conflict with the hypothesis, we used PLATHO for a cross-comparison of data sets from five contrasting experiments with juvenile beech. The PLATHO based analysis shows that differing responses of trees to similar treatments can be explained to a large extent by the experimental boundary conditions which lead to varying relative availabilities of carbon and nitrogen in the plants. Whether an experimental treatment, which enhances carbohydrate availability within the plant, induces increased concentrations of carbon-based defense-related compounds in pant tissues or not, depends primarily on the question whether growth conditions during the stage of leaf development were dominated by carbon rather than nitrogen limitation.

Zusammenfassung

Die Zuteilung von Kohlenhydraten und Stickstoff zu den verschiedenen und oftmals konkurrierenden energieverbrauchenden Prozessen in Pflanzen kann durch experimentelle Faktoren wie die Nährstoff- und Wasserverfügbarkeit, die Konzentration des atmosphärischen Kohlendioxids oder das Lichtangebot beeinflusst werden. Insbesondere die Balance zwischen Wachstumsprozessen und dem Metabolismus, der die Abwehrbereitschaft einer Pflanze gegenüber

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Parasiten und abiotischem Stress aufrechterhält, ist seit vielen Jahren ein Gegenstand der ökologischen Forschung. In diesem Zusammenhang wurden verschiedene, sich ergänzende aber auch teilweise sich widersprechende Hypothesen entwickelt, die Ideen liefern, wie sich ändernde Umweltbedingungen und experimentelle Behandlungen auf die Konzentration abwehrrelevanter, kohlenstoffbasierter Metabolite in pflanzlichem Gewebe auswirken. Unter diesen Hypothesen zum Abwehrvermögen von Pflanzen wurde die sogenannte „Growth-Differentiation-Balance“-Theorie als am weitesten ausgereift im Sinne der Theorieentwicklung identifiziert. Um zu überprüfen, wie zutreffend die Vorhersagen dieser Theorie über die Balance zwischen Wachstums- und Differenzierungsprozessen in der Pflanze sind, wurden während der letzten Jahrzehnte zahlreiche Experimente durchgeführt, in denen verschiedene Pflanzenarten den unterschiedlichsten experimentellen Bedingungen ausgesetzt wurden. Allerdings hat sich hierbei in zahlreichen Fällen noch kein konsistentes Bild ergeben, wie die Konzentrationen der abwehrrelevanten kohlenstoffbasierten Metabolite auf die einzelnen experimentellen Faktoren reagieren.

Die „Growth-Differentiation-Balance“-Theorie sagt für Nährstoffe und Wasser einen nichtlinearen Zusammenhang zwischen Ressourcenverfügbarkeit und Allokation von Kohlenhydraten in den abwehrrelevanten Metabolismus vorher, wobei die höchsten Allokationsraten im Fall mittlerer Ressourcenverfügbarkeit auftreten sollten. Für eine Evaluierung der „Growth-Differentiation-Balance“-Theorie ist es folglich notwendig, für ein gegebenes experimentelles Szenario die Verfügbarkeit der jeweils untersuchten Ressource auf einer Skala von „niedrig“ bis „hoch“ zu quantifizieren. Weitere Schwierigkeiten beim Testen dieser Theorie ergeben sich durch die Dynamik der Quellen- und Senkenstärke der beteiligten pflanzeninternen Ressourcenpools während verschiedener Stadien eines Experiments, die in einer konzeptionellen Hypothese nicht voll erfasst werden kann. Um mit solchen prinzipiellen Problemen bei der Theoriebildung zur pflanzlichen Abwehr besser umgehen zu können, wurde das numerische Pflanzenwachstumsmodell PLATHO entwickelt, welches die Allokation von Kohlenhydraten in den Metabolismus der abwehrrelevanten kohlenstoffbasierten Verbindungen simuliert.

Im vorliegenden Beitrag untersuchen wir, in welchem Maße Muster in den Konzentrationen der abwehrrelevanten kohlenstoffbasierten Verbindungen auf spezifische Wachstumssituationen zurückzuführen sind. Wir nutzen PLATHO für einen Quervergleich der Datensätze aus fünf kontrastierenden Experimenten mit jungen Buchen, um zu klären, unter welchen Umständen Pflanzen die Allokation von Kohlenhydraten in den abwehrrelevanten Metabolismus im Einklang mit der „Growth-Differentiation-Balance“-Theorie verändern, bzw. um Szenarien zu identifizieren, die in Konflikt mit den Vorhersagen dieser Theorie stehen. Die Analyse dieser Experimente zeigt, dass unterschiedliche Reaktionen der Bäume auf ähnliche Behandlungen weitgehend durch die experimentellen Rahmenbedingungen erklärt werden können, denen die Pflanzen ausgesetzt waren. Ob eine experimentelle Behandlung, die die Kohlenhydratverfügbarkeit in der Pflanze erhöht, auch die Konzentration der abwehrrelevanten Verbindung steigert, hängt hauptsächlich von der Frage ab, ob die Wachstumsbedingungen während des Stadiums der Blattentwicklung eher durch Kohlenstofflimitierung oder durch Stickstofflimitierung dominiert wurden.

1. Introduction

In natural as well as in managed ecosystems, plants have to compete with neighbors for the locally available resources light, water and nutrients. Thereby, the proportion of resources, which can be captured by a single individual within a plant canopy, depends strongly on its relative position to neighboring plants, on the spatial distribution of its resource-capturing organs, and on the spatial distribution of the resources in the surrounding of the plant. Once resources like carbon and nitrogen are captured and assimilated, plants have to balance their partitioning between different and sometimes conflicting demands such as organ growth and different biochemical functions. Carbohydrates, nutrients and energy are needed for feeding growth-related as well as defense-related metabolism, and for processes maintaining the plant in a living state. Such processes comprise re-synthesis of enzyme proteins, ribonucleic acids, and membrane lipids that undergo metabolic turnover, maintaining ion gradients across cellular membranes, and energy support for all kinds of metabolic activities. As the total demand of all such processes cannot be met simultaneously in most cases, resource allocation at the whole-plant level is also determined by a plant *internal* competition for carbohydrates and energy. Upon fulfilling the demand for processes (Fig. 1) which are indispensable for staying alive, plants have to trade-off their resources between the demand for growth to be competitive against neighbors and the demand for averting injury by parasites, e.g., pathogens (Matyssek et al. 2005).

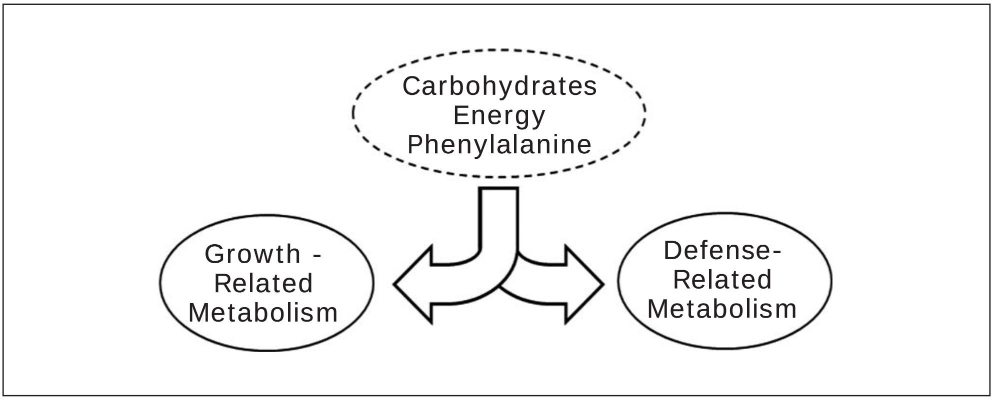

Fig. 1 The plant internal trade-off between carbohydrate allocation to growth-related metabolism or defense-related metabolism

A sizeable share of assimilated carbon is metabolized in plants *via* the phenylpropanoid pathway to soluble phenolic compounds, flavanoids, condensed tannins and lignin. These so-called carbon-based "secondary" compounds (in the following abbreviated as "CBSCs") are important for different functions and usages in plants. Many of the CBSCs are known to be involved in defense responses of plants against herbivores and pathogen diseases (Dixon 2001, Treutter 2005) or have important functions in wound sealing (Jones and Hartley 1999) or in the interaction between roots and soil organisms (Northup et al. 1998). Moreover, CBSCs are important for fruit and crop quality due to health promoting effects of antioxidative phenolics. CBSCs contents of plant tissues, in particular lignin, are also of interest in biofuel production, because lignin concentration is crucial in synthesizing ethanol from lignocellulosic biomass (Boudet et al. 2003). Partitioning of carbohydrates between growth-related and defense-related metabolism has therefore been the subject of ecological research since several decades.

2. Plant-Defense-Hypotheses

In many studies it was shown that environmental factors such as nutrient and water supply, temperature, light conditions or atmospheric carbon dioxide concentrations can influence the level of CBSCs in plant tissues. However, up to date, there is no clear picture of how these compounds respond under different environmental conditions, for example to elevated CO_2 or nitrogen fertilization (Luo et al. 2008). In apple trees flavonoid biosynthesis was inhibited by N fertilization resulting in a decreased pathogen resistance (Rühmann et al. 2002, Leser and Treutter 2005). Plant growth was negatively correlated with foliar phenolic concentrations in a study investigating effects of nutrient availability on constitutive herbivore resistance of poplar (Glynn et al. 2003). Glynn et al. (2007) also observed in a study across five fertility levels that medium nutrient availability results in highest total phenylpropanoid levels in two willow species. A study carried out by Blodgett et al. (2005) showed that fertilization decreases resistance of red pine. Stefanelli et al. (2010) looked within a review at the effect of nitrogen and water supply on quality of a wide range of fruits and legumes. They showed that in the examined studies maximization of carbon-based secondary metabolite concentrations is near the minimum level of nitrogen application. For water, in the same review, it was shown that limiting irrigation can increase the level of CBSCs in different plant species. A study with paper birch (*Betula papyrifera*) showed that CO_2 enrichment stimulated pathway-wide increase in carbon partitioning to phenylpropanoids (Mattson et al. 2005). Light-limited leaves are characterized by low concentrations of soluble phenolic metabolites in a study with different plant species carried out by Poorter et al. (2006). Additional nitrogen supply decreased phenolic concentrations in potato leaves (Mittelstrass et al. 2006).

Different complementary and, in some cases, contradictory plant-defense hypotheses were developed to explain the observed patterns and variations in concentrations of defense-related metabolites. Such conceptual approaches predict the impact of environmental resource availability on the balance of whole-plant resource allocation between growth and defense-related metabolism and, eventually, on the concentration of defense-related metabolites in plant tissues. The most prominent under the plant defense-hypotheses are the ‘Carbon-Nutrient-Balance hypothesis’ (Bryant et al. 1983, Tuomi et al. 1991), the ‘Protein-Competition model’ (Jones and Hartley 1999) and the ‘Growth-Differentiation-Balance hypothesis’ (Loomis 1932, Herms and Mattson 1992). The ‘Carbon-Nutrient Balance hypothesis’ explains that variation in plant defense is based on the environmentally available carbon and nitrogen supplies. This hypothesis predicts that plants growing in nitrogen-poor soils will favor allocation to CBSCs whereas such plants that grow under low carbon availability (e.g. low light conditions) more likely produce nitrogen-based metabolites of similar function. In addition CNB predicts that in plants growing in low-nutrient conditions the level of constitutive carbon-based defenses will decrease when the availability of nitrogen is increased (e.g. upon fertilization). The ‘Protein-Competition model’ focuses on the biochemical regulation in the synthesis of proteins and phenylpropanoids, both of which compete for the limiting resource phenylalanine, which is a branching point at the end of the shikimic acid pathway, being located between primary and secondary metabolism. As phenylalanine is an amino-acid and thus contains nitrogen, the growth-defense trade-off depends not only from competition for a limited pool of available carbohydrates, but also from competition for nitrogen as a component of common precursor compounds. The Growth-Differentiation-Balance theory (in the following abbreviated as “GDB”), in particular in its expanded form (Herms and Mattson 1992) was identified to mirror an advanced “maturing” stage in theory development about plant defense (Stamp 2003, Matyssek et al. 2005). The GDB predicts a parabolic relation between resource availability and allocation to defense-related compounds in plant tissues (Fig. 2) in the case of such resources whose increasing shortage reduces growth rate to a greater degree than photosynthesis. This prediction is founded on the presumption that the utilization of carbohydrates for growth processes (cell division and enlargement) has priority over the utilization for differentiation processes (i.e. chemical and morphological changes leading to qualitative differences between cells). Consequently allocation of carbohydrates to defense-related metabolites like the group of CBSCs takes place predominantly in situations where availability exceeds the demand for growth processes. Considering different levels of resource availability, therefore, the highest fraction of carbohydrates should be allocated to

CBSCs in plants that grow in an environment with moderate shortage of resources, which already limits growth while photosynthesis still sustains the accumulation of photosynthates. Typical resources of this type are water and nitrogen. An exception is the resource of light, which influences photosynthesis more than growth. In this latter case, CBSCs will increase in proportion to growth under increasing light (Herms and Mattson 1992). A more detailed discussion of presumptions, predictions and evidence of plant-defense hypotheses can be found in Matyssek et al. (2013a).

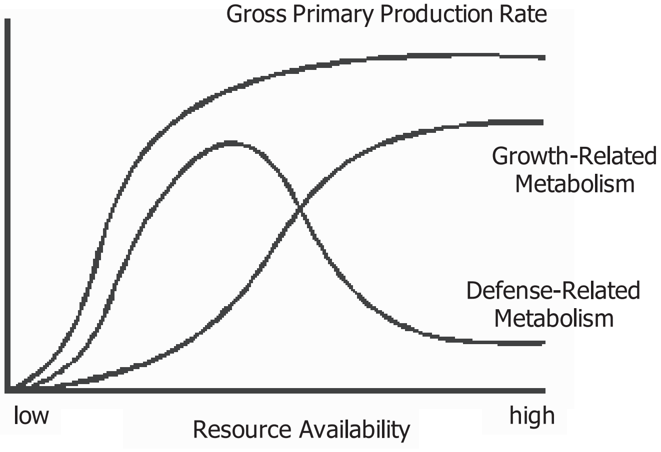

Fig. 2 Plant response across a resource gradient of nutrients or water as predicted by the Growth-Differentiation-Balance theory: Relationship of gross primary production rate, carbohydrate allocation rate to growth-related metabolism and carbohydrate allocation rate to defense-related metabolism (modified after Herms and Mattson 1992).

In many of the studies, which were conducted to investigate the effect of different levels of resource availability on growth and allocation to defense-related metabolism, the results are consistent with the predictions of GDB. Examples are given by Herms (2002) for the effect of fertilization on insect resistance of woody ornamental plants, by Mattson et al. (2005) for birch, by Mittelstrass et al. (2006) for potato, and by Le Bot et al. (2009) for tomato. But there are also many studies which yielded results which do not agree with predictions of GDB (Herms and Mattson 1992, Lindroth et al. 1993, Koricheva et al. 1998, Koricheva 2002a). As a consequence of these contradictory results considerable dissatisfaction with the importance and usefulness of GDB was raised. This is documented in several review papers and comments (Herms and Mattson 1992, Koricheva et al. 1998, Hamilton et al. 2001, Koricheva 2002b, Stamp 2003, Matyssek et al. 2013a, b). In particular the questions were discussed if it is resource availability at all which determines shifts in the balance between growth-related and defense-related metabolism (Hamilton et al. 2001) and if it is in fact possible to test the GDB in a rigorous way (Stamp 2004). There are a number of reasons which make it difficult to test the explanatory power of GDB directly by empirical experiments. Five most important constraints of hypothesis evaluation can be identified:

(*i*) It is hardly possible to quantify whether the plant-internal resource availability in a special experiment is "high", "intermediate" or "low" in the sense of GDB. Consequently, we do not know whether we are below, at, or above the optimum for resource allocation to defense-related metabolism. Due to competition effects between individuals in a stand or due to constrained functionality of the resource capturing organs, plant *external* resource availability and plant *internal* availabilities of nutrients and carbohydrates for metabolic processes can differ from each other considerably. Consequently, carbohydrate pools and nutrient levels in the plant should be measured rather than plant external availabilities of light, water and nutrients.

(*ii*) In general it is hardly possible in experiments to change the availability of a single resource without changing the relative availability of the other resources. Complex interactions of different factors hamper the precise analysis of the effect of a single resource on allocation to defense-related metabolism. For example, if CO_2 is increased in an experiment the demand of plants for nutrients also increases. Or additional nitrogen supply in mixed plant systems can change the competitive situation between individuals and hence the availability of light. Consequently, it is necessary to conceptually pursue more than one dimension, if GDB is tested, as for different treatment factors different non-linear effects are predicted by GDB (Matyssek et al. 2013a).

(*iii*) The fluctuating dynamics of carbon partitioning between growth-related and defense-related metabolism takes place at different temporal scales. Under the influence of circadian rhythms fluctuating transcript abundance of genes encoding enzymes in the phenylpropanoid pathway were observed in *Arabidopsis* (Harmer et al. 2000). Diurnal variations of secondary metabolism were also shown at the metabolite level in leaves of wild-type tobacco plants under nitrogen-deficient conditions (Fritz et al. 2006). In leaves of adult beech trees, a continuous seasonal decrease of concentrations of phenolic compounds was observed repeatedly over a time-span of three growing seasons (Bahnweg et al. 2005). Consistent observations were made for juvenile trees in a lysimeter study over a time-span of four growing seasons (Gayler et al. 2013). This indicates variable patterns of resource partitioning between growth-related and defense-related metabolism during different phenological growth stages at the organ level. Moreover, some of the secondary metabolites are continually catabolized and re-synthesized, and thus measurements of metabolite concentrations are just "snapshots". This means that observed products of defense-related metabolism do not necessarily reflect the actual costs of allocation to defense-related metabolism, unless the turnover rates of all single metabolites are known. Consequently, directly measuring total allocation flow to defense-related metabolism over a time period of weeks or month seems to be impossible, because metabolite assessments are restricted to net levels of resource allocation which neglect metabolite turnover.

(*iv*) Since a parabolic dependency of allocation on defense from resource availability is expected, a minimum of five resource availability levels, representing a resource gradient, is advisable in experimentation (Stamp 2004). However, in most experiments only two or three levels of resource supply have been realized. An exception is the study of Pizarro and Bisigato (2010), in which the GDB was tested on the response of allocation of photo-assimilates to five water supply regimes. As different plant behavior is claimed by GDB under severe relative to moderate resource shortage, effect prediction on carbon-based defense-related metabolism requires *a priori* knowledge of the physiological status of the plant and soil nutrient availability as depending on the ecological scenario.

(*v*) If results from different experiments are compared in evaluating the coherence of GDB over a wide range of experimental conditions, the problem arises on how to assess the resource availability levels which prevailed during the specific experimental situations of the individual studies. Due to the non-linearity of the expected relation between resource availability and allocation rates to defense-related metabolism, it is difficult to account for the actually differential resource supply across the single studies, which has consequences for adequately concluding about treatment effects. Consequently, for each experiment which is considered in such a study, the position of both the control plants and the experimental plants along the resource availability gradient (the x-axis in the conceptual model of Herms and Mattson [1992], see Figure 2) should be determined. Moreover, the determination of experimental resource gradients should be done separately for nutrients, water and light, if one cannot exclude that the availability of one of these factors is constant over all experimental situations.

Some of these limitations in evaluating GDB might be accomplished with considerable experimental efforts and by further elaborating experimental designs, as pointed out by Stamp (2004). However, barely any empirical study has fulfilled such advanced experimental standards. Therefore, Stamp (2003) and Matyssek et al. (2005) state that shortcomings in experimental design or failure in adequately testing conceptual presumptions bear the risk for arriving at contrasting or premature conclusions about the validity of GDB and other plant-defense related hypotheses. Consequently, modeling approaches are needed which provide reliably calculated assessments of the resource availability levels within plants over the duration of experiments or ecologically meaningful time periods.

3. Modeling the Growth-Defense Trade-off

Up to date, only few attempts have been undertaken to develop mathematical models which describe the growth-defense trade-off in plants (Coley et al. 1985, Gayler et al. 2008, Le Bot et al. 2009, Rötzer et al. 2013). The simplest of these models is the modification of the basic exponential growth equation as presented by Coley et al. (1985). Their model is also called the 'Growth-Rate hypothesis'. Despite the simplification, the model provides a distinct look at the relationship between costs and benefits of allocation to defense at the whole-plant level, explaining the relation between growth rate, allocation to defense and biomass loss due to herbivory. With the assumption that all resources which are not allocated to defense are available to growth, the model predicts that plants which allocate a high portion of their available resources towards the defense-related metabolism, inherently reduces growth rate to a larger degree than plants with lower investment into defense. However, a higher investment into defensive compounds potentially reduces injury by herbivores so that plants may benefit in habitats with high herbivore pressure.

There are two most important predictions of the model: (*i*) the existence of an optimum investment rate into defense, maximizing the growth rate under a given stress scenario, and (*ii*) decrease in the optimum investment rate along with an inherently increasing maximum growth rate of the plant. Consequently, the constitutive defensive potential of fast growing plants species with a high turnover of their organs should be lower compared to that of slow growing ones with low turnover. A comparison of saplings of 41 tree species, which were grown in light gaps of a rain forest, confirmed these predictions of the model. A negative correlation between growth rate and tannins was observed as well as a positive correlation between growth rate and the rate of herbivore damage to leaves (Coley 1988). Other examples do not agree with the Growth Rate Hypothesis. Cates (1996) for instance observed in fast-growing early successional pine species higher constitutive levels of defense compared to slower-growing late successional fir species. However, the potential for induced defense after wounding was markedly higher in fir compared to pine.

A new approach to simulate the effect of changing resource availability in the environment on carbohydrate allocation to defense-related metabolism in plants is realized in PLATHO, a dynamic plant growth simulation model (Gayler et al. 2006, 2008). The approach combines concepts of GDB and other plant-defense hypotheses, but in addition considers the volatile dynamics of source and sink strengths of carbon and nitrogen within the plant during different phenological growth stages. The assumption of GDB that a parabolic relation exists between nutrient availability and allocation to defense-related compounds is complemented by concepts of the 'Growth-Rate hypothesis' and the 'Protein-Competition model'. The idea underlying PLATHO is to estimate the dynamics of two key variables, φ_C and φ_N, which define the ratios between source and sink strengths of carbon and nitrogen within the plant:

$$\varphi_C(t) = \min\left\{1; \frac{\text{"C-source"}}{\text{"C-sink"}}\right\}; \quad \varphi_N(t) = \min\left\{1; \frac{\text{"N-source"}}{\text{"N-sink"}}\right\} \qquad [1]$$

In Platho, the sink-strengths of both resources are defined as the total amounts of carbon and nitrogen each, which are needed for supporting maintenance, for ensuring growth rates of plant organs and for meeting the demand for the synthesis of defensive compounds. During a given time step, they are calculated from the actual biomass of plant organs and its biochemical composition, from the phenological stage of the plant, from abiotic factors like temperature and, if present, from additional demands as inferred by defense against pathogens or detoxification of ozone (Gayler et al. 2004, 2009). The source strengths are defined as the amounts of carbon and nitrogen each, which are available for supporting all sinks within the plant during the considered time step. Source levels are calculated as the amount of resources which can be captured by the plant within the considered time interval as depending on the external resource availability and the competitive situation of the plant within the stand. Source-strengths further include the amount of carbon and nitrogen each, which can be drawn from mobile pools in the plant. Allocation rates of carbohydrates between biochemical pools at the whole plant level are then calculated as functions of these φ_C and φ_N. Four main pools are considered in the model: assimilates (temporarily existing products of photosynthesis and reserve remobilization), reserves (which can be mobilized if required), carbon-based defense-related compounds (CBSCs), and structural biomass. The simulation of resource partitioning between these pools is described in detail in Gayler et al. (2008). The key assumptions underlying the module for simulating carbohydrate allocation to the pool of CBSCs are adopted from GDB and the Protein-Competition model. The demand for maintenance, therefore, takes priority over all other processes in the model, growth takes priority over defense, and photosynthesis is less affected by moderate nitrogen deficiency than growth. This is in accordance with GDB, which predicts that additional assimilates may be converted into CBSCs if carbohydrates accumulate in excess over growth demands or if availability of nitrogen is lower than the nitrogen required for promoting growth processes. In addition, it is assumed that the formation of carbon-based defensive compounds, even though they contain no nitrogen, requires adequate levels of available nitrogen in the plant, being due to the requirements for biosynthesis of precursory compounds (Jones and Hartley 1999). Based on a study of Häberle et al. (2009), it is assumed that some CBSCs are always needed for plant tissue and only a restricted amount of assimilates is disposable between growth and defense-related metabolism. Consequently, a constant minimum baseline of carbohydrates is always allocated to the pool of CBSCs in the model whereas allocation of additional carbohydrates to CBSCs depends on plant internal carbon and nitrogen availabilities. Finally, recent photosynthetic assimilates are primarily directed to energy-consuming processes before reserves are remobilized (e.g. Lötscher and Gayler 2005). Given such a priority scheme in resource allocation, a function of the two variables φ_C and φ_N is derived, which is used in PLATHO to describe the assignment of carbohydrates to the pool of CBSCs (Fig. 3*A*). This function reflects a parabolic behavior of allocation to CBSCs with respect to nitrogen availability, which is in accordance to the predictions of GDB. With respect to carbon availability, the function reflects a linear increase of allocation to CBSCs with increasing carbon availability. This relation holds unless carbohydrate saturation is reached in the plant. As the internal carbon availability is closely related to light availability as long as a plant operates below photosynthetic light saturation, this behavior is also in accordance to the predictions of GDB. Consequently, the function realized in PLATHO (Fig. 3*A*) is a two-dimensional extension of the conceptual model of Herms and Mattson illustrated by the line for defense-related metabolism in Figure 2. Moreover, this numerical simulation model provides insights into the complex dynamics of resource allocation in time steps of hours to single days, which are hardly available from empirical studies alone.

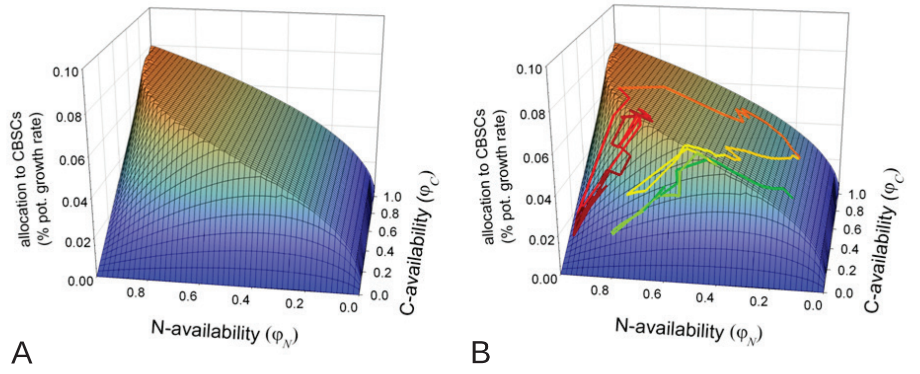

Fig. 3 (*A*) The shape of the function of φ_C and φ_N, which describes in PLATHO carbohydrate allocation to the pool of carbon-based defense-related compounds (CBSCs). (*B*) Simulated dynamic of $\varphi_C(t)$ and $\varphi_N(t)$ in an experimental scenario with juvenile beech trees and predicted allocation to the pool of CBSCs. The colored line joins daily mean values of the simulated variables. The scenario is starting in May and ending in September. The time course of the simulation is represented in by the color of the line from green (May) to red (September).

This performance of PLATHO is illustrated by Figure 3*B*, where simulated φ_C(t) and φ_N(t) and predicted allocation to the pool of CBSCs are exemplified for a scenario with juvenile beech trees. The simulation covers one growing season. The climatic forcing data and boundary conditions such as soil properties and fertilization rates are taken from an actual greenhouse experiment. Simulation results show that φ_C(t) and φ_N(t) can strongly fluctuate during one growing season, as processes such as phenological development, competition between individuals of different sizes (e.g. shading) or fluctuating supply of water and nutrients (e.g. precipitation, fertilization) strongly influence source and sinks strengths. Due to the large fluctuations in φ_C(t) and φ_N(t), the simulated trajectory meets areas of contrasting plant behavior for the same factorial setting. The model switches between a state, where the allocation to CBSCs is reduced with increasing nitrogen availability (e.g. the light green and dark red part of the trajectory), and a state where the opposite is the case (orange part of the trajectory). Apparently, the plant internal state of resources' availability can induce different types of system behavior during an experiment. Similar behavior can emerge, if experiments are compared, which were carried out under different environmental scenarios. Thus, different responses of plants to similar factorial scenarios are possible, depending on the plant's phenological stage and experimental constraints.

4. Cross-Sectional Analysis over Different Experimental Scenarios with Juvenile Beech

The adequacy of PLATHO to simulate growth of plants and variations in CBSCs was shown repeatedly by comparing simulation results with data from experiments, which were carried out with juvenile trees from different species (Gayler et al. 2004, 2006, 2008, 2009, 2013). These experiments were realized within the scope of the integrated research program "SFB 607 – Growth and Parasite Defense – Competition for Resources in Economic Plants from Agronomy and Forestry" (cf. Matyssek et al. 2002, 2005, 2013a) to investigate the trade-off between growth and defense over periods of several weeks up to four growing seasons. Amongst others, five experimental scenarios were realized with juvenile beech trees. In greenhouse, phytotron and lysimeter studies, conflicts in resource allocation were induced to learn about underlying mechanisms (Kozovits et al. 2005, Luedemann et al. 2005, Winkler et al. 2009). Such conflicts were mediated through contrasting levels of light and nutrient supply, chronic exposure to elevated ozone and carbon dioxide regimes or scenarios defined by competition amongst plants. Defense-related carbon-based compounds were measured once or several times throughout growing seasons and predictions by GDB were compared to experimental results.

To examine the extent to which response patterns in plants may relate to specific growth situations, we used PLATHO for a cross-comparison of data sets from the five experiments with juvenile beech. The aim of the analysis was to clarify the circumstances under which plants did enhance allocation to defense-related metabolism as predicted by GDB and to identify scenarios in conflict with the hypothesis. Only treatments were examined, where an increase in the carbon pool of trees compared to the pool of free nitrogen in the plants was to be expected. Such an increase was inferred if plants were exposed to elevated atmospheric carbon dioxide concentrations and increased light supply. After exposure to elevated concentrations of atmospheric ozone ("+O_3") a decrease of the pool of available carbohydrates was to be expected due to enhanced demand for energy and substrate for detoxification and repair. Consequently, compared to a "+O$_3$"-treatment, an analogous treatment with ambient ozone concentrations corresponds to an increase of carbohydrate availability in the plant. An increase in light supply of beech trees was mediated in these experiments indirectly by the planting scheme: Beech trees grown in mixed culture were shaded by dominating spruce trees, whereas higher irradiance prevailed when beech trees grew in mono-culture (KOZOVITS et al. 2005). According to GDB, increased carbon availability at constant nutrient supply should increase both growth and the plant internal pool of carbohydrates, the latter also inciting increased allocation to carbon-based defensive compounds. However, similar to the outcome from the reviews mentioned above, the results agree or disagree with GDB as depending on the specific case.

As a first step of the cross-sectional analysis the time course of plant internal availabilities of carbon and nitrogen, φ_C(t) and φ_N(t), were simulated for all experimental scenarios under consideration. Each simulation scenario was based on the respective experimental growth conditions which represented the inputs and constraints of the model. Simulation results from treatments, which increased the carbon supply to plant metabolism, were compared to the respective "controls", i.e., to the corresponding scenario but in the absence of the treatment (Fig. 4). In a second step, the means of $\bar{\varphi}_C$ and $\bar{\varphi}_N$ during the stage of leaf development were calculated from simulated φ_C(t) and φ_N(t). This stage is crucial for the observed plant response, when most of CBSCs are synthesized before underlying turnover, irreversible alteration or decomposition during subsequent phenological development. Aggregating $\bar{\varphi}_C$ and $\bar{\varphi}_N$ of corresponding "control" and "treatment" scenarios to

$$\tilde{\varphi}_C = \left(\bar{\varphi}_{C,control} + \bar{\varphi}_{C,treatment}\right)/2 \ \ and \ \ \tilde{\varphi}_N = \left(\bar{\varphi}_{N,control} + \bar{\varphi}_{N,treatment}\right)/2, \qquad [2]$$

then provides the data coordinates in a two-dimensional space of resource availability. The graphical treatment, thus, two-dimensionally positions the individual experimental scenarios along the resource gradients of nitrogen and carbon (Fig. 5).

Upon identifying the respective experimental scenarios along these gradients, treatment effects on allocation to growth and defense-related metabolism were assessed on the basis of measured plant growth rates and concentrations of phenolic compounds in plant tissues. These compounds were measured during late summer before leaf senescence. Due to turnover and decomposition, the late-summer measurements represent integrative information on the carbohydrate allocation to defense-related metabolism, reflecting the plant's history during the entire growing season. The different responses of trees under the individual experimental scenarios to enhanced availability of carbohydrates are indicated by different symbols in Figure 5 as a result of the cross-sectional analysis. Open triangles represent an increase of phenolic concentrations as well as of growth rates upon treatments increasing internal carbohydrate availability. Diamonds symbolize scenarios where phenolic concentrations were increased but growth rates were not. And closed triangles represent experimental scenarios where concentrations of phenolic compounds were not increased. The open symbols are separated more or less from the other symbols towards a direction, where $\tilde{\varphi}_N$ is increased but $\bar{\varphi}_C$ decreased. Apparently, allocation of carbohydrates to defense-related metabolism is enhanced by experimental treatments mainly in situations, where the growing conditions during the early stage of the vegetation period is dominated by low carbon availability rather than nitrogen shortage. Concurrently, in situations with low nitrogen availability relative to carbon

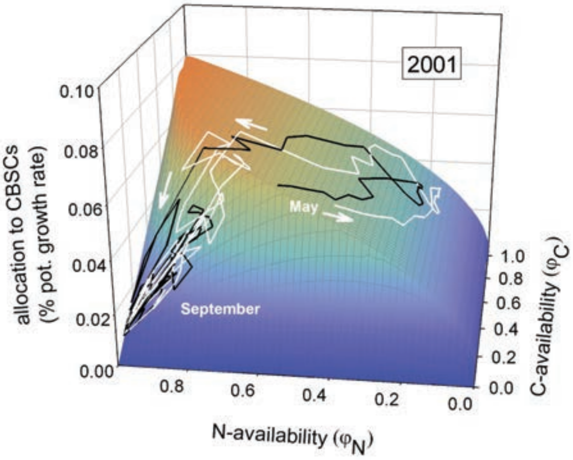

Fig. 4 Simulated time course of the plant internal availabilities of carbon (φ_C) and nitrogen (φ_N) and corresponding allocation to the pool of carbon-based secondary compounds (CBSCs) in a phytotron-experiment with juvenile beech trees during the vegetation period 2001. The black lines represent trees grown under ambient concentrations of atmospheric carbon dioxide (350 µl l^{-1}), white lines represent trees, which were exposed to an elevated concentration of atmospheric carbon dioxide (700 µl l^{-1}).

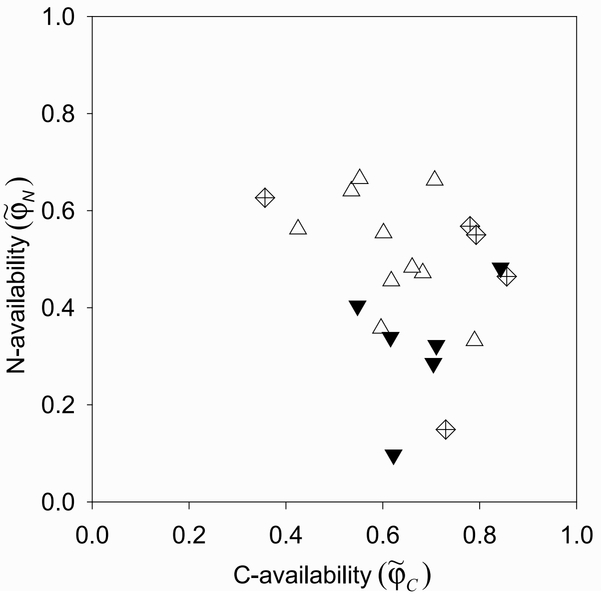

Fig. 5 Simulated position of different experimental scenarios with juvenile beech in the C- and N availability space. The reaction of plants on treatments which potentially enhance the availability of carbohydrates is indicated by different symbols. Open circles: defense and growth increased; diamonds: defense increased, growth not increased; half-filled circle: defense not increased, growth increased; closed circles: neither defense nor growth is increased.

availability, plants in most cases are not able to increase defense upon treatments increasing internal carbohydrate availability.

According to Figure 3*A*, increasing carbon availability drives allocation to CBSCs until a distinct saturation level is reached, preventing additional carbohydrates to be used for further CBSCs formation. Probably, surplus of carbohydrates remains in the assimilate pool, perhaps being used for reserve formation. Saturation is reached earlier in situations with low nitrogen availability in the plant, because in such a case the level of the nitrogen-containing amino acid phenylalanine, a precursor compound needed for both biosynthesis of growth-related and defense-related metabolites, is also low. If nitrogen availability is sufficiently high, both growth- and defense-related metabolism can be served with the effect of enhancing the saturation level. Thus, the model based analysis shows that the different responses of the trees can be explained to a large extent by the experimental conditions, which lead to varying relative availabilities of carbon and nitrogen in the plants.

5. Conclusions

Plant internal partitioning of carbohydrates and, hence, energy between growth-related and defense-related metabolism has been the subject of ecological research since several decades. Different hypotheses about environmental impacts on constitutive defense in plants were developed to explain patterns and variations in the concentration of carbon-based defense-related compounds in plant tissues. Among such conceptual frameworks GDB was identified as the one which is most advanced (i.e. “mature”) towards theory development (MATYSSEK et al. 2013a, b). However, testing the explanatory capacities by experimental approaches is difficult, and results seem to be contradictory in some cases. In particular, this is due to the presumed parabolic relation between resource availability and allocation to defense-related metabolism. A possible way out of this intricacy would be to estimate how strongly an experimental treatment changes the availabilities of all concerned resources and where an experiment is located along the respective resource gradients. However, so far this has hardly been achievable in a satisfactory way through experimental studies.

The dynamic plant growth model PLATHO was developed as a tool to support testing of plant-defense hypotheses and analyzing experimental results. The approach pursued in this model to simulate allocation to defense-related carbon-based metabolites is an extension of GDB, considering several factors that affect plant internal source- and sink-strength of carbohydrates and nitrogen simultaneously. The progress achieved compared to GDB is that the numerical simulation provides insights into the dynamics of resource allocation. This is because effects of fluctuating environmental conditions on source and sink strengths of plant internal resources are quantifiable at high time resolution. Such an outcome cannot be provided through empirical studies alone. Consequently, the model is useful for interpreting experimental results, for interrelating results from different experiments, and for analyzing complex interactions of experimental factors which change simultaneously.

Simulations show that in one individual experimental scenario different responses of plants to changing nutrient availability are indeed possible, depending on the actual ratios of plant internal source and sink strength of carbohydrates and nitrogen. These ratios can be highly volatile during a growing season, due to changing demands for carbon and nutrients during different phenological stages and processes such as competition between plants or fluctuating supply of water and nutrients. The simulated evidence is of high relevance in view of the debates on the value of GDB, contributing to reconcile seemingly conflicting information from empirical assessments.

Employing the PLATHO model allows quantitative positioning of experimental scenarios and treatments along the resource gradients, given that boundary conditions of experiments are known. In this way, a prerequisite is provided for pursuing cross-sectional analyses over different experiments, which has not been achieved by previous reviews on the adequacy of plant defense hypotheses. Such a cross-sectional analysis was carried out over five experiments with juvenile beech trees, which were grown under contrasting experimental scenarios and treatments. The PLATHO based analysis shows that differing responses of trees to similar treatments can be explained to a large extent by the experimental conditions which lead to varying relative availabilities of carbon and nitrogen in the plants. Whether an experimental treatment, which enhances carbohydrate availability within the plant, induces increased concentrations of carbon-based defense-related compounds in pant tissues or not, depends primarily on the question whether growth conditions during the stage of leaf development were dominated by carbon rather than nitrogen limitation.

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Plant Competition: Can Understanding Trait-Behavior Linkages Offer a New Perspective on Very Old Questions?

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With 2 Figures

Abstract

The study of competition has a long tradition among ecologists, driven in large part by the visible impacts of shading on plant growth. Not surprisingly, strong competitors for light tend to be larger than weak competitors. However, plants simultaneously interact both above- and belowground. Though we may understand how reducing shading by neighbors, or adding fertilizer may mitigate resource limitation, what is less obvious is that under natural conditions, competition for soil resources often more greatly limits plant growth than does competition for light. However, the traits associated with root competition are less well understood. In my lab, we have been exploring the above- and belowground traits of plants that are associated with both their ability to suppress the growth of other plants, and their ability to resist being suppressed. We are finding that though size influences competition for light, there are few consistent predictors for competition belowground. One possible reason is that root growth is extremely dynamic, and the outcome of a diversity of responses to local stimuli. The failure to recognize these responses as 'behaviors', and instead to maintain a focus on static 'traits' may be limiting to scientific progress in understanding the causes and consequences of plant competition. Despite the large number of researchers studying plant competition for many years, I suggest that a degree of certainty has been placed on long-established concepts that may not always be consistent with data. Though here I focus primarily on interactions among plant roots, these findings suggest there is a need for adopting new perspectives on the functional and evolutionary ecology of plant competition.

Zusammenfassung

Die Untersuchung der Konkurrenz zwischen Pflanzen hat für Ökologen eine lange Tradition, die in weiten Bereichen durch die erkennbaren Beschattungseffekte auf das pflanzliche Wachstum bestimmt ist. Nicht überraschend tendieren starke Konkurrenten um den Faktor Licht größer zu werden als schwache. Jedoch interagieren Pflanzen gleichzeitig sowohl oberirdisch als auch unterirdisch. Obwohl wir verstehen, auf welche Weise abnehmende Beschattung oder Zugabe von Dünger die Ressourcenlimitierung abschwächen können, ist es doch weniger offensichtlich, wie unter natürlichen Standortsbedingungen Konkurrenz um Bodenressourcen oftmals das pflanzliche Wachstum stärker limitiert, als es bei Konkurrenz um Licht der Fall ist. Jedoch sind Wurzeleigenschaften in ihrer Relevanz für die Konkurrenzinteraktion vergleichsweise wenig verstanden. In meinem Labor prüfen wir ober- und unterirdische Pflanzenmerkmale auf ihre Fähigkeit, das Wachstum anderer Pflanzen zu unterdrücken, aber auch auf die Fähigkeit, selbst der Bedrängung standzuhalten. Obwohl Größe oberirdisch die Konkurrenz um Licht beeinflusst, finden wir wenige konsistente, verlässliche Merkmale mit Aussagekraft für unterirdische Konkurrenz. Als Ursachen erscheinen die äußerst hohe Dynamik des Wurzelwachstums sowie die Vielfalt von Reaktionen auf lokale Umweltreize. Die fehlende Beachtung solcher Reaktionen als „pflanzliche Verhaltensweisen“ und, stattdessen, das Festhalten an Betrachtungen statischer Wurzelmerkmale limitieren ein Ursache-Wirkungs-bezogenes Verständnis pflanzlicher Konkurrenz. Trotz der großen Anzahl von Forschern, die seit vielen Jahren pflanzliche Konkurrenz untersuchen, zeigt sich, dass teilweise lang etablierte Deutungskonzepte als gesichert angesehen werden, obwohl nicht immer Konsistenz mit der Datenlage besteht. Wenngleich sich der Beitrag primär auf Interaktionen zwischen Pflanzenwurzeln konzentriert, legen die präsentierten Befunde generellen Bedarf an neuen Betrachtungsweisen zur funktionellen und evolutionären Biologie der pflanzlichen Konkurrenz nahe.

1. Why Study Competition?

The study of plant competition has long taken a central role in plant ecology. Many well-known books have focused on competitive interactions (Harper 1977, Tilman 1982, Grime 1979), and competition remains integral to major models of plant growth, population dynamics, and community organization (Craine 2005). Such a focus on competition has many causes, including the visually obvious effects of shading on the growth of many plants. Though there are exceptions, competition for light is typically size-asymmetric (Schwinning and Weiner 1998), such that larger plants are at a disproportionate advantage in competition for light over small plants. The logic is quite simple – a plant able to 'overtop' another, has unrestricted access to the resource pool (light), while the smaller plant only has access to a limited resource pool. Followed over time, these size differences become compounded and exagerated (Schwinning and Weiner 1998). Such size-dependent effects of competition are apparent to anyone that spends summer days weeding a vegetable garden – it is always best to get the weeds while they are small, before they overtop and shade out your fall harvest.

Moving beyond the individual garden, and looking instead at forestry and agriculture, competition again remains a critical focus of both research and management. Planting densities, thinning recommendations, and weed control are based upon economic calculations that indicate plant competition can result in lost income. However, harder to estimate, let alone directly observe, are the effects competition can have on species diversity and community structure.

In many cases, strong competition can cause the exclusion of weak competitors from a community, reducing overall species diversity (Grime 1979, Keddy 2001, Tilman 1988). Well-known examples exist in the context of invasive species, where native vegetation is slowly replaced by invaders from distant shores. Although a number of mechanisms are involved in this process, competition can play a central role (Davis et al. 2000). These patterns of species loss are consistent with the competitive exclusion principle (Hardin 1960), and are central to well-known ideas such as Connell's intermediate disturbance hypothesis (Connell 1978), Grime's theories of community organization (Grime 1979), and Keddy's theory of centrifugal organization (Wisheu and Keddy 1992). Such changes are potentially predictable if species are able to be ranked in terms of competitive ability – forming a competitive hierarchy (Keddy and Shipley 1989). However, there is substantial data contradicting this common narrative of plant competition, with much of it coming from the realization that competitive interactions belowground are fundamentally different from those that occur aboveground.

Though light is essential to plant growth, so too are water and a number of mineral resources. As a result, plants can compete both above- and belowground, with root-root interactions often being more limiting under natural conditions to plant growth than the effects of shading (Casper and Jackson 1997). Further, competition belowground is typically viewed as size-symmetric (Cahill and Casper 2000), rather than size-asymmetric, as occurs with competition for light. Under size-symmetric competition, initial differences in plant size may still influence competition (such that a large root system may have a larger competitive effect than a small root system), but these size differences will tend to be maintained, rather than compounded, over time (Gerry and Wilson 1995). As a result, increases in the strength of root competition do not necessarily result in changes in community structure (Lamb and Cahill 2008, Mitchell et al. 2009).

Because of both the fundamental importance of plant competition to society, as well as its consequences for the structure and function of natural systems, it is important to understand the mechanisms that determine and drive competitive interactions among plants. In this paper I will focus primarily on how plant traits – both structural and behavioral – influence plant competition. Further, I will tend to include both root and shoot interactions – providing more generality than occurs when only one competitive form is discussed.

2. Linking Traits to Competition

2.1 Competitive Ability

Though it is clear that species vary greatly in competitive abilities (Cahill et al. 2008), it is less clear as to why. In Figure 1, I present a simple diagram describing the proximate factors that influence a plant's competitive ability. At a most basic level, we are assuming that there is a genetic basis for plant competitive ability, traditionally expressed as variation in morphology and/or physiology of the organism. Surprisingly, few studies have demonstrated a specific genetic basis for a trait directly related to competitiveness, however, the existing evidence does support such an assumption. In a large comparative analyses, we found deep conservatism of competitive ability among eudicots and monocots (Cahill et al. 2008) implying a heritable component. In an artificial selection experiment using *Brassica rapa*, Miller was able to alter a plant's competitive ability (Miller 1995), further highlighting a heritable aspect to plant competition. At the genotypic level, my lab, and others, have found significant variation in competitive ability among genotypes within single species (Cahill et al. 2005). However, there is little expectation that 'genotype' is a direct mechanism driving competitive interactions, and instead genotype (along with environment and other factors) leads to differences in phenotype – which then influence competitive abilities. As an aside, studies measuring the additive genetic variance associated with plant competition evolution are generally lacking, and such evolutionary ecological studies are needed. Despite that lacking information, the idea that specific traits drive competitive interactions is intuitive, though the data supporting this assertion are fairly limited – particular when our attention focuses belowground.

The foundational work in linking plant traits to competitive ability came from Paul Keddy's research group (Gaudet and Keddy 1988). In this paper, the researchers used a phytometer method of comparative biology to measure the competitive ability of wetland species. They found that plant size was the dominant determinant of a species' competitive ability, a finding replicated in other studies (Keddy et al. 2002). However, trait-function research related to plant competition typically either uses high resource conditions, such that shading is likely strong, and/or measures are primarily of shoot, not root, traits. Though it is intuitive that larger plants will have a disproportionate advantage in size-asymmetric competition for light (Schwinning and Weiner 1998), it is less clear whether size positively influences competitive ability in size-symmetric competition for soil resources (Cahill and Casper 2000).

In my lab, we have use greenhouse experiments to explore the above- and belowground traits of plants that are associated with both their ability to suppress the growth of other plants (*competitive effect*), and their ability to resist being suppressed (*competitive response*), at both high and low resource levels (Wang et al. 2010). Overall, we found competitive effect and response are weakly positively correlated, though the main traits associated with each com-

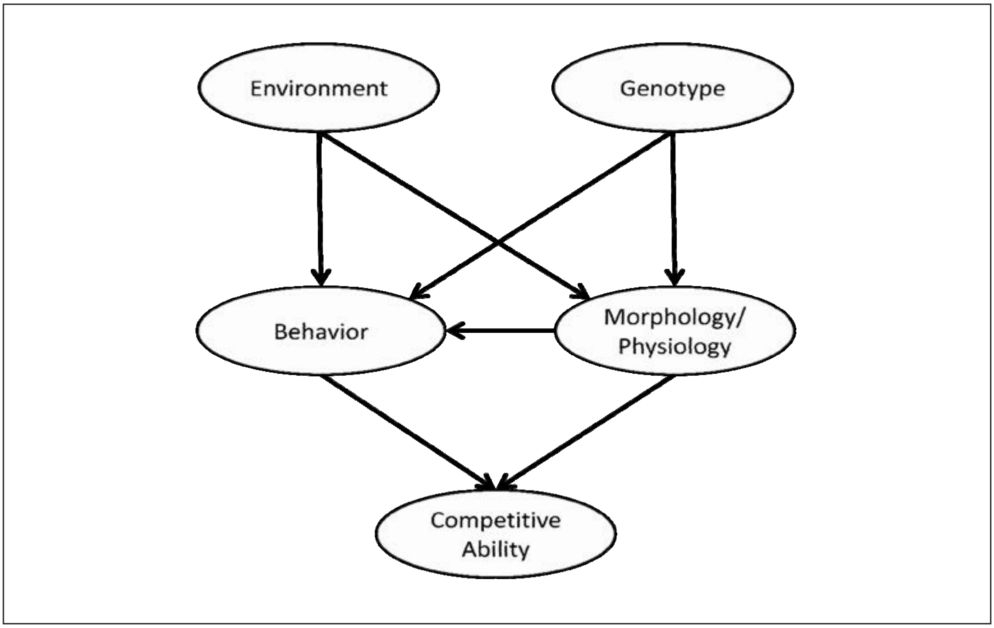

Fig. 1 Proximate factors influencing the competitive ability of a species. The traditional plant competition model would typically exclude the inclusion of behavior, focusing instead primarily on the static morphology and physiology of an organism.

petitive form differ. Competitive effect tends to be most strongly associated with size, even under low nutrient conditions where competition for light is limited. Trait-associations in competitive response are more complicated, such that no consistent set of root or shoot traits appear to be associated with a plant's ability to avoid competitive suppression. As a result, we suggest that competitive response is not a discrete aspect of a plant's competitive ability, and instead is a combination of a plant's stress-avoidance and competitive suppression abilities (WANG et al. 2010).

One result that was clear from our 'simple' greenhouse experiment was that there is no single trait that determines a plant's competitive ability. Thus, though size can be important, a focus on that single plant trait gives only a partial understanding of the factors that determine a plant's competitive ability. It is from this that my group has begun to question the value on focusing on single traits as indicators for ecological function, at least with regards to plant competition.

2.2 Competitive Struggle

Traits play a central role not only in efforts to understand variability in competitive ability among different species, but also in the 'competitive struggle' (sensu DARWIN 1859) that is experienced by individuals in the throes of competition. In *Origin of Species*, DARWIN asserted that more closely related species will be more similar in 'form and function' than distantly related species (DARWIN 1859). Consequently, the 'struggle' between such species will be most intense. This is inherently a trait-based argument, where DARWIN was suggesting that similarity in traits (due to shared descent) results in intense competition. The idea of similarity leading to strong competition (Fig. 2) is well entrenched in the community ecology literature, and forms the basis for concepts such as limiting similarity (MacArthur and Levins 1967) and character displacement (Dayan and Simberloff 2005).

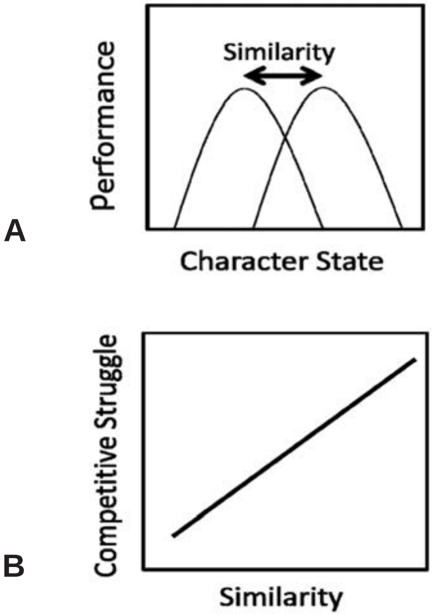

Fig. 2 In the traditional view of competition, (*A*) one can draw a hypothetical 'performance curve' for a given character, as a function of the state of the character. Different species may have similar curves, resulting in overlap. (*B*) In traditional competition theory, increased overlap in trait characters is associated with increased competitive struggle.

Despite the historic pedigree of this idea, it has been challenged by several groups in recent years. Violle et al. (2011) used an experiment to directly measure competitive struggle among different genotypes of protists, and found support for the competition-relatedness hypothesis. However, the data specifically regarding plant competition is less clear. The key issue is that as already discussed under certain conditions, competition can be driven by specific character states – such as being tall. Struggle within the community then emerges due to size differences and the directionality of light supply, as smaller individuals have access to a smaller light supply. In this model, it is a trait-state, rather than trait-similarity that may influence competition struggle (Cahill et al. 2008, Mayfield and Levine 2010). This idea has intuitive appeal, as the trait-similarity hypothesis would support an absurd position that a tall tree and a grass should not compete for light – as they have little 'similarity' in height. However, simple observation lets us know this is false, and instead our understanding of competition for light is based upon the idea that *size differences* result in compounded competitive effects over time (Schwinning and Weiner 1998). Aside from rhetorical arguments, there is beginning to emerge some data from plant systems directly testing these ideas.

The first comparative study on this issue was published by my lab in 2008 (Cahill et al. 2008). We used existing data sets that had measured competition among several sets of species, and related that information to the estimated phylogenetic distance of the species in competition. The relatedness-competition hypothesis predicts that as relatedness decreased, so too would the strength of competition. Our results found no indication of a strong relationship, and instead we found only a weak correlation between relatedness and competition. Overall, relatedness explained a very small proportion of the observed variation in competition, suggesting that even if 'significant', the relatedness-competition hypothesis was not likely 'important' in explaining much of the variation in competition among species. Following this study was a large field experiment from the Strauss lab (Burns and Strauss 2011). They found the effects of phylogenetic relatedness on a plant's overall performance were variable depending upon response variable and life-stage, at time supporting Darwin's assertion and other times not. Other studies testing this critical issue are lacking, and thus general principles cannot yet be drawn. It does, however, appear unlikely that a competition-relatedness relationship will be universal and invariant.

To help contribute to a resolution of this issue, my research group has recently conducted two separate experiments using (*i*) ecotypes of *Arabidopsis thaliana*, and (*ii*) 23 co-occurring grassland species. In both cases, we developed (or obtained) molecular phylogenies for the competitors, allowing us to estimate phylogenetic distance among ecotypes or species. We then conducted both pairwise and phytometer-based competition experiments measuring the strength of the 'struggle' (e.g. competition) between competitors. We also were able to measure a number of traits previously identified as important to competition (e.g. size, phenology). We are currently merging these trait, relatedness, and competition data sets – which should allow us to make meaningful contributions to this critical issue in plant competition and evolutionary biology.

As a result of our lack of success in explaining substantial amounts of ecological function despite a very exhaustive trait characterization (Wang et al. 2010), and reasonable skepticism of the logic underlying an argument of universal similarity-competition relationships for plants, my lab has begun to question the utility of a trait-based approach to understanding competition. Though it is attractive to imagine a direct connection between form and function (Fig. 1), I cannot help but see traits simply as a list of anatomical or physiological features. Rather than count the number of bones in each finger, as might a comparative zoologist, the botanist is focusing on the length and thickness of leaves, roots, or other organs. From these data sets one can describe broad patterns in the evolution of the hand, or gross variations in photosynthesis, but do either explain how individuals actually live within a specific community? In other words, does anatomy indicate individual function? I suggest not – instead it gives you the general constraints of what is possible (it CAN run fast, or it COULD dig), but it doesn't explain the fine details of how the organism actually lives. Or put another way – structure indicates constraints (one cannot fly without wings), but not the actual interactions of an individual in response to the abiotic or biotic aspects of their environment (e.g. some birds hide, while others fly away in response to a predator). What if the same is true for plants? What if traits are only the starting point for understanding function, not the endpoint. This issue is well resolved for animal biologists, as the discipline of behavioral ecology merges physiology and anatomy with social interactions. It is clear that the outcome of encounters are based not solely on 'what' an animal has, but also on how they use it. Plant ecophysiologists have also typically focused on the linkage between form and function, but I suggest that community ecologists often skip this integrative component. More specifically, without explicitly allowing for ‘behavior’, when studying trait-function relationships (Fig. 1), I suggest there may be little hope in understanding the functional ecology of species interactions. One of the first steps in this direction is to recognize that even for plants, time matters.

3. Integrating Time and Behavior into an Understanding of Plant Competition

A trait-function interpretation works well, if, and only if, plant interactions are completely deterministic. However, it has been suggested that such encounters can also be viewed analogous to ecological games (Gersani et al. 2001, O’Brien and Brown 2008), such that the optimal root growth strategies are contingent upon the distribution of neighbors within the soil environment. Or put another way, if root growth and function are variable due to the local nutrient and competitive environment, then we should not expect deterministic models of competition to be generally successful. Animal behaviorist has recognized this contingency in competitive interactions for decades, using game theory as an effective tool for describing alternative strategies and outcomes. Plant behaviorists have only begun to do the same, recognizing that plasticity and ‘contingent’ growth is more than a novelty of plant biology – instead it is fundamental to mechanisms of plant interactions (Cahill and McNickle 2011). Whether one chooses to embark on this path or not is likely dependent upon one thing – evidence that plants exhibit behavior.

4. Root Growth and Foraging Behavior

There are a number of papers demonstrating a diversity of plant processes typically referred to as behavior (Dudley and File 2007, Karban 2008). There are also a large number of papers debating the proper term (Trewavas 2009). Personally, I prefer the term ‘non-cognitive behavior’; in anonymous reviews I have been criticized for both implying a lack of cognitive action (by those that believe cognitive-like processes may be controlling plant growth) and for suggesting that non-animals may exhibit behavior (preferring ‘adaptive plasticity’). By being both too conservative and too liberal to reviewers, non-cognitive behavior seems to suggest a reasonable balance. As my research program has focused primarily on belowground processes, that will remain my focus here.

Root growth is (mostly) non-random, influenced by very fine scale distributions of resources and neighbors (Cahill and McNickle 2011). Or put another way, plants demonstrate non-cognitive behavior by altering where they forage as a function of resource and competitor distributions. There is substantial variation in species’ abilities to make the fine-scale adjustments, resulting in alternative ‘behavioral types’ among species (Cahill and McNickle 2011). Some of this behavioral variation is associated with deep evolutionary changes – such as eudicots being more able to proliferating roots in nutrients patch than are monocots (Kembel and Cahill 2005). The consequences of variation in behavior related to resource capture has obvious implications for plant-plant competition. However, there are few studies that have directly analyzed how variation in behavioral types of plants alter either the strength or importance of competition in communities (e.g. Day et al. 2003).

Root foraging behavior is exhibited also in the timing of growth, not only the degree of root proliferation. This can be seen in some recent work in my lab testing the applicability of the Marginal Value Theorem (MVT) to understanding root foraging. The MVT was developed by Eric Charnov, and represents a predictive model of 'patch use' by foraging animals (Charnov 1976). The underlying question is quite simply: How long should an animal remain in a patch, before moving to a new patch? The theory has been extremely influential, and been tested in a number of systems. A key recognition of the model is that the rate of cumulative resource gain diminishes as resources are removed – a reality that should occur regardless of whether the foraging organism is a coyote or a carrot. In general, the model predicts that organisms should stay in a patch longer as the value of the patch increases. Gord McNickle and I tested this prediction, using mini-rhizotrons allowing us to visualize root growth over time. The results were broadly consistent with theory – when presented with high and low value nutrient patches, plants reduced the lateral spread of their root systems, even though they had access to higher levels of resources than those plants encountering a lower quality patch (McNickle and Cahill 2009). Though this result is consistent with optimal foraging theory, it is not that which would be expected with a simple assumption that plants grow uniformly bigger in response to increased nutrient supply.

The issue of patch use, and its relevance to plant competition, can be seen in a related experiment conducted by my lab. Here, we again used mini-rhizotrons to allow us to visualize root growth in relation to nutrient distributions. However, we did this while also varying the presence or absence of a competitor. Amongst animal behaviorists, it is widely recognized that animals integrate information about patch quality and competitors (Brown et al. 1994), such that foraging decisions are not necessarily additive responses to these two factors. We found a similar pattern for plants (Cahill et al. 2010). Specifically, *Abutilon theophrasti*, a common weed in agricultural regions of central North America, showed high rates of avoidance of neighbor roots – resulting in a spatial segregation of the soil habitat. Further, when grown alone, root growth was agnostic with respect to soil nutrient distributions. However, when a nutrient patch was placed between the competitors, this neighbor-avoidance behavior was relaxed (Cahill et al. 2010). Recently, a second study has also found that plants appear to integrate information about nutrient and competitor distributions, resulting in novel patterns of root growth and foraging behavior (Mommer et al. 2011).

Demonstration of information integration has significant implications for understanding plant competition, in that it suggests that plants may alter the degree with which they compete with neighbors as a function of the resource environment. This is subtly, but importantly, different from the classic idea that the strength of competition may vary along resource gradients (e.g. Reader et al. 1994). In the classic model of plant competition, competition is simply an inevitable consequence of plants and the environment. In other words, at particular resource levels, one might expect a certain degree of root and shoot competition – a deterministic interaction. The behavioral data here indicate plants have the potential to actively alter the strength of competition they – as individuals – experience. The consequence is potentially profound, in that competition is not a deterministic outcome of the environment, and instead is dependent upon behavioral responses. As species differ greatly in behavior (Cahill and McNickle 2011), the null expectation is that there should be no consistent relationship between habitat productivity and the strength of competitive interactions.

My lab has shown nutrient distributions are one stimulus to which plants may alter their engagement in competitive interactions, though there are likely others yet to be described (Cahill and McNickle 2011). It is worth noting that traditionally competition experiments are conducted with homogeneous nutrient distributions, even though nature is notoriously patchy (Jackson and Caldwell 1993). Thus, these fundamental characteristics of a plant – the ability to integrate soil information – may be widely overlooked to a standard experimental technique. I encourage researchers interested in extrapolating their findings to natural systems to consider using incorporating soil nutrient distributions commonly found in natural systems into their experimental designs. Mixed soil in a greenhouse pot is unusual outside of agricultural systems.

None of these examples of foraging behavior would be predicted by traditional trait descriptions of plant and their root systems. There is nothing one can see and measure in the morphology of a root that would lead you to conclude that it will spend more time in patches of high quality than in those of lower quality. Further, there does not appear to be any morphological indicator that some species are more likely to exhibit this behavioral type, while others will not. In other words, the linkage between form and function is opaque. Further, there is nothing to be dissected out of a plant that we can point to as a center of 'information integration', yet we see plants responding to very fine-scale information in the soil. Despite a lack of understanding of all the mechanisms that drive these patterns (e.g. information integration), and despite it being substantially more difficult to study behavior than anatomy, I suggest a reconceptualization of the nature of plant interactions is needed.

5. Moving Forward

Despite the large number of researchers studying plant competition for many years, there remain a number of substantial holes of understanding. In particular, I believe that a degree of certainty has been placed on long-established concepts that may not always be consistent, nor even confronted, with data. Of particular concern is the emerging focus on plant traits (of which my own lab is party to!) – suggesting that the function of an organism can be accurately described by a list of its parts. However, my lab – and others – are showing that plants are substantially more dynamic than would be consistent with a mere additive outcome of traits, i.e. reflecting behavior as a bridge between anatomy/physiology and ecological process. There is substantial evidence to believe this will be particularly important in understanding competition for soil resources, and attempts to identify key 'traits' associated with competitive ability belowground have been largely unsuccessful. In animal ecology, it is standard that one needs to understand behavior if one wants to understand how individuals interact with other individuals. Plant ecology is further behind.

There remains substantial room for additional research in the functional and evolutionary ecology of plant competition. Such research is critical, as competition is typically a great influence on plant establishment and growth, with significant consequences for biodiversity conservation, agriculture, and forestry.

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Imaging and Integrating Heterogeneity of Plant Functions: Functional Biodiversity from Cells to the Biosphere

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Bildgebende Verfahren und Computertechniken spielen auch in der Botanik eine immer größere Rolle. Räumliche Heterogenitäten und zeitliche Dynamik von Strukturen und Funktionen sind essentiell, um das Verhalten von Pflanzen und ihre Wechselwirkung mit Böden und der Atmosphäre verstehen zu können. Ihrer quantitativen Analyse kommt deshalb eine Schlüsselrolle zu – sowohl für die Erforschung der Grundlagen pflanzlichen Verhaltens als auch für die Entwicklung innovativer Anwendungen in der Pflanzenproduktion. Neue Methoden der Bildaufnahme und die quantitative Bild(sequenz)analyse schaffen derzeit die Grundlage für ein völlig neues Verständnis der Bedeutung von Heterogenität und Dynamik in Pflanzen und Umwelt auf allen Skalen von der Zelle bis zum Ökosystem. Der Band verdeutlicht, wie dynamisch Wachstum, Photosynthese und Transport wirklich sind und welche Bedeutung räumliche und zeitliche Muster für Pflanzen haben. Die Beiträge belegen ein hohes Maß an Interdisziplinarität und Kommunikation zwischen Entwicklern von Verfahren, Anwendern aus den Pflanzen- und Umweltwissenschaften sowie Modellierern und Theoretikern. Es erweist sich, dass die Integration von modernen Methoden, innovativen experimentellen Ansätzen und Theoriebildung unser Verständnis von Pflanzen und von ihrem Verhalten in ihrer sich ständig verändernden Umwelt grundlegend wandeln wird. Alle Beiträge sind in englischer Sprache verfasst.

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A Space-Related Perspective on Plant-Plant Competition

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With 2 Figures

Abstract

Plants are exposed to a plethora of environmental influences that are affecting the life cycle. This contribution discusses some of these influences, i.e. the effects of atmospheric changes by elevated carbon dioxide (CO_2) and tropospheric ozone (O_3) concentrations. The focus is on growth and resource allocation of two important central European tree species, Norway spruce (*Picea abies* (L.) Karst.) and European beech (*Fagus sylvatica* L.). Mechanisms of inter- and intraspecific competition between the two tree species are explored and the question is raised how plant-plant competition interacts with the effects of elevated CO_2 and O_3. To this end, competitiveness of plants is quantified and mechanistically interpreted as space-related resource investments and gains. Competition for aboveground space was found to have a decisive influence on the development of juvenile spruce and beech trees grown in mono- or mixed cultures. In this respect, space in itself was interpreted to have the function of a resource for plant life.

Zusammenfassung

Pflanzen sind einer Vielzahl von Umwelteinflüssen ausgesetzt. Dieser Beitrag diskutiert beispielhaft die Einflüsse von erhöhten Kohlendioxid (CO_2)- und Ozon (O_3)-Konzentrationen. Der Schwerpunkt liegt hierbei auf der Beeinflussung von Wachstum und Ressourcenallokation zweier wichtiger zentraleuropäischer Baumarten, der gemeinen Fichte (*Picea abies* (L.) Karst.) und der Rotbuche (*Fagus sylvatica* L.). Mechanismen der Konkurrenz um Ressourcen zwischen diesen beiden Baumarten werden erörtert und der Frage nachgegangen wie erhöhte CO_2- und O_3-Konzentrationen diese Mechanismen beeinflussen. Hierfür wird das Konkurrenzverhalten der Pflanzen als raumbezogene Ressourceninvestition und Ressourcengewinn quantifiziert und mechanistisch interpretiert. Die oberirdische Konkurrenz um Raum hat einen entscheidenden Einfluss auf die Biomasseentwicklung junger Buchen und Fichten in Mono- und Mischkultur. In diesem Sinne hat der Raum selbst eine Funktion als Ressource für die Pflanzen.

1. Plant Resource Competition and Effects of Elevated CO_2 and O_3

Resource competition between plants has been recognized as a driving factor in evolution (Grace and Tilman 1990). Although plant responses to their neighboring individuals have been studied quite intensively, much less effort was put into linking these reactions to evolution and rethinking of community theory (Lortie et al. 2004, Thorpe et al. 2011). Likewise, interactions of abiotic stressors such as drought or elevated temperatures with plant strategies in resource competition has attracted much less attention and stimulated less research. This is particularly surprising as competitive interactions are known to mediate plant responses to global change factors such as elevated CO_2 or O_3 concentrations (Karnosky et al. 2003, Körner 2006, Grams and Andersen 2007). Giving an example from the research of SFB 607, this type of interaction has been studied in a three-factorial experiment with mono- and mixed cultures of juvenile European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.) grown under experimentally increased CO_2 and O_3 concentrations. In this experiment biomass development of plants was dominated by the type of competition and not by the gaseous treatments: Juvenile beech trees grown in mixture with spruce displayed strongly limited biomass development irrespective of the gaseous treatment (Kozovits et al. 2005a, b). In contrast, growth of spruce was hardly affected by any treatment, reflecting the low phenotypic plasticity of this species. The high responsiveness of juvenile beech to neighboring spruce is also reflected at the foliage level (Fig. 1). Total foliage area of juvenile European beech trees grown isolated as single potted trees or in monoculture remained unaffected by the applied CO_2 or O_3 treatments (Kolb and Matyssek 2001). In contrast, inter-specific competition with spruce strongly limited the total foliage area of beech. Moreover, mixture with spruce evoked negative effects on beech foliage area with reductions of 30 and 50 % under elevated CO_2 and O_3, respectively (Fig. 1). The reduction of foliage area under elevated CO_2 in beech is most likely not a direct CO_2 effect but interpreted to result from intensified resource competition with neighboring spruce trees. Hence, the competitive scenario plants are exposed to, e.g. if growing in isolation or in mono- or mixed culture, has a high potential to evoke plant responses to abiotic stressors (Navas et al. 1999, Poorter and Navas 2003, Grams and Andersen 2007).

2. Quantifying Competitiveness by Space-Related Concepts

Because of their long life spans, studying resource competition in trees is challenging as experimental and observation periods are often limited to a couple of years. In this case, approaches that quantify the trees' competitiveness as a measure of individual fitness become relevant. To this end and as stated by Küppers (1989) "[...] an integrated view of C gain, increment of biomass and its architectural arrangement in space" is needed. Such an approach of cost-benefit relations may reveal efficiencies in resource turnover that unveil the competitive performance amongst neighboring individuals (Connolly et al. 2001). In other words, resource competition of plants is conceived as the integral of spatio-temporal resource use (Matyssek and Schulze 1987, Schwinning 1996, Daigo et al. unpublished). Along this line, Grams et al. (2002) tested an approach to quantitatively relate the competitiveness of woody plants to space-related efficiencies in resource investments and gains. These efficiencies comprise:

- space occupation as the ratio of occupied crown or soil volume per unit of biomass investment,
- space exploitation as the resource return form the occupied space and
- maintenance costs per unit of occupied crown or soil volume as a space-related respiratory or transpiratory cost for sustaining the plant structure involved in resource competition.

Subsequently, this approach has been employed and promoted for quantification and mechanistic interpretation of tree competitiveness (Kozovits et al. 2005b, Luedemann et al. 2005, Luedemann et al. 2009, Rodenkirchen et al. 2009, Grams and Matyssek 2010). This space-related view on analyzing competitive interactions among trees was supported by studies stressing the importance of the spatial arrangements of canopies and plastic responses of canopy volume in approaching a mechanistic understanding of competitiveness (Tremmel and Bazzaz 1993, 1995, Muth and Bazzaz 2002). In the following section, this space-related concept for quantifying plant resource competition is exemplified by competition studies on juvenile beech and spruce trees from SFB 607.

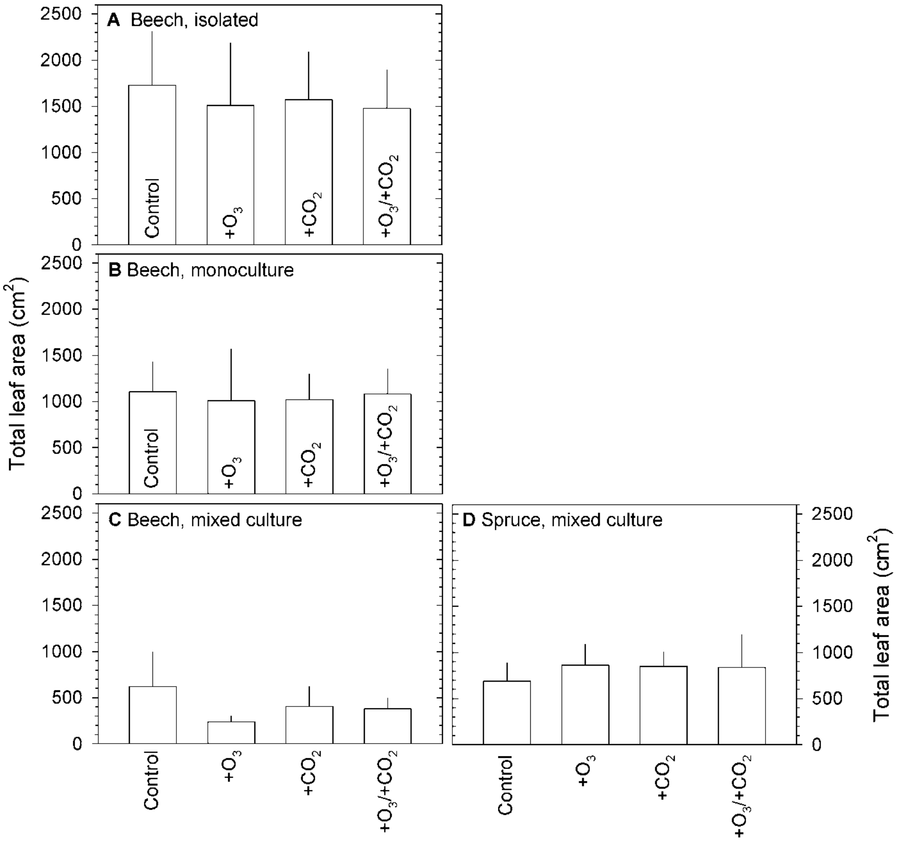

Fig. 1 Total foliage area at the end of the growing season of juvenile beech individuals grown (*A*) as single potted trees, (*B*) in monoculture or (*C*, *D*) in mixed culture with spruce, depicting the performance of beech (*C*) and spruce (*D*), respectively. Growth in mixed culture with spruce induced significantly reduced foliage area in beech under elevated O_3 and CO_2. Data taken from Kolb and Matyssek (2001) and Kozovits et al. (2005a).

3. Competition for Space: Examples from Experiments of SFB 607

The following examples originate from a series of growth chamber experiments that were performed during the years 1999 through 2005 in the phytotron facility of the Helmholtz Zentrum München – German Research Center for Environmental Health. Experimental design and procedures are detailed elsewhere (Kozovits et al. 2005a, Luedemann et al. 2005, Ritter et al. unpublished). In brief, 20 juvenile beech or spruce trees were grown for 2–3 years together in one container filled with untreated forest soil (dystric cambisol, pH of about 4.5) in either mono- or mixed cultures (soil volume of 62 l, with a surface area of 0.56 m × 0.37 m, soil depth of 0.30 m). The climate conditions and O_3 regimes throughout entire growing seasons were reproduced from a natural forest site. CO_2 from a gas tank was used to generate the "elevated CO_2" treatment (ambient CO_2 concentrations plus 300 µl l^{-1}).

After canopy closure and intense aboveground competition for light, competitiveness of tree individuals was related to the efficiency of above-ground space occupation, i.e. the ratio of occupied crown volume per unit of foliage and stem/branch axes biomass (Kozovits et al. 2005a, b, Ritter et al. unpublished). In mixture with spruce, beech individuals formed smaller crown volumes per unit of shoot biomass compared with beech plants grown in monocultures. Conversely, spruce displayed a somewhat increased efficiency in aboveground space occupation when grown in mixture with beech (Grams et al. 2002, Kozovits et al. 2005b). As mentioned above, gaseous treatments, i.e. elevated CO_2 and O_3, were only of secondary importance for the trees' competitive success. The relative growth rate (RGR) of beech individuals was significantly correlated with the efficiency in aboveground space occupation (Fig. 2). Saturation of this relation at high space occupation efficiency may reflect inefficient light harvesting when the occupied crown volume is only coarsely filled with leaves. As a result, shading of neighboring individuals is less pronounced reducing the trees competitiveness (see Section 4). No correlation was found between RGR and the aboveground efficiency of space exploitation, i.e. C gain per unit of occupied crown volume. The fact that the efficiency of space occupation was the only efficiency parameter that quantitatively related to RGR underlines the importance of this efficiency parameter in competition for the resource light (Weiner 1990, Grams and Andersen 2007, Daigo et al. unpublished). This confirmed earlier findings by Küppers (1985) and Schulze et al. (1986) that in a dense canopy, high competitive ability is related to increased efficiencies of aboveground space occupation and the shading of competing neighboring individuals. As growth performance of juvenile spruce trees was largely unaffected by the type of competition, i.e. growth in mono- or mixed culture, it is not surprising that no significant correlation of RGR with the efficiency of aboveground space occupation or exploitation was found. This further stresses the low phenotypic plasticity of spruce. The decline in the efficiency of aboveground space occupation of juvenile beech trees grown in mixture with spruce results from a lower investment into foliage per unit of shoot axes biomass in mixed compared to monoculture. This change in C allocation was found to be size-independent and thus to be a true regulation of the canopy architectural arrangement in response to intense aboveground competition (Kozovits et al. 2005b).

More recently, Ritter et al. (unpublished) performed an experiment to follow the allocation of recently fixed photoassimilates of plants in response to elevated O_3 and competition. To this end, juvenile beech and spruce trees were grown in mono- and mixed cultures under the same experimental setup as detailed above. Plants were ^{13}C-labeled for five days towards the end of the growing season by increasing the $\Delta^{13}C$ of CO_2 inside the growth chambers from the natural background of about –9 ‰ to +111 ‰. At the time of labeling, trees had already been grown for two years under elevated O_3 and intra- or interspecific competition, although tree biomass had not been affected yet by those treatments. Nevertheless, allocation of recent photoassilimates to beech stems as indicated by the ^{13}C label distribution was significantly lowered under elevated O_3. This reflects the initiation of reduced stem growth as observed in the previous experiments that extended through a total of three years (Kozovits et al. 2005a, Luedemann et al. 2005). In addition, these findings corroborate the generally high O_3 sensitivity of beech (Grams et al. 1999, Matyssek et al. 2010a, b). Conversely, spruce increased allocation of recent photoassilimates to stems when grown in mixed culture which reflected the higher competitiveness compared to neighboring beech individuals. Hence, allocation of recent photoassilimates indicated incipient tree responses to elevated O_3 and inter-specific competition. In this way, the mechanistic basis was provided for the biomass development

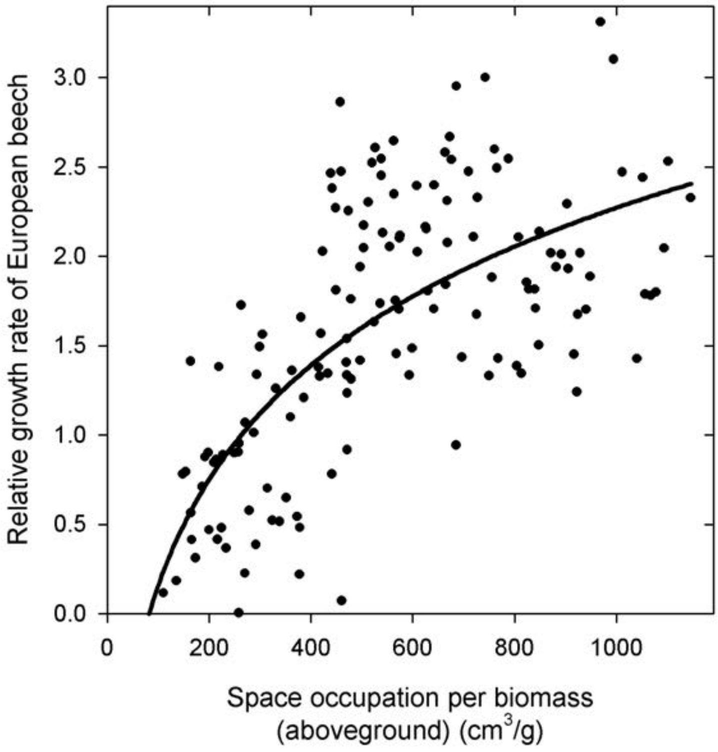

Fig. 2 Relative growth rate (RGR) of juvenile beech trees *versus* the trees efficiency in aboveground space occupation, i.e. the ratio of crown volume per unit of aboveground biomass (adopted from GRAMS et al. 2012) ($R^2 = 0.52$, $P < 0.001$). Together with the starting biomass of trees, the annual whole-tree RGR was calculated as (HUNT et al. 2002):

$$RGR = \frac{\ln(Biomass_{t1}) - \ln(Biomass_{t0})}{t_1 - t_0}, \qquad [1]$$

where Biomass_{t_0} and Biomass_{t_1} represent the biomass at the end of two subsequent years, i.e. the years t_0 and t_1, respectively.

as previously observed in experiments of longer duration on tree response to O_3 and intense aboveground competition.

4. The Resource “Space”

In the previous section the efficiency of space occupation was identified as a crucial factor in the aboveground competition between juvenile beech and spruce individuals. This raises the question of whether space *per se* is a resource to plants as recently discussed by GRAMS and LÜTTGE (2011). They conclude that indeed sheer space has the function of a resource to plants. This conclusion is supported by another example from SFB 607, the aboveground competition between adult beech and spruce trees at the research site “Kranzberg Forest”. Reiter et al. (2005) found beech trees to support branches in the deep shade that displayed negative annual C balances for at least five years. On the short run this may curtail the whole-tree C balance and conflicts with the paradigm of branch C autonomy (Sprugel et al. 1991). However, on the long run the value of the occupied space that is currently in the deep shade may increase, e.g. after gap formation, with resulting increase of the light level reaching the previously shaded branch. Thus, the support of those branches may be interpreted as a “sit-and-wait” or “gambling” strategy (Falster and Westoby 2003). Competing for and keeping this crown space occupied may pay back over time. Another example may even further illustrate that space occupation may be beneficial for plants irrespective of the resource return from that space. In the case of “pre-emptable” resources that allow for shading effects, such as the unidirectional resource light, space occupation may significantly reduce the resource availability to a competing neighboring plant. This effect alone can result in a competitive advantage, although resources available from the occupied space may be low. Hence, competing for and keeping space occupied may be an advantageous strategy of plants in resource competition, irrespective of the resources gained from this space.

In summary, interspecific competition of plants for resources has the potential to induce effects of elevated CO_2 or O_3 concentrations that may not be present under growth in monoculture or in isolation, i.e. as single potted plants. In the case of juvenile beech and spruce, such responses were more pronounced in beech individuals as this species displayed a much higher phenotypic plasticity than its competitor spruce. The lower competitiveness of juvenile beech in intense aboveground competition was related to a lower efficiency in aboveground space occupation compared to spruce. This was supported by a direct correlation between RGR and this efficiency parameter of competitiveness in juvenile beech. The reduction of aboveground space occupation in beech was brought about by a size-independent change in C allocation aboveground with lower investment in leaves versus shoot axes. Conversely, spruce displayed higher allocation of recent photoassimilates to stems when grown in mixture, reflecting its higher growth and competitiveness compared to its competing neighbor beech.

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Effects of Growth Conditions on Carbon Allocation in a Perennial Grass – the Role of Stores in Supplying Growth and Respiration

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With 1 Figure and 1 Table

Abstract

Sustaining individual fitness in a continuously changing environment requires plants to allocate photosynthate efficiently among competing sinks. This article is concerned with the role of carbon stores as substrate for respiration and leaf growth in *Lolium perenne* L., an important C_3 grass in temperate humid grasslands. It is based on experiments in controlled environments. Three scenarios are compared: plants growing in 'day/night' cycles at high nitrogen fertilization, and plants growing in continuous light with either 'high nitrogen' or 'low nitrogen' supply. Plants were labeled with $^{13}CO_2/^{12}CO_2$, and the changes in tracer contents with time were measured in respired CO_2, and in the flux of carbon into the zones of leaf growth. The tracer time courses, analyzed with compartmental modeling, showed a remarkable variation in the size and the turnover of stores. The adjustment of carbon storage deposition and mobilization fluxes seems to be an important ability for achieving high growth rates and individual fitness in a fluctuating environment.

Zusammenfassung

Um die Fitness unter ständig wechselnden Umweltbedingungen aufrechtzuerhalten müssen Pflanzen ihren Kohlenstoffgewinn aus der Photosynthese effizient auf verschiedene Senken verteilen. Dieser Artikel beschreibt die Rolle von Kohlenstoffspeichern für die Versorgung von Respiration und Wachstum in *Lolium perenne* L., einem wichtigen C_3-Gras der feucht-gemäßigten Breiten, das unter verschiedenen kontrollierten Umweltbedingungen gezogen wurde: in Tag/Nacht-Zyklen und hohem Stickstoffangebot sowie in Dauerlicht mit entweder hoher oder niedriger Stickstoffversorgung. Die Pflanzen wurden über Zeitintervalle von unterschiedlicher Länge mit $^{13}CO_2/^{12}CO_2$ markiert, die Erscheinungsraten der Markierung im respirierten CO_2 sowie im Substratfluss ins Blattwachstum gemessen und mittels kompartimenteller Analyse untersucht. Die Markierungskinetiken zeigten eine ausgeprägte Variabilität in Größe und Umwälzungsrate der Speicher für Wachstum und Respiration. Die beobachtete Dynamik scheint ein wichtiges Mittel zu sein, Wachstumsraten und individuelle Fitness in unterschiedlichen Umweltbedingungen zu maximieren.

1. Introduction

Plants are sessile organisms. Once a seed germinates and the roots penetrate a substrate, the seedling has to face the prevailing environment in its habitat in all weathers. The above and below ground space of the habitat needs to be explored by shoot and root growth and exploited for available resources to ensure growth and reproductive success.

In order to achieve and maintain a 'functional equilibrium', a balanced acquisition of resources (including CO_2, nutrients, water and light) is required for warranting the species' particular elemental composition and physiological functioning (Poorter and Nagel 2000, Ågren 2008). Resource acquisition and the capacity to resist biotic and abiotic stress are indispensable for the plant to stay competitive against neighbors and to sustain or increase overall fitness (Herms and Mattson 1992, Stamp 2003, Matyssek et al. 2012).

The successful development of the plant involves the concerted functioning of many substrate-demanding processes (terminal sinks) in different organs (leaves, [pseudo]stem, roots) that all rely on the limited amount of carbon assimilated in photosynthesis. Growth of shoot and root as resulting in fully functional biomass, able to promote further resource acquisition (Chapin et al. 1990), can dominate carbon demand. However, respiration, a complex process that provides energy and metabolic intermediates (Cannell and Thornley 2000, Plaxton and Podestá 2006), can at times consume up to 80 % of gross primary production (Amthor 1989, Van Iersel 2003). In addition, root exudation and symbiosis with mycorrhizal fungi and other micro-organisms that assist plant nutrition may account for a significant fraction of carbon consumption, too (Koide 1991, Grayston et al. 1996, Kuzyakov and Cheng 2001, Farrar et al. 2003, Grimoldi et al. 2006). Formation of carbon-based secondary defense compounds may further lead to a substantial demand for photosynthate (Dixon 2001, Gayler et al. 2008).

As a consequence, the plant faces a tremendous challenge: to allocate the valuable carbon resource amongst various competing sinks and balance resource use efficiency in a continuously changing environment. For example, the circadian day/night rhythm creates an unbalance in the photosynthetic carbon supply and sink demand. In plants of the C_3 and C_4 photosynthetic pathway (Larcher 2003), photosynthetic carbon supply during daylight hours mostly exceeds the concurrent carbon demands by sinks, although the sink demands at night can still be high in the absence of photosynthetic activity. Such kinds of unbalance can be buffered by the use of transient carbon stores (Chapin et al. 1990).

Many plants store a carbon surplus during daylight hours as carbohydrates, primarily as transitory starch in the chloroplasts or, as in C_3 cereals and grasses, as sucrose and/or fructan (fructose-based oligo- and polysaccharides) in cell vacuoles. Stores act both as kind of an overflow basin to prevent inhibition of photosynthesis by end product accumulation and as a carbon source when sink demand exceeds photosynthetic supply. Therefore, stores may be considered both as intermittent sinks and sources of carbon (Lüttge 2012).

Although the importance of carbon stores in the physiology and ecology of plants has been acknowledged in research for decades, the understanding of regulatory source-sink coordination has progressed only recently (see reviews by Smith and Stitt 2007, Zeeman et al. 2007, Walter et al. 2009, Graf and Smith 2011). Regarding mechanistic understanding of the role of stores in plant performance and resource use efficiency, it is indispensable to assess their participation in source-sink relationships and alterations in their contribution to the supply of terminal sinks under environmental impact.

The present article first provides a methodological outline for assessing respective research questions, and then summarizes recent findings about the carbon supply for respiration and leaf growth. Growth conditions will be highlighted as affecting substrate availability, exemplifying own work conducted with perennial ryegrass (*Lolium perenne* L.). Research on this important C_3 forage species in grasslands of the temperate-humid climate zone was performed within the framework of the DFG-supported Sonderforschungsbereich 607 “Growth and Parasite Defense – Competition of Resources in Economic Plants from Forestry and Agronomy”.

2. Materials and Methods

2.1 Investigating Substrate Pools with Stable Isotope Labeling Techniques

Assessing of whether two sinks like respiration and leaf growth consume the same or different substrate pools requires the characterization of the functional/biochemical identity of the metabolic carbon pools which may serve as potential carbon sources. One common feature of metabolic carbon pools is their turnover. That is, there is a flux of carbon through these pools, driven by the supply of new carbon from current photosynthesis and the carbon drain by sinks that are to be served. Pools of different functional identity turn over at different rates. For instance, carbon in pools closely related to photosynthetic pathways is exchanged within minutes (Bassham et al. 1954, Heber and Willenbrink 1964). Sugars in the vascular transport system of herbaceous plants show a half-life (i.e. the time it takes until 50 % of a pool is exchanged with new carbon) of a few hours (Geiger et al. 1983, Farrar 1989). Half-lives of one to several days were found regarding storage carbohydrates like chloroplastic starch or vacuolar fructan (Gibon et al. 2009, Lattanzi et al. 2012). That is, information about the half-lives of pools supplying particular processes allows to draw conclusions about biochemical and functional pool identities (e.g. currently produced assimilates vs. stores), even in the absence of direct biochemical analysis. Labeling with the stable carbon isotope ^{13}C is one adequate means of gathering the required information.

When a plant is grown under constant environmental conditions and an atmosphere with constant isotopic signature ($\delta^{13}C_{air}$, see Farquhar et al. 1989, Coplen 2011), the δ^{13}C of the substrate pools as well as that of the measured processes (e.g. leaf growth or respiration) stay constant, too. As a consequence, the δ^{13}C of the considered processes reflect isotopic equilibrium with $\delta^{13}C_{air}$.

When the isotopic composition of CO_2 is changed to a new, different $\delta^{13}C_{air}$ then the δ^{13}C of the photosynthetic CO_2 fixation becomes 'labeled'. The substrate pools in the plant are then gradually exchanged with new (labeled) carbon. This is translated into a gradual change in the δ^{13}C of the process supplied by such pools until a new isotopic equilibrium with $\delta^{13}C_{air}$ is reached. The higher the contribution of slowly turned over substrate to a process, the longer it takes to reach the new equilibrium. The rate of tracer change from the old isotopic equilibrium ($\delta^{13}C_{old}$) to the new isotopic equilibrium ($\delta^{13}C_{new}$) can be observed by periodic measurements of the isotopic signature of the process during labeling ($\delta^{13}C_{sample\ (t)}$). Using a two-member isotopic mass-balance equation, the fraction (proportion) of new, labeled carbon in the supply of the process at different times during labeling ($f_{new\ (t)}$) is then obtained as:

$$\text{fraction of labeled carbon } (f_{new\ (t)}) = (\delta^{13}C_{sample\ (t)} - \delta^{13}C_{old}) \,/\, (\delta^{13}C_{new} - \delta^{13}C_{old}). \qquad [1]$$

By definition, f_{new} is 0 before the start of labeling, and it eventually approaches 1, when the new equilibrium is reached (see Fig. 1). The rate(s) of change of f_{new} with time is what actually carries the information about the properties of the substrate supply system. This information can be extracted by compartmental analysis as described in the next section. For a more detailed description of tracer techniques using stable and radioactive carbon isotopes to study biological processes at various scales of time and complexity see Schnyder et al. (2012).

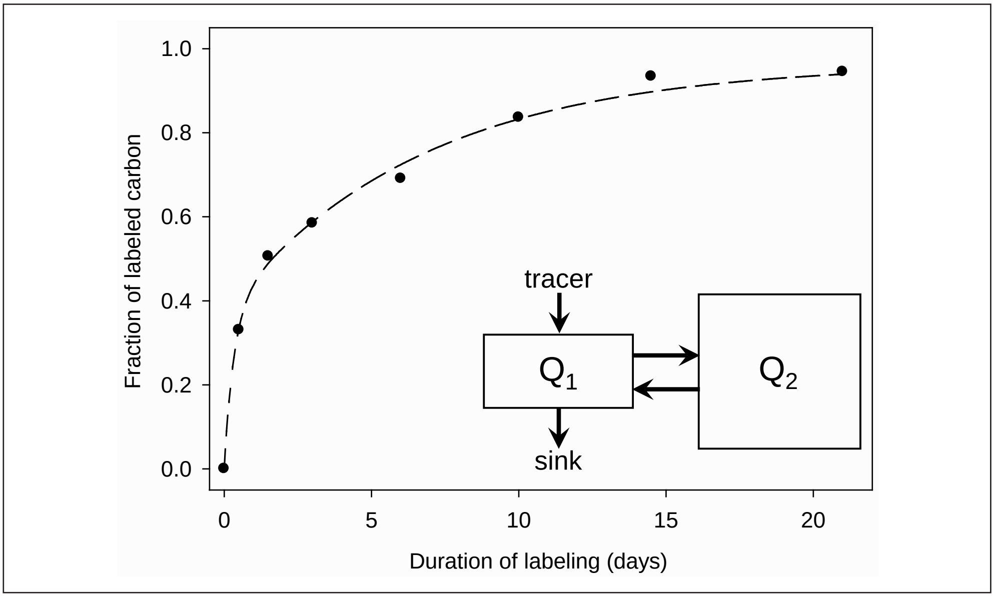

Fig. 1 Evolution of the fraction of labeled carbon in a process with duration of labeling (black circles). The dashed line is the prediction of the two pool compartmental model fitted to the data. See sections 2.1 and 2.2.

2.2 Analysis of Tracer Kinetics with Compartmental Models

Tracer (or labeling) kinetics (Fig. 1) can help to resolve a series of questions: How many pools are supplying a process? How are these pools interconnected? What are their sizes and half-lives? How much carbon do the individual pools contribute to the process? Such questions can be addressed by analyzing the tracer kinetics with compartmental models.

In a first step, the number of participating pools is explored by fitting exponential functions to the tracer kinetics. In our example in Figure 1, the tracer time course shows the fraction of labeled carbon increasing rapidly during the first days of labeling. At around day 3, the rate of tracer increase slows down and continues at a slower rate until the end of measurements at day 21. A function with two exponential terms of the form $y = 1 - (a{*}e^{-{*}t} + c{*}e^{-d{*}t})$ provided a good fit to the tracer kinetics. This function was statistically superior to a single-term exponential function; more than two exponential terms did not provide a statistically sharpened fit to the data, indicating that more than two pools are not necessary to simulate the given tracer kinetics. Hence, in accordance with the principle of parsimony (Ockham’s razor) the carbon supply system of the considered process is suggested to consist of two pools.

Exploring the interrelationship between the two pools, namely, as to whether only one or both of them receive tracer and supply the process, is more of a challenge. Several model arrangements may exist that provide equivalent fits to the measured data, which impedes a decision purely based on statistics. However, the pool characteristics assessed by the model do depend on its structure, and thus, it is important to consider the biologically most meaningful pool arrangement (SCHNYDER et al. 2012). A priori knowledge of biochemical pathways or cellular compartmentation is therefore essential for designing a plausible compartmental model with valid parameter estimation. For instance, it is reasonable to assume that a store (Q_2, Fig. 1) like a fructan pool in the cell vacuole exchanges carbon with a pool Q_1 that receives carbon from current photosynthetic assimilation, and comprises, for instance, cytosolic sugars.

Having made such a decision about the model, the further analysis of pool characteristics is straightforward. The fraction of tracer in the pools Q_1 and Q_2 (Fig. 1) is given by:

$$dQ_1/dt = \text{Tracer} + k_{21}{*}Q_2 - k_{10}{*}Q_1 - k_{12}{*}Q_2 \quad [2a]$$
$$dQ_2/dt = k_{12}{*}Q_1 - k_{21}{*}Q2 \quad [2b]$$

The rate constant k_{12} governs the flux from pool Q_1 to Q_2 and k_{21} that from Q_2 to Q_1. The flux out of Q_1, that is either the respiration rate or the carbon flux into the leaf growth zone, is governed by k_{10}. It is assumed that the system is in a steady-state, that is, pools sizes and fluxes do not change with time, and so, the flux into the system (tracer) equals either the respiration rate or the carbon flux out of the leaf growth zone into newly formed tissue. The differential equations [2] may then be either implemented in commercially available software (e.g. LATTANZI et al. 2005) or in a custom-made computer program (e.g. LEHMEIER et al. 2008). The rate constants are optimized to find the best fit to the tracer time course (the measured values; Fig. 1).

The optimized rate constants are used to calculate the pool sizes as

$$Q_1 = \text{Tracer}/k_{10} \quad [3a]$$
$$Q_2 = \text{Tracer}/k_{10} * k_{12}/k_{21} \quad [3b]$$

and the pool half-lives ($t_{1/2}$) as

$$t_{1/2}(Q_1) = \ln(2)/(k_{10}+k_{12}) \quad [4a]$$
$$t_{1/2}(Q_2) = \ln(2)/(k_{21}). \quad [4b]$$

The probabilities that tracer moves either directly through Q_1 without visiting Q_2, or that it is first deposited and remobilized in the store Q_2 are interpreted as the fractional contribution of a pool in the supply of the sink and defined as

$$\text{Contribution of } Q_1 = (k_{10}/k_{10}+k_{12}) \quad [5a]$$
$$\text{Contribution of } Q_2 = (k_{12}/k_{10}+k_{12}). \quad [5b]$$

The analysis of a tracer time course with compartmental models as exemplified here is based on several assumptions which are stated and discussed in LATTANZI et al. (2005) and LEHMEIER et al. (2008). As shown above, a major one is that the system is in a physiological/metabolic steady-state and tracer is supplied with constant isotopic composition.

2.3 Steady-state Growth Conditions in Controlled Environments

Perennial ryegrass was sown individually in plastic pots, which were filled with quartz sand, and distributed in growth chambers at a density of 378 plants m^{-2}. We applied three different treatments (see LEHMEIER et al. 2008, 2010a, b): plants were either grown under continuous 24-hour light exposure (275 µmol photons m^{-2} s^{-1}) with high or low nitrogen fertilization (“High N” and “Low N”, respectively), or with alternating 16-hour light (425 µmol photons m^{-2} s^{-1}) and 8-hour dark periods (“Day/night”). Every three hours, all plants received a modified Hoagland nutrient solution that contained either 1 mM NO_3^- (“Low N”) or 7.5 mM NO_3^- (“High N” and “Day/night”). All other experimental conditions were the same in the three treatments, including constant air temperature of 20 °C, relative air humidity of 85 % and ambient-air CO_2 concentration of 360 µL L^{-1}. Total daily irradiance was 24 mol photons m^{-2} in all treatments.

The growth chambers were part of a $^{13}CO_2$/$^{12}CO_2$ gas exchange facility (Schnyder et al. 2003). CO_2-free air was mixed with commercially available CO_2 of given $\delta^{13}C$ from gas cylinders and supplied to the chambers. The isotopic composition of the CO_2 in the growth chambers was monitored with a continuous-flow isotope-ratio mass spectrometer (CF-IRMS), and was kept constant by adjusting the rate of air flow through the chambers. Within each treatment, half of the plants were grown under ^{13}C-enriched CO_2, the other half under ^{13}C-depleted CO_2. Other growth conditions were identical.

Once closed stands were established, labeling was performed by switching the $\delta^{13}C$ of the CO_2 supplied to the plants (from constant ^{13}C-enriched CO_2 to constant ^{13}C-depleted CO_2 or *vice versa*), while all other environmental factors remained unchanged.

2.4 Respiration and Leaf Growth Measurements

Plants remained in the presence of the labeling CO_2 for intervals of different length, ranging from 1 h until almost 1 month. At the end of the target duration of labeling, plants were taken out of the growth chambers and either used for respiration measurements or harvested for leaf growth analysis.

Shoot and root respiration of individual plants was monitored for 5 – 8 h in the dark using a custom-made automated gas-exchange measurement system as described by Lötscher et al. (2004) and Lehmeier et al. (2008). The system served to determine the rates of shoot and root respiration (as mg C h^{-1}) as well as the $\delta^{13}C$ of shoot- and root-respired CO_2, and to calculate the rate and $\delta^{13}C$ composition of whole-plant respiration. Thereafter, the plants were weighed and frozen, and the contents of total carbon and nitrogen, and of water-soluble carbohydrates (including sucrose and fructan) were analyzed in the freeze-dried shoot and root biomass as described by Lehmeier et al. (2010a).

For leaf growth analysis, plants were dissected and the leaf growth zones at the bases of individual tillers were harvested as small, functionally distinct segments as outlined by Lattanzi et al. (2005) and Wild (2010). Freeze-dried tissue samples were combusted in an elemental analyzer, and carbon content as well as $\delta^{13}C$ of the biomass were assessed with the CF-IRMS, yielding rate and $\delta^{13}C$ of the carbon influx into the leaf growth zone as described by Lattanzi et al. (2004, 2005).

The same protocol of respiration and leaf growth measurements was followed for non-labeled control plants that experienced the same $\delta^{13}C$ exposure throughout growth until sampling. Analysis of these plants provided the end-members, $\delta^{13}C_{old}$ and $\delta^{13}C_{new}$, of the two-member mixing equation (see Equation [1]), which served to partition the fractions of old and new carbon in shoot and root respiration as well as in the carbon influx into the leaf growth zone. In that way, the kinetics of tracer incorporation into respiration and leaf growth during the labeling period were obtained.

3. Results and Discussion

All plants remained in vegetative growth throughout the entire experimental periods in all treatments (High N, Low N and Day/night, see Section 2.3). Measurements of leaf elongation of individual tillers and the analysis of carbon contents in shoot and root biomass with time showed that growth proceeded at constant specific rates. Also, specific shoot and root respiration rates did not change during the experiments. Thus, the ratio of photosynthate incorporated into new biomass to the carbon costs for new biomass synthesis plus biomass maintenance costs were also constant (Lehmeier et al. 2008, 2010a, b, Wild 2010). Such a performance underlines that the particular growth chamber conditions in each treatment provided steady-state conditions for plant growth with constant allocation patterns. That is, the only parameters that changed significantly with time were the fractions of tracer in the carbon supply of the sinks.

Tracer kinetics of plant respiration of all three treatments, and of the substrate flux into the leaf growth zones in the High N treatment showed a similar pattern: respiration and leaf growth was supplied by carbon from two sources, namely from current photosynthetic assimilation and from carbon that first underwent storage before it arrived at the sinks. The mechanistic features of source pools serving respiration and growth were extracted by compartmental analysis of the tracer kinetics using the two-pool model as shown in Figure 1 (see Section 2.2).

3.1 Day-length Effects on Carbon Stores for Respiration

The only difference in growth conditions for plants in the "Day/night" and the "High N" treatment was how the (same) amount of daily irradiance was distributed over the day: either it was provided during a 16 h photoperiod (with high irradiance) or it was supplied continuously (with low irradiance, Section 2.3). The difference in light regime had little influence on the growth performance of plants (Lehmeier et al. 2010b). Specific growth rates were about 8 % per day, and the photosynthetic carbon use efficiencies [calculated as growth rate/(growth rate + respiration rate)] were high and similar (approximately 0.7) in both light regimes. There were, however, fundamental effects on the turnover and possibly also on the biochemical identity of the storage pool supplying respiration.

While the pool of current assimilates (Q_1) was turned over at similar, rapid rates (half-life 5 h for Day/night and 6 h for High N; Tab. 1), the half-life of the store (Q_2) in Day/night was almost 4 times faster than the store in the High N treatment with continuous light. The store's half-life of 13 h in Day/night is close to half-lives reported for vacuolar storage sucrose (Farrar 1989), while the half-life of 48 h in the continuous light treatment is closer to half-lives for vacuolar fructan in leaf blades of perennial ryegrass (Lattanzi et al. 2012). Furthermore, plants in the Day/night treatment had a much lower fructan to sucrose ratio in total plant biomass (3:1 vs 10:1 in continuous light; Lehmeier et al. 2010b). These findings indicate that sucrose was a more abundant biochemical substrate in the respiratory store of Day/night plants, whereas plants in continuous light drew on a greater proportion of stored fructan for respiration.

3.2 Nitrogen Deficiency Effects on Carbon Stores for Respiration

The carbon mass of plants in the Low N treatment increased only at 4 % per day, that is, at half the rate of the plants in the High N and Day/night treatments. The specific respiration rate of Low N plants, however, was only reduced by about one third (Lehmeier et al. 2010a). As a result, the photosynthetic carbon use efficiency was reduced to 0.6. At least part of this lower efficiency was probably due to a lower shoot to root ratio of 3:1 as compared to 4:1 in the other treatments, and to higher specific costs of nitrate assimilation under conditions of nitrogen deficiency (Cannell and Thornley 2000, Lehmeier et al. 2010a).

While the storage pool supplying respiration comprised only 13 % of total carbon in the biomass of High N plants, the proportion was 20 % in nitrogen-deficient plants (Lehmeier et al. 2012). Also, the half-life of the store increased from 48 h at High N to almost two weeks at Low N (Tab. 1). A half-life of almost two weeks is much slower than the half-lives reported for storage carbohydrates in leaf blades of grasses (Farrar 1989). Rather, such slow turnover suggests that carbohydrates, mainly fructans, in basal leaf sheaths posed a significant fraction of substrate in the respiratory store of Low N plants (Borland and Farrar 1988). Leaf sheaths of grasses are known to be important storage organs under conditions of limited nitrogen availability (Pollock and Cairns 1991). Hence, growth conditions may not only affect the biochemical identity of the stored substrate for respiration (see 3.1) but also the spatial location amongst functionally distinct parts of the plant.

Tab. 1 Half-lives and fractional contributions of substrate pools (Q_1, Q_2, see Fig. 1) supplying respiration and leaf growth of *Lolium perenne* plants grown either in continuous light with High or Low nitrogen (N) supply, or in Day/night cycles with high N supply. Total daily irradiance and all other growth conditions were the same in the three scenarios (see Sections 2.2 and 2.3). Data are adopted from Lehmeier et al. (2010a, b) and Wild (2010).

	Leaf growth	Respiration		
	Continuous light			Day/night
	High N	High N	Low N	High N
Half-life (h)				
Q1	1	6	20	5
Q2	68	48	288	13
Fractional contribution (%)				
Q1	69	44	67	36
Q2	31	53	33	56
Unclassified old carbon	0	3	0	8

3.3 Shoot and Root Respiration are Supplied by the Same Substrate Pools

In all three treatments, the tracer kinetics of CO_2 respired from whole shoot and root systems, and consequently, the model structures and half-lives of pools serving shoot and root respiration were nearly identical (Lehmeier et al. 2008, 2010a, b). Furthermore, as it is typical for grasses (Sullivan and Sprague 1943, Davidson and Milthorpe 1966), more than 90 % of all water-soluble carbohydrates – as the most likely major constituents of respiratory substrate – were located in the shoot biomass. Consequently, most of the storage-derived carbon supplying root respiration must have resided in the shoot biomass (Lehmeier et al. 2008). These findings dictate the conclusion that also the control of assimilate supply to root respiration resided in the shoot. Consequently, the supply of substrate to root symbionts and soil microbes feeding on plant root exudates seems to be largely influenced and controlled by allocation changes in the shoot organs of the plant (see Grayston et al. 1996, Kuzyakov and Cheng 2001, Farrar et al. 2003, Thornton et al. 2004).

3.4 Differences in Substrate Supply to Leaf Growth and Respiration

The substrate pools supplying leaf growth were characterized for the same population of plants that served to assess the substrate pools for respiration at High N. The two substrate supply systems showed considerable differences. The pool of current assimilates supplying leaf growth (Q_1) was exchanged much faster with new carbon than the corresponding pool supplying respiration (half-life 1 h *versus* 6 h, Tab. 1). Indeed, both half-lives are close to those reported for sucrose, the major transport sugar in the vascular system of herbaceous plants (Geiger et al. 1983, Farrar 1989). However, it is reasonable to assume that growth was mainly supported by sucrose that rapidly passed through the cytoplasm, apoplast, and sieve tubes and companion cells of the phloem in actively photosynthesizing and exporting leaves before arriving at the basal growth zones of a tiller (Geiger et al. 1983, Wild 2010). Conversely, respiration was measured at the whole-shoot level and thus integrated all shoot tissues, including mature, exporting leaves as well as growing and senescing organs. Possibly, the turnover of the sucrose pool differs between functionally and developmentally distinct tissues.

About 50 % of all respired carbon cycled through the store before it arrived at the centers of respiration and it was, most likely, mainly present in carbohydrates. In contrast, the fractional contribution of the store to leaf growth was only 30 % (Tab. 1). Combined labeling with ^{13}C and ^{15}N (Lattanzi et al. 2005, Wild 2010) suggested that the store supplying leaf growth included both carbohydrate-carbon and amino-carbon derived from protein turnover, and that each of the two components supplied only about 15 % to total carbon flux into the leaf growth zone (Wild 2010). This indicated that leaf growth of the grasses under steady-state conditions in continuous light relied to a much lesser degree on storage carbohydrates than did respiration.

4. Conclusions

The combined approach of $^{13}CO_2/^{12}CO_2$ labeling techniques and compartmental analysis of tracer time courses enabled the identification of major substrate pools supplying leaf growth and respiration of perennial ryegrass. The partitioning into functionally distinct components (current assimilates *versus* stores), and the assessment of their response to environmental conditions proved to be a meaningful way to clarify key controls of carbon allocation.

Experimental alteration of growth conditions unveiled great functional plasticity in plants to adjust the use of stores, which appears to be an important capacity to support high growth rates and individual fitness in a fluctuating environment (Smith and Stitt 2007, Walter et al. 2009, Graf and Smith 2011; and references therein).

The concerted use of labeling with stable isotopes and analysis of tracer kinetics can be employed at different scales of biological integration, for instance, to study resource fluxes on the level of single leaves or the ecosystem scale (Gamnitzer et al. 2009, Epron et al. 2011, Lattanzi et al. 2012). The proven effectiveness of such an approach may stimulate further research to identify the substrate sources supplying other sinks like the synthesis of carbon-based secondary defense compounds. Such new insights into source-sink relationships of plants can help to advance new concepts about the controls of carbon allocation between growth, storage and defense and to quantitatively balance related costs and trade-offs in plant carbon allocation (Lüttge 2012, Matyssek 2012).

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Facilitation, Competition and the Organization of Plant Communities

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With 2 Figures

Abstract

Facilitation is one of many processes that determine the distribution and abundance of species and the organization of communities. Therefore, it is profitable to explicitly consider how facilitation works in the context of the other processes. Here I review examples of how competitive and facilitative mechanisms operate at the same time to create "net" effects, and how understanding this balance of interactions can contribute to predicting when and where different facilitative and competitive net effects or mechanisms might predominate. For the most part, predictions have been experimentally studied as shifts in the relative importance of facilitative *versus* competitive interactions along gradients of productivity and abiotic stress in a number of different systems. Such experimental evidence for conditionality in interaction intensity and importance helps us to better conceptualize how interactions *might* affect species coexistence and community diversity, but empirical evidence for the importance of facilitation on biodiversity has emerged most convincingly in the last decade. Now a number of studies have explored whole-community patterns to show how key species in desert and alpine systems can have strong positive effects on species richness and functional richness at multiple scales. Also, paleobotanical, ecological, and phylogenetic evidence has been synthesized to suggest how facilitation may have preserved biodiversity at geological time scales. Continuing to expand our understanding of facilitation in the context of other ecological processes provides new insight into mechanisms that sustain diversity and that determine the effects of diversity on ecosystem functions.

Zusammenfassung

Die im Gegensatz zur Konkurrenz koexistenzfördernde Fazilitation ist einer von vielen Prozessen, welche die Verteilung der Arten und deren Häufigkeit sowie die funktionelle Struktur von Artengemeinschaften bestimmen. Daher erscheint eine Eruierung der Wirkungsweise von Fazilitation im Kontext anderer Prozesse lohnend. Ich will hier einen exemplarischen Überblick geben, wie koexistenzlimitierende (infolge Konkurrenz) und koexistenzfördernde Mechanismen (infolge Fazilitation) zeitgleich agierend in „Netto-Effekte“ münden und wie ein Verständnis des Interaktionsgefüges zu Vorhersagen beiträgt, welcher Effekt wann und wo zu erwarten ist. Meist werden Vorhersagen experimentell in verschiedenen Systemen auf veränderte Gewichtungen zwischen koexistenzfördernden und -limitierenden Interaktionen entlang abiotischer Stressgradienten und damit Produktivitätsgradienten gegründet. Experimentelle Erkenntnisse zu funktionellen Voraussetzungen von Interaktionen sowie deren Intensität und Auswirkung führen zu verbesserten konzeptionellen Vorstellungen, auf welche Weise Artenkoexistenz und Diversität bestimmt sind. Auf dieser Basis wurde im Verlauf des vergangenen Jahrzehnts die Bedeutung der Fazilitation für die Biodiversität auf überzeugende Weise deutlich. Inzwischen hat eine Anzahl von Untersuchungen das funktionelle Strukturgefüge gesamter Artengemeinschaften analysiert und gezeigt, wie Schlüsselarten in Wüsten und alpinen Systemen auf verschiedenen räumlich-zeitlichen Skalen Artenreichtum und Vielfalt an ökologischen Funktionen positiv beeinflussen. Durch Zusammenführung paläobotanischer, ökologischer und phylogenetischer Erkenntnisse wurde zusätzlich erkennbar, wie Fazilitation über geologische Zeiträume hinweg Biodiversität bewahrt haben kann. Fortgesetzte Erkenntnismehrung zur Fazilitation als Komponente des ökologischen Prozessgefüges bietet neue Einblicke in diversitätserhaltende und damit die Ökosystemfunktionen bestimmende Mechanismen.

1. Introduction

Facilitation occurs when the presence of one plant enhances the growth, survival, or reproduction of another plant (Callaway 2007). Much like "competition", "facilitation" is usually used in a relatively inexact way, and the positive effect of one species on another can be met in response by reciprocal negative, positive, or neutral responses. Furthermore, as for any biological interaction among organisms, facilitation is best understood in the context of other types of interactions and abiotic processes. Such interacting drivers of community organization have often been conceptualized as filters of regional species pools that determine the species that are present in a community (Fig. 1) and these filters are the subject of this review.

As a filter, facilitation operates differently than other processes. While antagonistic and abiotic factors are usually thought of as processes that limit species and constrict the niche, facilitation is a process that counters limitation and expands the niche (Baumeister and Callaway

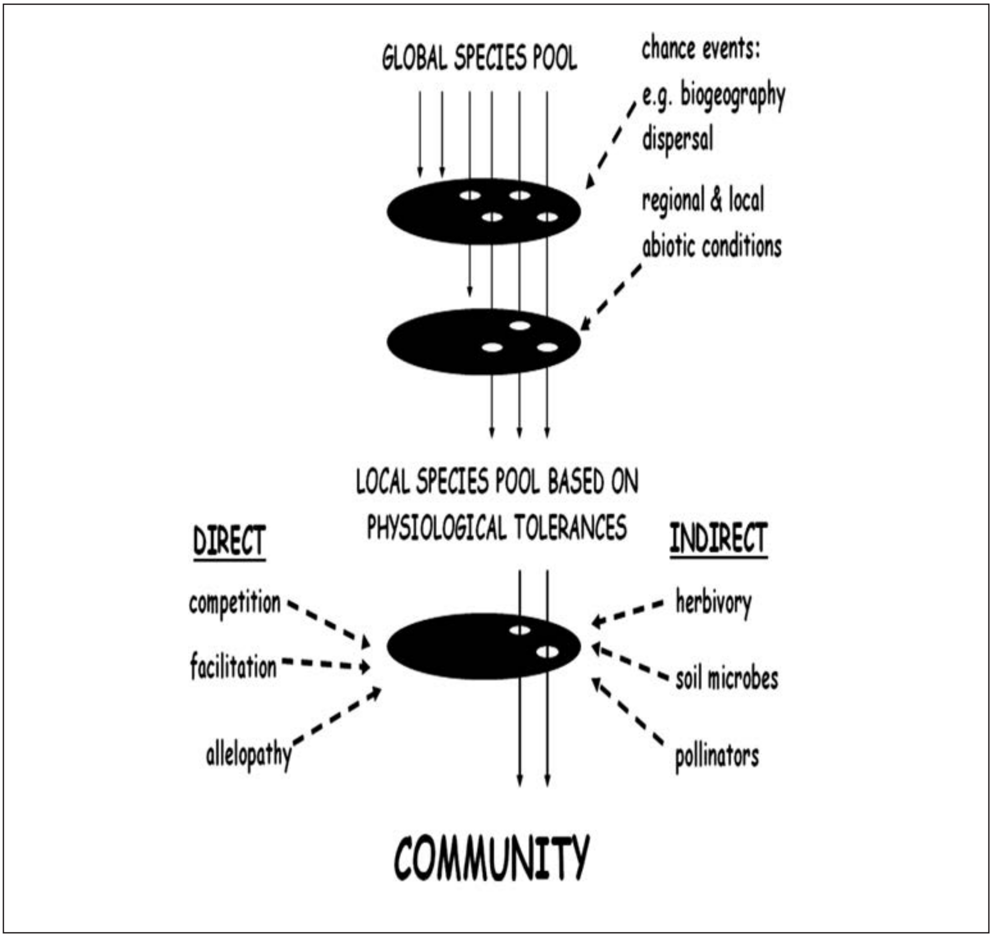

Fig. 1 Schematic illustrating how different might "filters" determine the organization or composition of local communities.

2005). Conventionally, the performance of a species along a suite of relevant environmental variables has been assumed to be enough to explain the fundamental niche, and discrepancies between realized and fundamental niches have historically been attributed to resource competition (Austin 1989). In contrast, van der Maarel et al. (1995) discussed the realized niche in both the context of "niche limitation" and "niche facilitation", and examined deviations from an expected variance in species richness in plots in grasslands, finding evidence for niche facilitation. There are now many examples of such niche facilitation (Callaway 2007).

One of the consequences of the recent syntheses of direct and indirect facilitation into community theory (e.g. regarding the structure of the niche) has been a challenge to strict "individualistic" interpretations of community organization (Callaway 1997). Most general conceptual models of community structure are either explicitly or implicitly based on competition, and this perspective is historically connected to individualistic theory. But recently Lortie et al. (2004) made the case that facilitation among plants is so common that individualistic theory is inadequate as a general foundation for plant community ecology. If so, it is valuable to explore this change in our paradigms for how communities are organized, and some of the specific cases in which non-individualistic processes appear to be at work.

To my knowledge, the first field experiment to demonstrate facilitation was published in 1914 by G. A. Pearson. He observed higher regeneration of conifers after fires within clonal groups of *Populus tremuloides* trees than outside such groups, along with much more productive understory growth under the poplar trees. Pearson then planted seedlings of *Pseudotsuga menziesii* under *P. tremuloides* clones and in openings and found greater survival under the poplar trees. Until the last 20 years or so, early findings such as these (also see Shreve 1910, Niering et al. 1963) were not interpreted as being conceptually important in ecological theory. However, empirical results have continued to yield insight into the balance between facilitative and competitive interactions (Callaway et al. 1991), shifts in facilitative and competitive interactions along abiotic gradients (Bertness and Callaway 1994), and the broad effects of facilitative processes on community diversity (Cavieres and Badano 2009). As a consequence, facilitation and more broadly related cooperative processes are being better integrated into community theory (Bruno et al. 2003) and evolutionary theory (Kikvidze and Callaway 2009). Here I review the following three aspects of how facilitation has been integrated into community ecology by focusing on specific examples from the literature.

- The first, and foundational to the others, is that facilitative and competitive mechanisms occur at the same time and thus the relative importance of particular mechanisms under particular conditions determines the net outcomes of interactions.
- The second aspect is how the balance between competitive and facilitative mechanisms and their net effects generally shift in relative importance along gradients of productivity and abiotic stress, with facilitative interactions either stronger or more common at the high stress ends of gradients.
- Finally, I discuss the role of facilitation in relationships of diversity with ecosystem function and how facilitation affects community diversity.

2. The Balance Between Facilitation and Competition

During the 1950's and 1970's large areas of the California endemic *Quercus douglasii* were cleared because trees at some locations suppressed grassland forage for livestock (Murphy and BERRY 1973). However, HOLLAND (1980) demonstrated that many *Q. douglasii* trees and stands not only did not suppress understory productivity, but were associated with a large increase in the productivity of the understory. This facilitative effect of *Q. douglasii* on understory productivity corresponded with higher levels of soil nutrients under trees. MCCLARAN and BARTOLOME (1989) measured the effect of *Q. douglasii* on understory productivity at five sites over two years, and found the effects of the tree were primarily neutral at xeric sites but competitive at mesic sites in both years. They found no evidence for positive effects. But RATLIFF et al. (1991) also found that the effect of *Q. douglasii* on understory productivity varied with habitat, and was often strongly facilitative.

In one of the rare California grasslands that has been free of livestock grazing for decades, the Hastings Natural History Reservation near Carmel, California, CALLAWAY et al. (1991) measured productivity under a suite of *Q. douglasii* trees and found that some trees had far more productivity in the understory than immediately outside the edges of the canopies (positive trees), whereas others had virtually bare understories (negative trees). However, soil from under positive *and* negative trees had much higher nutrient concentrations than soil collected in open grassland, and greenhouse experiments resulted in all understory soils producing larger target plants than soils from the open. Thus even though positive and negative trees elicited diametrically opposite results in the field regarding the actual extent of facilitation, all *Q. douglasii* trees measured appeared to have the potential to facilitate other species through their effects on soil nutrients. The key contrast between positive and negative trees was that in the upper 50 cm of soil, root biomass was ≈ 5 times greater under trees with low understory biomass than under trees with high understory biomass. Concomitant with these differences in root biomass in the surface soils, the predawn xylem pressure potentials of positive trees were much higher at the end of the long dry Mediterranean-climate summer than those of negative trees, indicating that the former utilized groundwater or some other more permanent soil water source. Finally, root exclosures reduced the competitive effects of *Q. douglasii* roots under negative trees, but not under positive trees. The crucial finding was that although all trees appeared to have facilitative effects, the positive effect of fertilization produced by "negative" trees was not manifest because of the negative competitive effects of the dense surface roots. The competitive effects of the surface roots overrode the positive effects of fertilization through canopy litter and throughfall. Thus, facilitative and competitive mechanisms operated simultaneously, and the balance of both interactions determined the overall effect of overstory tree on understory productivity.

Long before experiments with the facilitative and competitive effects of *Q. douglasii*, ELLISON and HOUSTON (1958) experimented with the effects of *Populus tremuloides* on herbaceous understory productivity. As noted earlier, PEARSON (1914) observed higher productive understory growth under *P. tremuloides* than in the open. ELLISON and HOUSTON (1958) established plots in open areas away from the influence of aspen canopies, and then set up plots under the aspen canopies where the tree roots were either excluded by trenching or not. In each of the plots they planted native herbaceous species and after three years they harvested the plots. Three of the four herbaceous species were much smaller under *P. tremuloides* canopies than in open meadows near the trees, indicating strong competitive effects of the trees on the herbaceous species. But when plots were trenched to exclude *P. tremuloides* roots the biomass of some herbaceous species substantially exceeded that in the open. The authors noted that the "principal factor in depressing yields under the aspen is root competition", and it appeared that strong facilitative and competitive effects were functioning at the same time in their system. Similar to the *Q. douglasii* system, root competition suppressed strong facilitative canopy effects.

In an another experiment explicitly designed to tease apart the facilitative effects of canopies from the competitive effects of roots, Callaway (1994) manipulated the canopies and root systems in the upper zones of a California salt marsh of a sub-shrub that appeared to have facilitative effects on spring ephemeral species. In this system very few species are able to tolerate the hypersaline conditions of the dry summer, and one of these is *Arthrocnemum subterminale*, a small shrub in the Chenopodiaceae. *Arthrocnemum* decreases soil salinity under its canopy by ≈ 30 %, which may provide a mechanism for the facilitative effect as this difference in salinity corresponds with increases in the abundance some ephemeral species that grow during the brief time in early winter when rains dilute the salts in the upper marsh. *Hutchinsia procumbens* and *Parapholis incurva* were found primarily under *Arthrocnemum* canopies and not in the open, whereas *Spergularia marina* was found primarily in open spaces. Survival of *Hutchinsia* was 4–7 times higher under *Arthrocnemum* canopies, and there was no effect of experimental exclosures of *Arthrocnemum* roots. The survival of *Parapholis* was two times greater under canopies when roots were not excluded, but four times greater when roots were excluded. In contrast, survival of *Spergularia* doubled when canopies were removed regardless of whether or not roots were excluded. In sum, facilitative and competitive mechanisms operated simultaneously but in different ways for different species in the highly stressful saline conditions of the upper salt marsh. Competition was important, but was weak relative to facilitation.

In one of the few cases that I know of in which facilitative and competitive mechanisms were experimentally disentangled, Maestre et al. (2003) explored the positive, negative, and net effects of *Stipa tenacissima* on the shrub *Pistacia lentiscus* in southern Spain. They used three manipulative treatments with *Pistacia* planted upslope and adjacent to isolated *Stipa* tussocks, in the open between tussocks, or under dead canopies of *Stipa* which were left intact as another level of control. They also removed the potential facilitative effects of shade but left strong root competition by bending *Stipa* canopies away from target *Pistacia*, and diverted runoff by inserting metal sheets in the ground upslope of experimentally manipulated *Stipa*. The removal of *Stipa* canopy shade significantly decreased *Pistacia* seedling performance; whereas removal of root competition increased *Pistacia* seedling performance. The net effect of *Stipa* on soil moisture and *Pistacia* was always facilitative and the magnitude of the net effect increased with the stressfulness of conditions in both space and time. Interestingly, net effects were not determined by the weakening of the intensity of any stress effect. Instead, the intensity of competitive effects, the facilitative effects, and the net effects all increased over the summer but to different extents. Thus, inherent mechanisms responded in different ways to environmental variation, but in a balanced way that created variation in net effects.

Understanding the simultaneous effects of competitive and facilitative mechanisms is helpful for exploring the shifting roles of competition and facilitation along abiotic gradients, conditional facilitative effects, and species-specificity in facilitative effects, all of which are crucial components of the effects of facilitation on community organization.

3. Shifts in Facilitative and Competitive Interactions on Abiotic Gradients

The balance between competitive and facilitative effects in *Quercus douglasii* savannas and in *Populus tremuloides* forests provides examples of how different mechanisms interact at the same time to produce a *net effect* of species on other species (Hunter and Aarsson 1988). Understanding this balance of interactions can contribute to predicting when and where different facilitative and competitive mechanisms might predominate. The most widely utilized conceptual model for such predictions is that proposed by Bertness and Callaway (1994). Their ideas were derived to a large degree from Grime's (1977) hypotheses about the relative importance of competition on productivity gradients, but mainly from field experiments conducted by Bertness (1991a, b) along gradients of salt and anaerobic stress in salt marshes. The basic hypothesis is that non-stressful conditions result in high productivity, and when consumer pressure is low to moderate then competitive interactions should be the most common way in which plants interact. In contrast, facilitative interactions should become more important when abiotic stress is high or when consumer pressure is intense. The "abiotic stress" component of this idea is commonly referred to as the "stress gradient hypothesis".

The *Quercus douglasii* system described above provides a good example of how shifts in the relative strength of facilitative and competitive interactions might generally correspond with the abiotic stress hypothesis. Ratliff et al. (1991) measured herbaceous productivity under various tree species and in open grassland for eight years and over a wide range of physical habitats. Across all years and sites the overall effect of *Q. douglasii* was facilitative; however, in mesic swales, where annual productivity was by far the highest, the effect of *Q. douglasii* on its understory was negative. In a re-analysis by regressing the relative effect of the tree against mean open habitat productivity, facilitation is much stronger where productivity was low, whereas competition is strong where productivity was high (Fig. 2). Similar effects have been reported in the much more arid Sonoran Desert, where Tewksbury and Lloyd (2001) examined the effects of *Olneya tesota* (the desert tree, ironwood) on the structure and diversity of understory plant communities. In xeric sites, *Olneya* canopies had strong positive effects on plant richness and abundance, and small positive effects on the size of understory plants, emphasizing the role of facilitation in extreme environments. In contrast, *Olneya* canopies in mesic sites had very little effect on understory perennials and a negative effect on ephemeral richness, suggesting that competitive effects predominated in this less stressful environment. Overall, *Olneya* canopies increased biological diversity through facilitation where abiotic stress was high, but did not increase diversity in mesic areas.

Shifts between competition and facilitation have also been shown to occur between the same species over time, thus providing insight into species interactions in systems where abiotic conditions fluctuate over time. Greenlee and Callaway (1996) explored abiotic stress as a factor affecting the relative importance of competition and facilitation in grasslands on calcareous substrates in western Montana. On open, south-facing aspects, the herbaceous *Lesquerella carinata* was three times more common under the canopies of the bunchgrass species, *Psuedoroegneria spicata*, *Festuca idahoensis*, and *Koeleria macrantha*, than expected by chance, a pattern suggesting facilitation. In an experiment in a relatively wet and cool growing season, *Lesquerella* seedlings that had been planted in the open survived much better than seedlings under bunchgrass canopies or with artificial shade, a result that indicated competition, and one that conflicted with the interpretation of spatial pattern. However, when the experiment was repeated in the much drier and hotter subsequent year the results were different. First, seedling survival across all treatments was over 10 times lower than in the wet year, and survival in the open was virtually non-existent. Survival was much higher under bunchgrass canopies and under artificial shade. The strong spatial associations of seedlings and adults with bunchgrasses suggest that facilitation is predominant over the lifespan of *Les-*

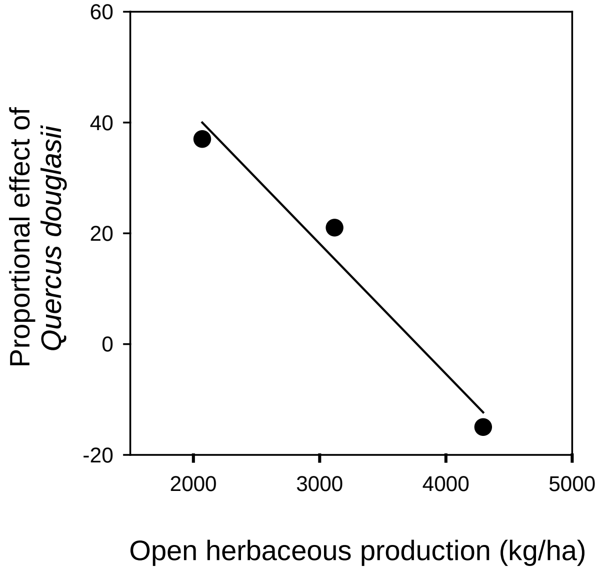

Fig. 2 The relationship between productivity in open grassland sites in central California grassland and the proportional effect of *Quercus douglasii*. Calculated from results reported in Ratliff et al. (1991) and as presented in Callaway (2007).

querella plants, and that the two years of experimental results are consistent with the general idea for how facilitation is more likely to be expressed under conditions of relatively high abiotic stress.

Altitudinal gradients in mountains provide other excellent opportunities for studying shifts in the occurrence and intensity of competition and facilitation. For example, Antonsson et al. (2009) studied the effect of *Silene acaulis*, a circumpolar cushion forming alpine plant species, on other species along an altitudinal transect in northern Sweden. They found that at high elevations, more species were found inside *S. acaulis* cushions than in paired control plots in the open, whereas at lower elevations *S. acaulis* tended towards having negative effects on other species. In northern Sweden, this cushion species appears to be an important promoter of plant diversity at the abiotically stressful end of elevation gradients. Similarly, Callaway et al. (2002) conducted an experiment in subalpine and alpine plant communities of 11 different mountain ranges and found that competition generally, but not exclusively, dominated interactions at lower elevations where productivity was higher and abiotic conditions were less stressful. In contrast, at high elevations where abiotic stress is high the interactions among plants were predominantly positive. In a similar experimental analysis, Choler et al. (2001) found highly significant shifts from strong competitive effects in low and sheltered sites to strong facilitative responses in high and exposed sites. But in this latter study, the responses of particular alpine plant species to neighbor removal corresponded with the species' position along elevation and micro-topographic gradients. When neighbors were removed from around target species at sites that were lower in elevation than the distributional mean of the target species in ordination analyses of large scale community sampling, competition was evident. In contrast, when neighbors were removed from around target species at experimental sites that were higher in elevation than the distributional mean of the target species, facilitation was evident. They interpreted this as facilitation allowing species from lower elevations to move up the gradient, but competition at lower elevations restricting high-elevation species from moving down the gradient.

4. Facilitation and Community Diversity

Tewksbury and Lloyd (2001), as noted earlier, provided a good example of how dominant species can facilitate companion species at the scale of whole communities, and thus gained insight into the effect of facilitation on biological diversity. They found that *Olneya* canopies were correlated with increased biological diversity where abiotic stress was high in the Sonoran Desert, whereas canopies had no effect on diversity in mesic areas. Similar research was conducted by Holzapfel et al. (2006) along a gradient of aridity ranging from very dry sites in the Negev Desert to mesic Mediterranean areas in Israel, along which the spatial relationships between annual plants and shrubs were examined. They found evidence for interactions ranging from competition to facilitation, in that above-ground productivity, species richness, seedling density, and fecundity of annual plants were higher under shrubs than in areas between shrubs at the arid end of the gradient, indicating facilitation, but significantly lower at the humid end, indicating competition. The "net effects" of shrubs on herbaceous plants demonstrated a "steady and consistent shift from net positive or neutral effects in the desert to net negative effects in the mesic part of the gradient".

Of course, habitat building such as described above for *Olneya* and desert shrubs may occur in many other systems, although in ways that are not as apparent as in deserts or similar places where patterning allows easy and clear measurements of spatial relationships among many species. As another example of utilizing systems with strong spatial patterning, Cavieres and Badano (2009) sampled 11 alpine plant communities in the Andes Mountains, ranging from 25°S to 55°S in latitude, that were dominated by "cushion plants", species with very compact and low growing morphologies that have been commonly shown to act as nurse plants for other plant species with different growth forms (Nuñez 1999, Cavieres et al. 2002, 2006, LeRoux and McGeoch 2008, Antonsson et al. 2009, Lortie and Reid, in press). Using rarefaction curves to quantify species richness they found that samples taken within cushions always contained more species than equivalent samples from open areas. By using ordination techniques they found that species assemblages in cushions were structurally different from those found in open areas. Most importantly, the presence of cushions consistently increased species richness at the entire community level. Consistent with the idea that facilitative interactions are more common in abiotically stressful conditions, Cavieres and Badano (2009) also found that the magnitude of these facilitated increases in species richness were the greatest at the very arid and at the very cold extremes of their latitudinal gradient.

Because beneficiaries are often quite morphologically and physiologically different from the species they nurse, facilitation is also highly likely to increase community *functional* diversity. In support of this, Butterfield and Briggs (2010) examined functional traits in Sonoran Desert communities and found that beneficiaries exhibited "conservative functional strategies" that adapted species to dry, low light, but cooler, conditions beneath canopies.

In contrast, the facilitators that colonized open space exhibited "opportunistic strategies" thought to be advantageous in the much more dynamic open conditions. Most importantly, these sharp contrasts in functional traits between benefactors and beneficiaries resulted in increased total functional diversity in plant communities. Over far longer time frames, VALIENTE-BANUET et al. (2006) explored the effects of facilitation on diversity in the context of the transition from the wet Tertiary period to the unusually dry Quaternary, when a wave of new plant species emerged, presumably in response to the drier climate. They integrated paleobotanical, ecological, and phylogenetic analyses to show that a large number of ancient Tertiary species in Mediterranean-climate ecosystems appear to have been preserved by the facilitative effects of modern Quaternary species. They suggested that hat these interdependent relationships among plants have played a central role in the preservation of global species richness and functional diversity over evolutionary time scales.

Facilitative interactions are also relevant to the large body of experimental research on positive relationships between plant diversity and ecosystem attributes (TILMAN et al. 1997, HOOPER et al. 2005). For example, MULDER et al. (2001) tested the idea that "species that seem to be functionally redundant under constant conditions may add to community functioning under variable conditions" using experimental communities built with bryophytes (MULDER et al 2001). Communities containing 1, 2, 4, 8, 16, 24, or 32 species were grown in shady humid conditions for a year and then different replicates were either kept in mesic conditions or exposed to much drier and brighter conditions. When humidity was high and light intensity was low species richness did not correlate with community productivity. However, when exposed to harsher conditions species richness increased community productivity. MULDER et al. (2001) found that increased biomass under drought conditions was associated with increased survivorship for most species, suggesting facilitation as a mechanism. Also, the species that showed the greatest increases in biomass under dry bright conditions in multi-species systems were the least resistant to drought at low species richness, indicating facilitation. Finally, they found that for a given species pool, the number of species that contributed to increases in biomass also increased with diversity, which also supported the idea that facilitation was an important driver of the relationship. CALDEIRA et al. (2001) experimented with vascular plants in the Mediterranean climate of Portugal and found that the total biomass and total cover in species-rich plots was significantly higher than in monocultures, and that facilitation affected this relationship of diversity-ecosystem function. Importantly, diversity-enhanced community performance did not appear to occur only through the inclusion of species that were each better at exploiting resources but also because a number of *individual species* performed better in mixtures.

5. Conclusions

There are many ways in which positive interactions can contribute to the structure and organization of plant communities, and the fundamental processes reviewed here are among the most important. Because we now know how facilitative and competitive mechanisms occur in additive or synergistic ways among plant species we can better understand conditionality in species interactions and net outcomes of interactions. Quantifying net effects and the balance between competitive and facilitative mechanisms along gradients of productivity and abiotic stress helps us to understand conditionality and to make predictions about where specific interactions might become important. Finally, measuring the role of facilitation in relationships of diversity and ecosystem function, including the effects of facilitation on community diversity, helps us to understand interdependence among species and how species loss may have cascading effects on natural systems.

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Der Begriff der Natur
Wandlungen unseres Naturverständnisses und seine Folgen

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Das Verhältnis des Menschen zur „Natur“ ist in seiner Geschichte durch unterschiedliche Beziehungen geprägt. Seit der Aufklärung wird die Natur dem Menschen zu seiner Nutzung untergeordnet und zunehmend ausgebeutet. Natur wurde zum Objekt technischen, ökonomischen und politischen Handelns. Spätestens seit Mitte des vorigen Jahrhunderts wissen wir um die akute Gefährdung natürlicher Lebensräume.

Die Gaterslebener Begegnung 2009 widmete sich daher dem Thema „Der Begriff der Natur“ und untersuchte Wandlungen des Naturverständnisses sowie die Folgen der gegenwärtigen Auffassungen von Natur. Behandelt werden unser Bild vom Leben, die Frage „Was ist Natur?“ aus verschiedenen Perspektiven und die philosophische Analyse der Stellung des Menschen in der Natur. Beiträge zum Naturverständnis in der Gegenwartskunst und zum Problemkomplex Naturrecht und Bioethik sowie eine Diskussion „Frieden mit der Natur“ ergänzen den Band.

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Facilitation and Competition in Mixed-Species Forests Analyzed along an Ecological Gradient

Hans Pretzsch (München)

With 8 Figures

Abstract

Long-term experiments, with many of them under survey since the 1880's, are applied for analyzing the extent of over- and underyielding of mixed *versus* pure stands. Firstly, a method for quantification of over- and underyielding and for indication of effects of facilitation and competitive reduction in mixed stands is introduced. Secondly, the extent of over- and underyielding in terms of productivity and the relevance of positive and negative interactions is presented for the mixtures of Norway spruce (*Picea abies* (L.) H. Karst.) with European beech (*Fagus sylvatica* L.) as well as for beech with oak (*Quercus petraea* (Mattuschka) Liebl. and *Quercus robur* L.). Thirdly, the mixing reactions are analyzed in dependence on site conditions. The discussion is focused on the relevance of the revealed effects for forest practice and ecological theory.

Zusammenfassung

Versuchsflächen, von denen viele seit den 1880er Jahren unter Beobachtung stehen, werden für die Analyse von Mehr- bzw. Minderzuwächsen von Mischbeständen gegenüber Reinbeständen verwendet. Zunächst werden Methoden für die Quantifizierung von Mehr- und Minderzuwächsen und Indikation von Facilitation und Konkurrenz eingeführt. Anschließend wird das Ausmaß der Mehr- und Minderproduktion quantifiziert und die Relevanz von Interaktionen zwischen den Mischbaumarten Fichte (*Picea abies* (L.) H. Karst.) und Buche (*Fagus sylvatica* L.) bzw. Buche und Eiche (*Quercus petraea* (Mattuschka) Liebl. und *Quercus robur* L.) aufgedeckt. Schließlich werden Mischungseffekte in Abhängigkeit von den Standortbedingungen untersucht. Die Diskussion konzentriert sich auf die Relevanz der aufgedeckten Mischungseffekte für die Praxis und die ökologische Theoriebildung.

1. Introduction

The strong influence of agronomy on forest practice resulted in extended forest monocultures in the past, however, mixed-species stands are receiving more attention at present (Scherer-Lorenzen et al. 2005). The reason for this increasing interest is that close-to-nature mixed species stands are widely held to supply ecological, economical and socio-cultural forests goods and services in a way similar to or even better than far-from-nature monocultures (Hector and Bagchi 2007, Hooper et al. 2005). A crucial question regarding the progressive currency of mixed stands is about how the productivity of poly-cultures compares with that of monocultures. Knowledge on the advantages or disadvantages of mixed *versus* pure stands with respect to productivity decisively influences the forest owner's decision in favor or against tree species poly-culture (Olsthoorn et al. 1999).

However, sound knowledge about mixing effects even for the most common tree species combinations is rather rare and scattered. Just in the last few years, after failure of lots of monocultures and rethinking on risk distribution (Knoke et al. 2005), resource efficiency (Richards et al. 2010), functional significance of species diversity (Scherer-Lorenzen et al. 2005) and mixed stand dynamics returned into the focus of forest science (see e. g., Forrester et al. 2006, Pretzsch et al. 2010, 2013). Most available works on mixed stand's growth and yield were searching for overyielding of mixed *versus* pure stands. In Norway spruce-European beech (*Picea abies* (L.) H. Karst. respectively *Fagus sylvatica* L.) mixtures in northern Germany Wiedemann (1942, 1943, 1951) found approximately the same dry biomass as in corresponding pure stands on sites of mediocre quality. However, on poorer sites with moderate growth of European beech the mixed stand biomass production is about 19 % less than in pure stands on neighboring sites. Kennel (1965) studied mixtures of Norway spruce and European beech in the Bavarian alpine foothills, the Bavarian Forest and the Harz in Lower Saxony as Burger (1941) did in Switzerland. Their results range from slight overyielding to underyielding of mixed *versus* pure stands. Zöhrer (1969) provides evidence that the biomass production of European larch-Norway spruce mixtures in the Salzburger Land is superior to that of neighboring pure stands of both tree species. The European larch-Norway spruce mixture therefore surpasses the pure Norway spruce stand by 22 to 28 % and the pure European larch stand by 2 to 13 %. In mixed stands of Sessile oak and European beech, Scots pine and Norway spruce and Scots pine European beech Bonnemann (1939) and Wiedemann (1943, 1951) found similar beneficial effects from species interactions after 50 years of observation. In the case of long-term Scots pine and European beech experimental areas in the Dübener Heide, Dittmar et al. (1986) note beneficial interaction effects compared with the pure stands of 7 to 25 % depending on the age and structure of the mixture. Burger (1941) and Wimmenauer (1941) note similar superiority in European larch-European beech mixtures.

Only a very few works deal with the dependency of mixing effects on site conditions: The study by Jensen (1983) along a West-East-transect through Jutland/Denmark gives a model example of site condition effects on the growth relationship between Norway spruce and silver fir. In the coastal dune belt silver fir is superior to Norway spruce, the adjacent Riss-glacial landscape leads to equivalent growth in silver fir and Norway spruce, whereas on the old inland moraines of the Würm-glacial period silver fir is inferior to Norway spruce in dry biomass production. The probable factor for the inland superiority of Norway spruce is its adaptability to low water supplies on acidic sites. By contrast, silver fir profits from better water availability and the more favorable nutrient supply in the coastal region. Frivold and Kolström (1999) studied silver birch (*Betula pendula*), Scots pine and Norway spruce growth in Finland, Sweden and Norway. Like Jensen (1983) they emphasize that the over- or underyielding of mixed *versus* pure stands is related to site conditions. In Southern and Central Finland Scots pine-silver birch mixtures surpass pure Scots pine and pure silver birch stands by 10 % and 14 %, respectively (Mielikäinen 1980). For Norway spruce-silver birch mixtures a 10 to 15 % increase in production compared to corresponding pure stands of these species may occur depending on the site (Mielikäinen 1985). In the oceanic regions of Norway and Sweden silver birch loses some of its increment capacity compared with coniferous species. There, Scots pine-silver birch mixtures do not achieve greater yield than pure stands whereas Norway spruce-silver birch mixtures show a beneficial effect only during early stand development (Frivold and Frank 2002).

The objective of this paper is to analyze the effect of species mixing on forest productivity at the stand level, exemplifying the two very common species mixtures of European beech (*Fagus sylvatica* L.) with Norway spruce (*Picea abies* (L.) H. Karst.) and of European beech with oak (*Quercus petraea* (Mattuschka) Liebl. and *Quercus robur* L.). To this end, first a method for quantifying over- and underyielding is introduced. Second, long-term mixing experiments are analyzed for indicating the extent of over- or underyielding of mixed versus pure stands. Third, mixing experiments located along an ecological gradient through Europe reveal how mixing effects are modified by site quality.

2. Methodological Considerations

The results in this paper base on long-term experimental plots in forest stands. From repeated measurements by 5 to 10-year intervals, the mean annual increment in stem volume and biomass growth (as "m^3 ha^{-1} yr^{-1}" and "t ha^{-1} yr^{-1}", respectively) can be derived for pure stands and neighboring mixed stands. The presentation encompasses analysis of two-species mixture where the results are based on triplets of plots (species 1 in monoculture, species 2 in monoculture, species 1 and 2 in mixed culture) and three-species mixtures based on quadruples (species 1, 2, and 3 in pure stand and species 1–3 in mixture). The comparison of the growth in mixed *versus* pure stands provides information of whether the mixed stand's productivity is higher, equal or lower relative to that of respective pure stands. Such a comparison for a great number of stands enables statements on the extent of over- or underyielding of mixed *versus* pure stands. Comparison for stands along an ecological gradient from rich to pure sites indicates the way by which mixing effects are modified through site conditions.

In the following, I will focus on such variables, algorithms and graphs which are essential for understanding the analyses of the triplets and quadruples of the included plots with respect to mixing effects. For a more detailed introduction into the nomenclature and the quantification of over- and underyielding in mixed *versus* pure stands, see PRETZSCH (2003, 2009) and PRETZSCH et al. (2010).

2.1 Quantification of Over- and Underyielding

First, the relative productivity $RP_{1,2}$ for a mixed stand as a whole will be considered as a ratio of observed productivity of the mixed stand $p_{1,2}$ divided by the productivity expected for the mixed stand $\widehat{p}_{1,2}$ in the absence of net mixing reactions $RP_{1,2} = p_{1,2} / \widehat{p}_{1,2}$. The expected productivity $\widehat{p}_{1,2}$ is derived from the productivity of both species in the neighboring pure stands, p_1 and p_2, and their mixing portions m_1 and m_2 ($\widehat{p}_{1,2} = m_1 \times p_1 + m_2 \times p_2$). The mixing portions m_1 and m_2 are calculated on the basis of the species' share of the stock of dry mass ($m_1 = W_1 / (W_1 + W_2)$, $m_2 = W_2 / (W_1 + W_2)$).

Second, the relative productivity *RP* of species 1 and 2 in mixed *versus* pure stands is of interest. For species 1, the relative productivity in mixed *versus* pure stand is $RP_{1,(2)} = pp_{1,(2)} / m_1 / p_1$, with the share of productivity of species 1 in the mixed stand, $pp_{1,(2)}$, mixing portion, m_1, and productivity of the pure stand, p_1. Regarding species 2, $RP_{(1),2} = pp_{(1),2} / m_2 / p_2$ applies accordingly. Notice, that $pp_{1,(2)}$ and $pp_{(1),2}$ are the parts of the productivity of species 1 and 2 in the mixed stand which add up to $p_{1,2}$ ($p_{1,2} = pp_{1,(2)} + pp_{(1),2}$). By contrast $p_{1,(2)}$ and $p_{(1),2}$

are the parts of both species in the mixed stand scaled up to 1 ha using their mixing portion ($p_{1,(2)} = pp_{1,(2)} / m_1$ and $p_{(1),2} = pp_{(1),2} / m_2$).

Third, the relationships $RPP_{1,(2)} = pp_{1,(2)} / p_1$ and $RPP_{(1),2} = pp_{(1),2} / p_2$ are of interest. The relative productivity on the basis of the portions (RPP) result from division of the parts of the productivity of species 1 and 2, $pp_{1,(2)}$ and $pp_{(1),2}$, respectively, by the productivity of the same species in the pure stand. Note that $RP_{1,2} = RPP_{1,(2)} + RPP_{(1),2}$.

2.2 Graphical Representation of Growth Reactions in Mixed Stands

For visualization of the mixing reactions, cross-diagrams according to Harper (1977, pp. 776–778) and Kelty (1992) are indicative. In such diagrams (Fig. 1) the productivity of species 1 in the pure stand is plotted on the left-hand ordinate, while that of species 2 is plotted on the right-hand ordinate. The broken straight lines represent the expected productivity of the mixed stand in total (horizontal 1.0-line) as well as the share of species 1 and 2 in the stand productivity (descending line connecting (0|1) with (1|0), and accordingly, rising line connecting (0|0) with (1|1)) in dependence on the mixing portion (scaled on the abscissa). The observed productivity is represented by the following three drawn functions: The observed productivity at the total stand level by the upper curve, the share of species 1 by the lower curve descending from left to right, and the share of species 2 by the lower curve rising from left to right. As Figure 1 shows cross-diagrams for the relative productivity, in analogy, cross-diagrams are common also for comparing absolute productivity (see Fig. 2*B*).

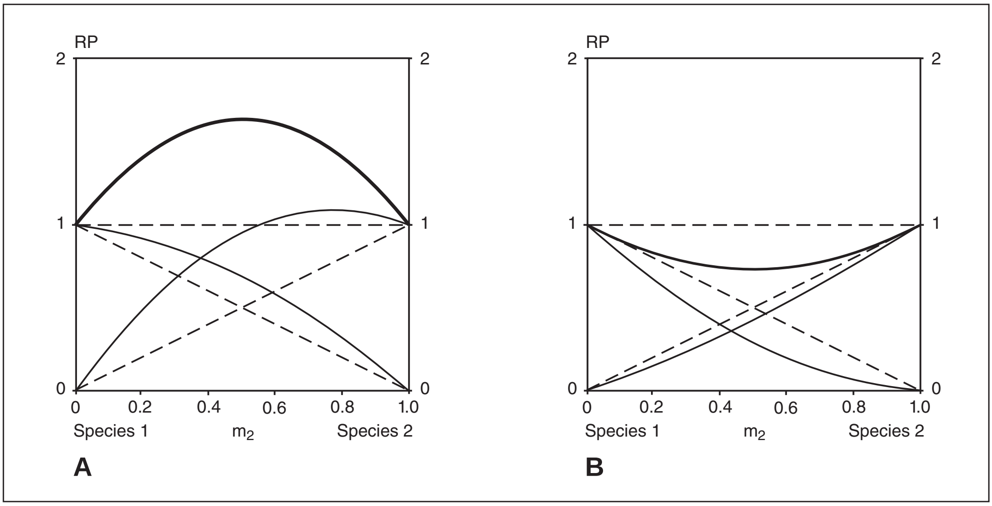

Fig. 1 Cross diagrams for display of mixing effects on productivity in two-species mixtures with overyielding (*A*) and underyielding (*B*). The left respectively right ordinates represent the relative productivity of the species in the pure stand. The range in between represents the relative productivity in the mixture depending on the mixing portion. Broken lines represent the productivity expected for neutral mixing effects on stand level (horizontal 1.0-line) and species level (decreasing resp. increasing lines). The solid lines show the observed productivity on stand level (upper bold curve) and species level (lower thin curves).

With growth-neutral mixture effects, the production of the mixed plot will lie on the straight horizontal lines connecting the two pure stands (Fig. 1). Positive or negative deviations from the reference lines reveal whether the mixed stand gains or losses in productivity. The concave (seen from below) upper lines in Figure 1*A* indicate positive mixing effects at the stand level, i.e. overyielding. In Figure 1*A* the species-specific functions are concave as well and indicate that both species contribute positively to the overall positive mixing effect. Hence, both species have a mutual benefit from the mixture: Species 1 in mixture lies above the species specific reference line, and species 2 in mixture even exceeds the productivity of the pure stand of species 2. In contrast, Figure 1*B* indicates antagonism. The convex functions (seen from below) at the stand as well as at the species level indicate mutual inhibition of growth in mixture. Species 1 suffers more in mixture than species 2 do. While the profit in relation to the pure stand in terms of productivity amounts to 60–70 % in the case depicted in Figure 1*A*, the mixture shown in Figure 1*B* reduces productivity by 20–30 %. The individual species lines (forming a cross) indicate whether a species gains or losses in productivity by mixing. Again concavity indicates benefit, and convexity loss due to the mixing. When the stand productivity of species 1 in a mixed stand comprising species 1 and 2 exceeds the productivity of species 1 in the adjacent pure stand, such a phenomenon is then interpreted as facilitation of species 1 by species 2. When the performance of a species in the mixed stand is lower than expected on the basis of productivity in the neighboring pure stand, this indicates that inter-specific competition exceeds intra-specific competition. Hence, the stand productivity of mixed *versus* pure stands is used as an indication for facilitation or competition at the stand level and separately for species 1 and 2.

Analyses of mixing effects based on the above shown algorithms and cross-diagrams are widely available for tree species mixtures were one species can fix atmospheric nitrogen (e.g. Forrester et al. 2006) and make mixing effects on productivity evident. For example, Figure 2 shows overyielding in mixed *versus* pure stands of *Eucalyptus globulus* Labill and *Acacia mearnsii* De Wild., the N-fixing species, stressing the difference between the *relative* productivity of a species in mixture (Fig. 2*A*) *versus* its performance in a pure stand (and the *absolute* gain or loss of productivity of a species or stand under conditions of mixed culture *versus* such of neighboring monoculture; Fig. 2*B*).

Forest science is primarily interested in species productivity in mixture *relative* to the pure stand productivity and to which extent two or more species benefit or lose under mixture. Conversely, forest practice is mainly interested in the amount of biomass (t ha^{-1} yr^{-1}) or volume (m^3 ha^{-1} yr^{-1}) by which mixed stands exceed pure stands. While the absolute outcome determines management decisions (production economy), the relative outcome contributes to the understanding of species-specific competitiveness and fitness at a given site (production ecology). As in central-European forests, combinations with atmospheric nitrogen-fixing species are still rare, it will be examined in the following as to whether stimulation of similar extent may be found in mixed stands of beech, oak and spruce which occur across large areas and are of considerable ecological and economical importance.

3. Extent and Relevance of Mixing Effects in Central-European Forest Stands

The prevailing reductionism in science continuously advances towards detailedness and tends to overwhelm us with amazing and often breathtaking mosaic pieces of facts on species mixing at the organ, tree and stand level (e. g., Richards et al. 2010, Rothe and Binkley 2001).

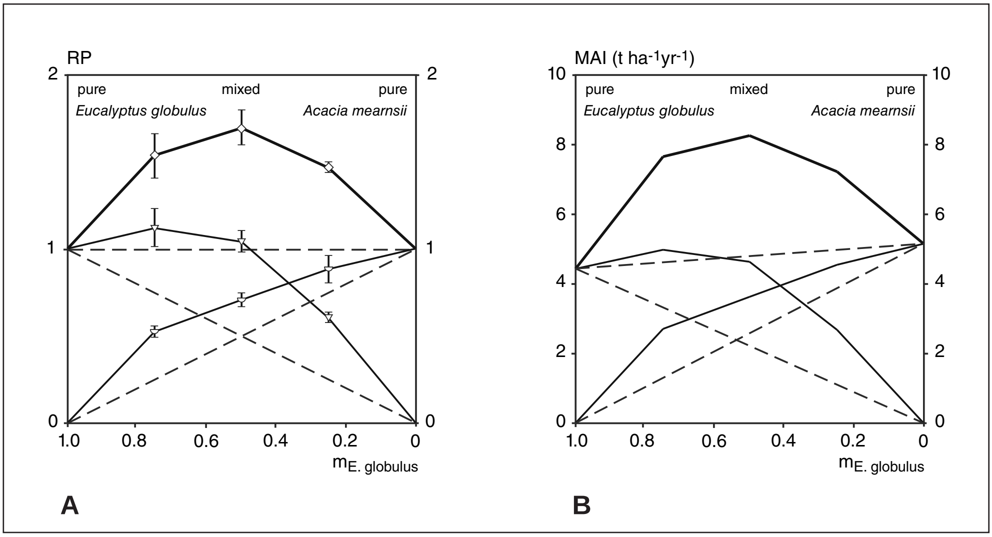

Fig. 2 Overyielding in mixed *versus* pure stands of *Eucalyptus globulus* Labill and *Acacia mearnsii* De Wild in terms of (*A*) the *relative* productivity of a species in mixture *versus* its performance in a pure stand and (*B*) the *absolute* gain or loss of productivity of a species or the stand as a whole in mixed compared with a neighbouring pure stand (courtesy of David Ian Forrester, see also Forrester et al. 2006)

However, the relevance of the observed high resolution in mixing effects in terms of the productivity of stands is hardly being addressed. In the following, the extent will be demonstrated to which mixing effects, which are evidenced by reductionistic research, are relevant for stand productivity.

3.1 Extent of Mixing Effects on Productivity

In order to unify the somewhat scattered sources of information about mixing effects, Pretzsch et al. (2010) compiled and analyzed existing growth and yield plots of pure and mixed stands of Norway spruce (*Picea abies* (L.) H. Karst.) and European beech (*Fagus sylvatica* L.). The database contains information from 23 long-term plots, covering an ecological gradient from nutrient-poor and dry to nutrient-rich and moist sites throughout Central Europe. Depending on site condition, dry mass growth in mixed stands can range from –46 % to +138 % of the growth yielded by neighboring pure stands. Figure 3 shows the observed relative volume productivity of mixed *versus* pure stands. On average the relative productivity of the mixed stand amounts to 120 % of the productivity expected on the basis of the neighboring pure stand (Fig. 3*A*). The gain of productivity in terms of absolute biomass productivity amounts to 1.5 t ha^{-1} yr^{-1}. The analysis at the species level (Fig. 3*B* and *C*) shows, that Norway spruce can profit but also suffer from mixture and comes off neutral on average (Fig. 3*B*). European beech also shows broad variation in performance, however, on average the outcome is positive. The latter result is indicated in Figure 3*C* by the transgression of the concave solid model line above the level of the increasing broken straight reference line. The functions representing the mean observed relative productivity in dependence of the mixing share result from model fitting by nonlinear regression analysis.

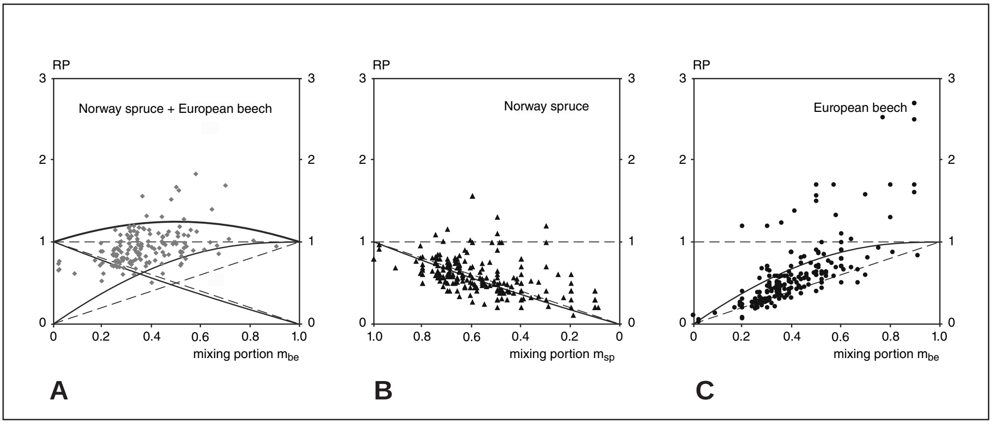

Fig. 3 Relative productivity of volume growth of (*A*) the mixed stand in total, (*B*) spruce and (*C*) beech in relation to the neighboring pure stands. The points represent the observed relative volume productivity of mixed *versus* pure stands. The curves represent the average mixing reactions of spruce, beech and total stand according to Pretzsch et al. (2010).

The species combination of oak (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.) and beech (*Fagus sylvatica* L.) is of considerable importance at present, but will become even more relevant in forests under climate change. Data from 37 long-term mixing experimental plots in Poland, Germany and Switzerland were pooled for analysis of mixing effects on stand productivity, as depending on mixing share and site conditions. On average mixed stands of oak and beech produce 30 % or 1.7 t ha^{-1} yr^{-1} more than the respective pure stands, as both species profit from the mixture (Pretzsch et al. 2013).

Based on 15 long-term experiments in mixed stands of Norway spruce (*Picea abies* (L.) H. Karst.), silver fir (*Abies alba* Mill.), and European beech (*Fagus sylvatica* L.) in the mountainous areas of the Bavarian Forest and the Bavarian Alps comprising a total of 46 plots, mixing effects of combinations of three species can be evaluated. The mean relative productivity amounts to 124 % of the neighboring pure stands while the absolute gain in productivity on average amounts to 1.60 t ha^{-1} yr^{-1}. Figure 4 shows a considerable variation around the mean mixing effect. A similar evaluation for 2 experiments with 6 plots and 3-species mixtures of Sessile oak (*Quercus petraea* (Matt.) Liebl.), European beech (*Fagus sylvatica* L.) and Scots pine (*Pinus silvestris* L.) in the hill country of Steigerwald and Spessart yielded, on average, a productivity in relation to the pure stands of 143 % and an absolute gain of productivity of 1.89 t ha^{-1} yr^{-1}. Although based on a rather small database, these results are indicative, given the scarce information to date on the effect of 3-species mixtures. The extent of mixing reactions (on average 124 to 143 % in relation to the neighboring pure stands) stresses, that in three-species mixtures the absolute gain as well as the variation is even higher than in 2-species mixtures. Hence, such outcome is attractive to forest science as well as to sustainable forest ecosystem management.

3.2 Dependency on Site Conditions

The above mentioned 37 long-term mixing experimental plots of oak (*Quercus petraea* (Matt.) Liebl and *Quercus robur* L.) and beech (*Fagus sylvatica* L.) in Poland, Germany

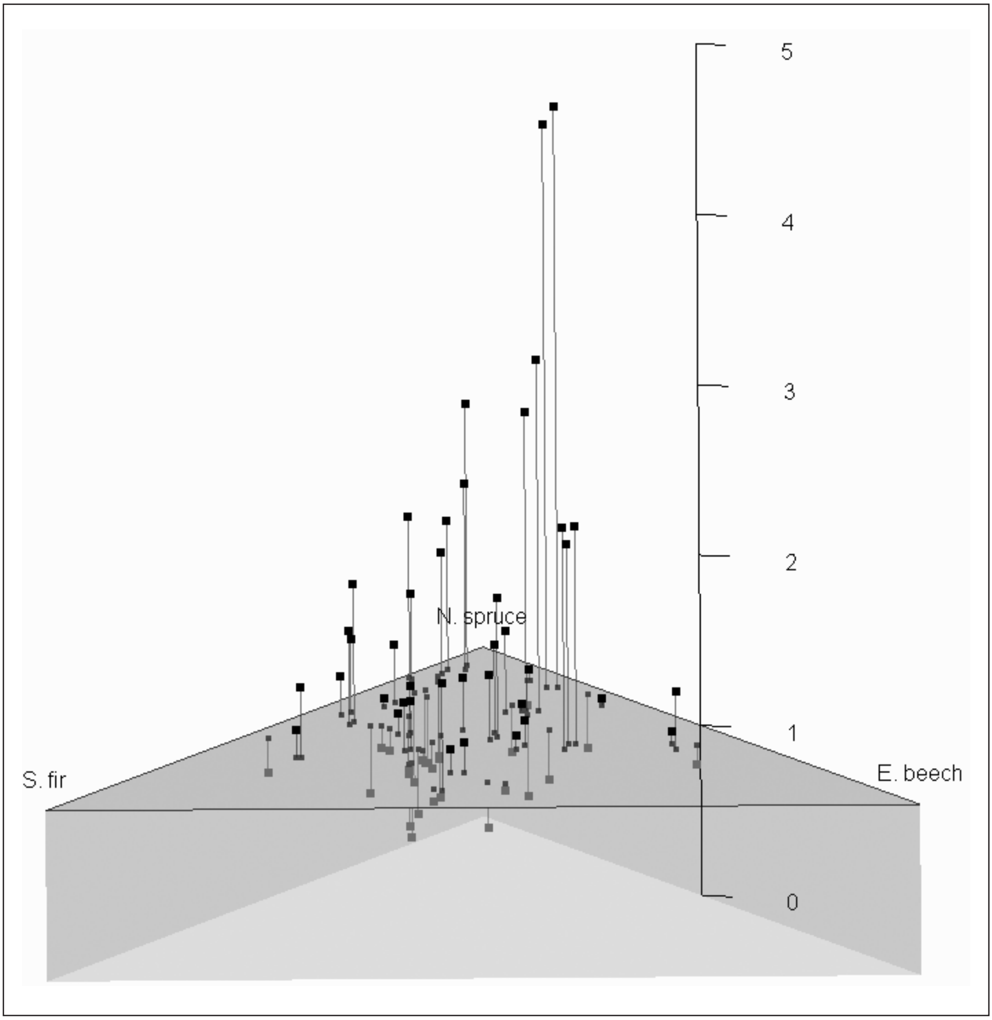

Fig. 4 Productivity of mixed stands with Norway spruce (*Picea abies* (L.) H. Karst.), silver fir (*Abies alba* Mill.), and European beech (*Fagus sylvatica* L.) *versus* pure stands. The horizontal lines framing the upper surface of the grey box represent the expected productivity in the pure stands (1.0-line). Each vertical bar in the center indicates the relative productivity of one mixed stand in relation to the pure stands. Overyielding is indicated by bars which reach out the 1.0-plane, while underyielding is evident when the bars remain under the plane. The relative productivity of the mixed stand as a whole amounts to 1.24 (+24 %) and the absolute gain of productivity amounts to 1.60 t ha^{-1} yr^{-1}.

and Switzerland cover a broad range of site conditions. For analyzing any dependencies of mixing effect on site quality, the quadratic mean height of oak and beech was used at an age of 100 years, being the so-called site indices hq_{oak} and hq_{be}, respectively (see Pretzsch 2009, pp. 200–203, for the definition and calculation of h_q) and surrogates for site quality. As most of the survey series included species up to the age of 100 years, observed hq_{oak} and hq_{be} were available from the pure-stand plots. Height of oak at age 100 ranges from 20 to 35 m, in the case of beech the range is between 20 and 45 m and even beyond. Based on the relative productivities (i.e. mixed *versus* pure stands), the mixing portion and the site index Pretzsch et al. (2013) fitted the model shown in Figure 5. Volume growth in mixed stands is revealed as changing from gains of 50 % to losses of 10 % in dependence on site condition and mixing share. In both species an increase in site index has a significantly negative effect on the relative productivity. Figure 5 shows the model functions against the scattergrams for oak and beech (Fig. 5*A* and *B*) in mixture. In order to demonstrate the site effect we inserted height values of 10 to 40 m into the model. On sites of low quality (hq < 25 m) both species profit considerably from the mixture. Their relative productivity even exceeds the 1.0-lines and indicates a facilitation effect. On mediocre sites (hq = 25 m – 30 m) the species still show a positive mixing effect, but do not exceed the level of the neighboring pure stands. On fertile sites (hq > 35 m) mixing causes a loss of productivity in relation to neighboring pure stands, reflecting exacerbating inter-specific competition.

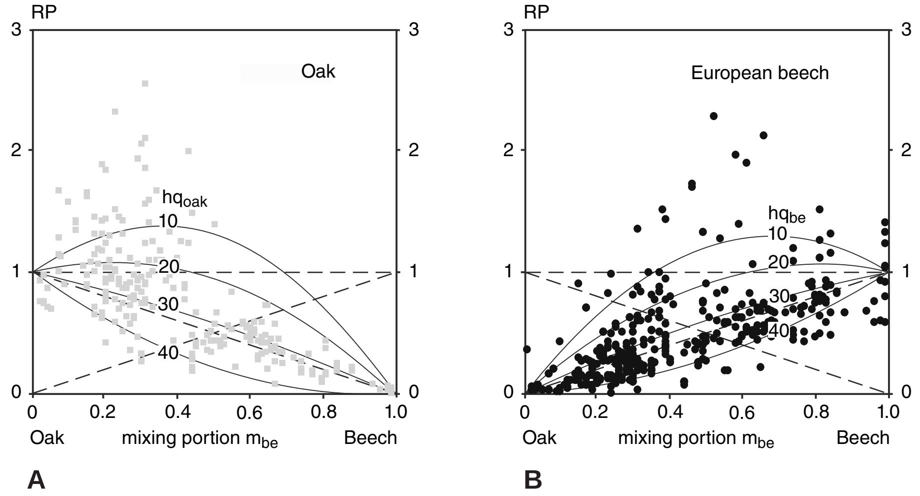

Fig. 5 Species-specific relative productivity in mixed *versus* pure stands of (*A*) oak (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.) and (*B*) European beech (*Fagus sylvatica* L.) depending on mixing portion, m, and quadratic mean height at age 100, hq, as indicator for site fertility. (*A*) Observed relative productivity for oak plotted over mixing portion of beech, m_{be}, (filled rectangles) and model prediction in dependence on admixture of beech and site fertility (curves with h_q= 10 … 40 m). (*B*) Observed relative productivity for beech plotted over mixing portion of beech, m_{be}, (filled circles) and model prediction in dependence on admixture of oak and site fertility (curves with h_q= 10 … 40 m).

Figure 6 makes the relationship between site quality and mixing reactions of oak and beech (Fig. 6*A*) and the mixed-stand performance (Fig. 6*B*) even more apparent. The dependency of the relative productivity (mixed *versus* pure stand) is displayed on the quadratic mean height at age 100 of oak hq_{oak} and beech hq_{be} as fitted by a simple linear equation. In the case of oak as well as of beech, the relative periodic annual volume increment decreases significantly with increasing site quality, indicated by significantly negative slopes. Insertion of hq = 20 m in the linear model yields $RP_{oak,be}$ = 1.49 and $RP_{(oak),be}$ = 1.38, being equivalent to a surplus of productivity by mixing of 49 % in relation to the pure stand in the case of oak and 38 % in the case of beech. Mediocre site conditions (indicated by hq = 30 m) yields + 11 % and 15 % in the productivity of oak and beech in mixture *versus* that under pure-stand conditions. On nutrient-rich site (hq = 40 m) the model predicts a loss of 27 % for oak and 8 % for beech. At the stand level the gains and losses in total for hq_{oak} of 20, 30, 40 m result in + 32 %, +7 % and –18 %, respectively. The mostly positive total stand reaction shown in Figure 6*B* can be interpreted as a mutualistic mixing reaction between oak and beech on poor sites, a neutral reaction on mediocre sites and an antagonistic reaction along with a reduction in productivity on fertile sites.

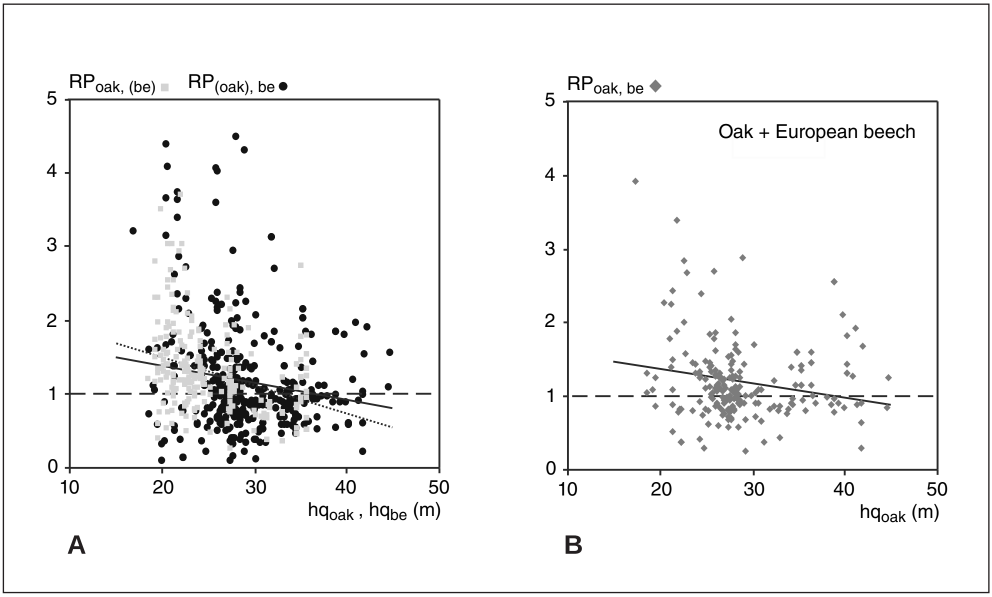

Fig. 6 Relationship between (*A*) species-specific relative productivity and (*B*) whole stand relative productivity of mixed *versus* pure stands of oak and beech (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. respectively *Fagus sylvatica* L.) in dependence on site fertility, indicated by quadratic mean height at age 100, hq. The graph shows the relative productivity expected for neutral mixing reactions (broken 1.0-line), the observed relative productivity of oak (rectangles), beech (circles) and the stand as a whole (rhombi), and the regressions lines for oak (Fig. 7*A*: broken line), beech (Fig. 7*A*: solid line) and total stand (Fig. 7*B*: solid line).

4. Discussion

Systematic overyielding up to 50 % reported by Caspersen and Pacala (2001), Hector et al. (1999), Loreau et al. (2001) and Pfisterer and Schmid (2002) for grasslands can hardly be found in managed forests in Central Europe. Presumably, in temperate forests of Central Europe niche differentiation is comparatively low due to species reduction in the course of the ice ages and due to the much slower evolutionary and co-evolutionary processes of long-lived trees. This may be a reason why the surplus of productivity of mixed stands compared with pure stands is much lower in long-lived forests than in short-lived herbaceous stands. Many of the European forest stands are “artefacts” designed with very productive species such as Norway spruce and Douglas fir cultivated outside their natural habitats. Often, genetic variation in these species no longer reflects natural selection but is a consequence of commercial selection criteria. Managed forests are therefore not designed for optimum niche utilization by the species in mixture. Compared with overyielding found in the subtropics and tropics (DeBell et al. 1989, Forrester 2006, 2007, Kelty 1992) the reported mixture effects of about ± 30 % for commercial tree species in temperate and boreal zones appear to be rather moderate. However, beyond the potential of direct increase or decrease of productivity, species mixing may indirectly change productivity by risk distribution and enhanced stand resilience in view of a broad set of forest functions and services (Hector and Bagchi 2007, Pretzsch 2005, Scherer-Lorenzen et al. 2005).

4.1 Site Conditions as Modifier of Mixing Reactions

As shown for the mixture of oak (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.) and beech (*Fagus sylvatica* L.), scattered and seemingly contradictory findings on mixing reactions may fit into an ecological continuum from poor sites with mainly positive interaction (overyielding) to rich sites with neutral or even negative mixing reactions (underyielding). In order to stress the site-specificity of relative mixing effects, cross-diagrams were introduced as demonstrated in Figure 1. Use of statistical relationships between mixing effects and site conditions allow to predict interaction between oak and beech on a poor site (represented by quadratic mean height at age 100 of hq_{oak}= 21 m, hq_{be}= 25 m), on a mediocre site (hq_{oak}= 26 m, hq_{be} = 29) and on a rich site (hq_{oak} = 33 m, hq_{be} = 36; Fig. 7). The reaction at the stand level (bold curve in the upper part of the cross-diagrams) results from the sum of the species-specific functions. Depending on site conditions, mixing can trigger a strong mutual facilitation with a relative productivity on stand level of about 1.66 (+ 66 %) on poor sites, a moderate mixing effect of 1.35 (+ 35 %) on mediocre sites, or a negative effect indicated by a relative productivity of 0.88 (–12 %) on fertile sites (Fig. 7*A*, *B* and *C*, respectively). While on the poor and mediocre site both species contribute to the productivity gain, on the rich site both react slightly negative and contribute to the overall loss. Accordingly, parts of the variation of the mixing effects observed to date can be explained by the site conditions of the analyzed stands.

4.2 Correspondence with the Stress-gradient Hypothesis

The observed mixing reactions correspond with the stress-gradient hypothesis (Callaway and Walker 1997) which predicts, that facilitation dominates on poor rather than rich sites whereas it is the latter sites where competition might prevail (Fig. 8*A*). In mixture both oak (*Quercus petraea* (Matt.) Liebl and *Quercus robur* L.) and beech (*Fagus sylvatica* L.) are more productive than in neighboring pure stands on poor sites and less productive on fertile sites. Only on mediocre sites mixed and pure stands are similar in productivity. Facilitation and competition between neighboring trees may occur simultaneously (Vandermeer 1989), however, the net effect is indicated by productivity gains or losses at the tree and stand level. The analysis of the productivity relationships suggest that on poor sites facilitation prevails, even mutual facilitation. The observations represented by data above the broken 1.0-line in Figure 5*A* and *B* indicate mutual facilitation. Such data reflect that the productivity of a fully stocked, closed oak stand can be increased in the presence of beech, and *vice versa* with oak

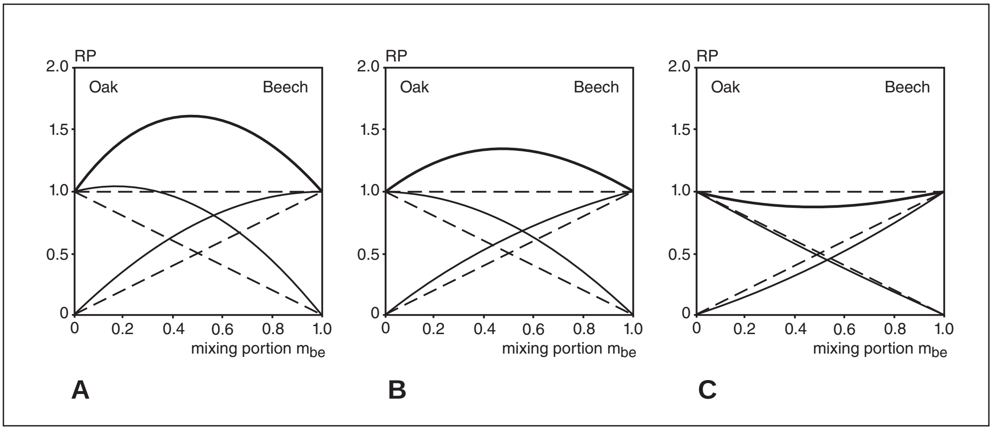

Fig. 7 Essential mixing reaction patterns of oak and beech observed along a gradient from poor to fertile sites. The site indices of oak resp. beech (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. respectively *Fagus sylvatica* L.) are 25 m and 21 m (*A*), 29 m and 26 m (*B*), and 36 m and 33 m (*C*). The cross-diagrams reflect that the relative productivity is modified by site conditions.

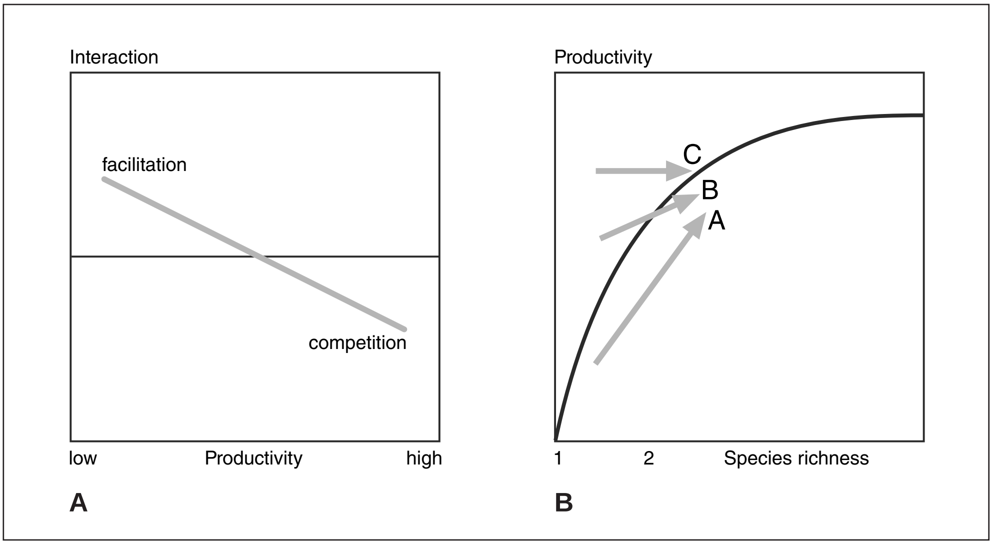

Fig. 8 Essential results of this study in schematic representation. (*A*) Change from facilitation dominated interactions to competition along the gradient from low to high productivity sites. (*B*) Site dependent relationships between productivity and species richness observed for oak and beech (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. respectively *Fagus sylvatica* L.) in this study (broken lines A, B, C for poor, mediocre and rich sites) and expected relationship (solid saturation curve) according to Körner (2002).

in beech stands. In other words, the competition effect by adding the other species is overcompensated by a supportive effect, yielding facilitation at the tree and stand level.

Other tree species mixtures behave differently, e.g. in mixed stands of Norway spruce (*Picea abies* (L.) H. Karst.) and European beech (*Fagus sylvatica* L.) the former conforms with the stress-gradient hypothesis, with beech profiting most from mixture on fertile sites (Pretzsch et al. 2010). The response behavior probably depends on covariates such as species traits, kind of limiting resources, and environmental factors. Scrutiny in testing the stress-gradient hypothesis for forest stands requires integrative analyses of species mixture, covering different functional groups of tree species, environmental conditions and resource availability (Callaway and Walker 1997, Holmgren et al. 1997).

4.3 Contribution to the Relationship between Species Richness and Productivity

It is an ongoing debate as to whether the relationship between productivity and species richness is represented by an increasing straight line, a saturation curve, an optimum curve or even a stepped, non-continuous trajectory (Körner 2002, p. 985). Figure 8*B* shows the saturation curve assumed according to Hector et al. (1999). In addition the graph reflects the relationships between mixing and productivity observed on our oak-beech long-term experimental plots on poor, mediocre and rich sites. The transition from pure to two-species stands results in strong increase of productivity on poor sites (*A*), moderate increase on mediocre sites (*B*), and constant, or even slightly decreasing productivity on fertile sites (*C*). This finding means, that experiments striving for relationships between productivity and species richness may bring different results depending on the initial site conditions and that the apparently contradictory findings might converge when the site conditions are taken into consideration as a modifier and third dimension. The phenomenon that the more fertile the initial site conditions the shallower the slope of the observed trajectories (A > B > C) reflects the predominantly positive but attenuating feed-back between stand and local environment: The mixture improves the site conditions, so that the additional benefit gradually becomes smaller. This kind of attenuating feed-back effect supports the hypothesis that the species richness-productivity curve follows a saturation curve.

4.4 Perspectives

Growth rates change with plant size. Pretzsch and Schütze (2005, 2009) show species mixing to alter tree size and as a consequence the expected growth rates of mixed *versus* pure stands. The analysis of this study is restricted to stand-level data, aims at providing a first overview on the extent of over- and underyielding, but neglects the above said effect of tree size on growth rate. Although descriptive, the outcome summarizes the existing knowledge on effects of tree species mixing at the stand level. The data of the long-term experimental plots used here has the potential for advanced analyses of mixing effects on productivity: Ontogenetic size effects on growth are to be separated from complex mixing effects. Furthermore, mixing effects will have to be decomposed into stand density and growth rate components of trees. Such components will be traceable to the individual tree level, using respective data on tree growth, crown size and competition. Although neither resource supply, nor resource uptake, nor resource use efficiency were measured directly on the long-term plots further detailed analysis of, e.g., density, growth rate, crown allometry and leaf area will functionally clarify the mixing reactions.

Compared to effects of thinning, tending, or fertilization the shown productivity increase by mixing is easy to achieve. Just knowledge is required about which species on which sites may pay off in terms of mixing. This article underlined the relevance of tree species mixing in forestry and the potential of further clarifying underlying mechanisms.

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Synthesis of Section II: The Processes – Competition *versus* Facilitation

Rainer Matyssek (München)

With 1 Figure and 1 Table

1. Introductory Considerations on Plant Growth Theory

"Growth and Defense in Plants", the topic of the symposium, spans the range of resource allocation in sustaining two crucial functions of plant persistence. These are (*i*) to prevent resource loss upon stress impact for (*ii*) ensuring resources to stay available to growth as a pre-requisite of plant competitiveness – and in addition, to reserve storage (Herms and Mattson 1992, Matyssek et al. 2005). The latter is to back allocation needs in decoupling external resource availability from use as e.g. for warranting reproduction and, hence, plant fitness. The coverage of such a span in allocation may cause regulatory trade-offs, in particular, between defense needs and other functions, although plants apparently do have means of mitigating or circumventing such conflicts (Rennenberg and Simon 2013, this volume, Matyssek et al. 2013, this volume). Section II highlighted the process level of competition for external resources as mediated through the internal resource allocation to growth, without neglecting, as an intrinsic component in regulation, however, the gradual transition between competition and facilitation. The latter phenomenon is to be conceived as an effect of the plant's allocation that alleviates rather than limits (as through competition) the co-existence of neighboring plants. Understanding the external exchange of resources between plants during competition and/or facilitation requires unraveling the internal allocation process to growth (Matyssek et al. 2005), with the associated consequences for the competition-facilitation continuum. This means, however, that resource allocation to growth is subject within the plant already to some kind of internal competition, namely, the metabolic conflict at least with the resource needs of defense.

After this introductory account on plant growth theory, it is conducive to comprehending the subject of Section II – in accordance with the "magic tripod of research" (see synthesis of Section IV, Lüttge 2013, this volume) – to examine the related grounds of modeling theory *prior to* highlighting the empirical knowledge. Modeling based on available knowledge can give guidance to experimentation through posing hypotheses to be falsified. In turn, the knowledge gained from hypothesis evaluation promotes modeling theory and, hence, the posing of new hypotheses as one further stimulus to research (cf. Popper 1989).

2. Plant-Internal and External Competition for Resources

It is the above rationale which is pursued by Gayler and Priesack (2013, this volume, Tab. 1) introducing PLATHO, a numerical model which simulates the internal competition for resources as a three-dimensional allocation trade-off between C and N availabilities and defense. This latter function is represented by the fluctuating pool of defense-related carbon-based secondary compounds (CBSCs). PLATHO is driven by the Growth-Differentiation-Balance Theory (Herms and Mattson 1992), being mechanistically most advanced amongst plant defense theories (Stamp 2003). However, no consistent plant response patterns prevail in the three-dimensional factorial relief, neither in modeling nor experimentation. Allocation is rather driven by source/sink dynamics. These are caused by growth stages and environmental impact, which results in complexity beyond presumed conceptual theory. On such grounds, numeric plant growth modeling becomes a process-based analytical tool that can extend explanatory horizons beyond the limitations of empirical approaches. The extent to which consistencies of allocation trade-offs may exist across spatio-temporal scales becomes accessible. The dominating challenge, however, remains to be tackled as to whether plant plasticity in stress response will be understood eventually in mechanistic terms.

Tab. 1 Section II: The Processes – Competition *versus* Facilitation

Speaker (in the order of referencing in the text)	Title of Presentation
Sebastian Gayler	Carbohydrate allocation to growth and defense-related metabolism – a modeling approach at the whole-plant level
Christoph Lehmeier	Stress effects on carbon allocation in a perennial grass – the role of stores in supplying growth and respiration
Thorsten Grams	A space-related perspective on plant-plant competition
Hans Pretzsch	Facilitation and competition in mixed-species forests analyzed along an ecological gradient
Ragan M. Callaway	Positive interactions and interdependence in plant communities
James F. Cahill Jr.	A trait-based approach to understanding competitive interactions

Lehmeier (2013, this volume, Tab. 1) extends the view on sink formation related to carbon as the substrate of respiration, in particular, by considering respiratory carbon costs under the multi-factorial influences of field sites. Under such conditions, little is known about the stores supplying and the underlying mechanisms controlling respiration and growth. For clarification, an integrated approach is demonstrated which unifies experimentation and modeling. On such grounds, high plant responsiveness became evident to external resource supply, regarding variability in respiratory activity and turnover associated with resource storage. Remarkable flexibility – i.e. plasticity – emerged related to store sizes and their kinetics, apparently reflecting a high degree in adaptability to variable environment for sustaining growth. An issue to be examined here is, as to whether the “real” allocation trade-offs between growth and defense are actually known in view of associated respiration costs. How far we are still away, both conceptually and methodologically, from assessing “full-cost analyses” of allocation trade-offs, covering respiration costs. The latter are related, in particular, to defense, with synthesis costs for metabolites as well as for their storage (in particular structures), turnover and transport (Lerdau and Gershenzon 1997). To which extent may trade-offs be buffered or even masked by interactions with resource storage and retrieval? Storage and respiration need to complement, as crucial determinants, the analysis of the plant-internal competition for resources, both in empirical and modeling approaches (Fig. 1).

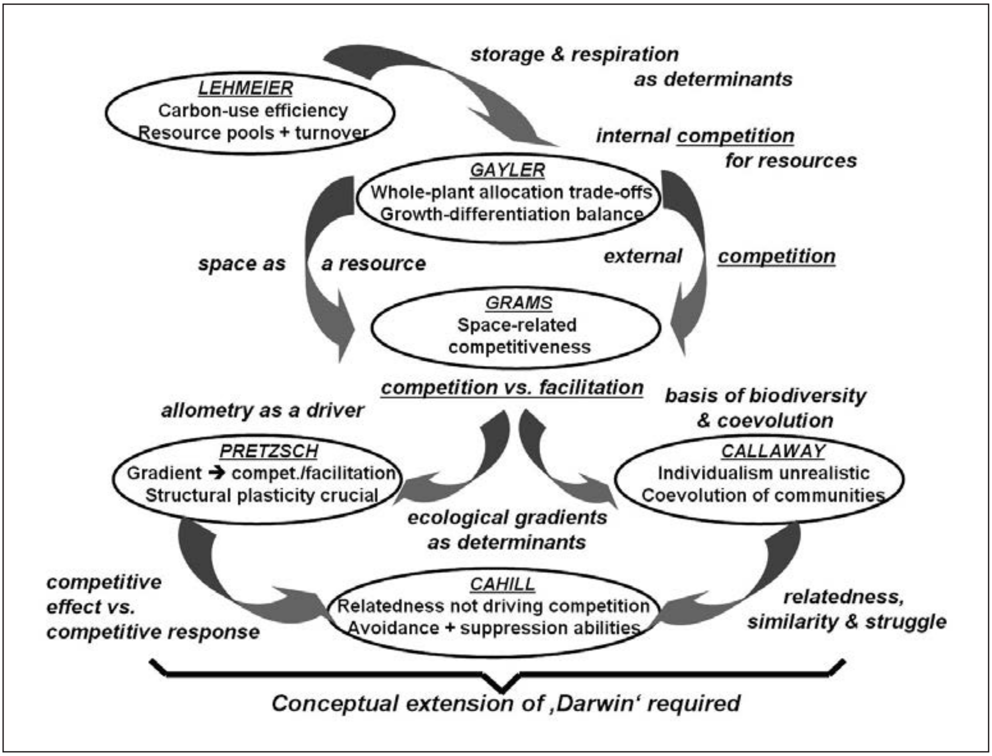

Fig. 1 Rationale of Section II “The Processes – Competition *versus* Facilitation”: Keywords and conceptual interrelationships of presentations (speakers in uppercase lettering).

At this stage, the plant-internal competition for resources between growth and defense (and other plant functions) may be conceived as a continuum with the external competition amongst neighboring plants (Fig. 1). This is where space – aboveground and belowground – comes into play, appearing as a resource by itself for competition in providing the peculiar ecological quality of enabling access to the basic resources, being light energy, water and nutrient elements. It is space primarily which vascular plants compete for (Schulze et al. 1986, Schwinning 1996, Grams and Lüttge 2010), to constrain or even expel neighboring plants in or from competition, respectively. Grams (2013, this volume, Tab. 1) points out that competitiveness is quantifiable as space-related resource investments and gains relative to plant neighbors. In particular, the mechanistic basis of plant competitiveness is apparently formed by efficiencies of resource investment into above and below-ground space occupation and resource gain from occupied space. It is concluded that resource-efficient competitiveness for space is one basic determinant of the plant's persistence. Exerting and tolerating competition has turned out as crucial modifiers of the plant's responsiveness to abiotic stress.

3. Competition *versus* Facilitation – Two Sides of the Same Medal?

In view of the preceding considerations, is reference to space use fundamental also to mechanistically defining and understanding facilitation? This is an issue yet to be examined. Use of stable isotope analysis may be an appropriate tool in resolving the issue. As demonstrated by Grams and Matyssek (2010) for competition, perhaps links can be established in analogous ways between space-related efficiency measures of facilitation and spatio-temporal trends in stable isotope patterns. The stage is set for ecologically valuating the phenomenon of facilitation *versus* that of competition at the stand level, as highlighted by the contributions of Pretzsch (2013, this volume) and Callaway (2013, this volume, Fig. 1, Tab. 1). The common observation in mixed forest stands of over and under-yielding relative to pure stands formed by given forest tree species appears to reflect the ecological significance of facilitation *versus* competition, respectively (Pretzsch 2013, this volume). As a general rule, the stress gradient hypothesis appears to be substantiated (Callaway et al. 2002), as on poor sites some species (e.g. beech) tend to facilitate companion species (like e.g. spruce or oak) under increasing climatic limitation, whereas on fertile sites competition amongst species typically prevails. Structural plasticity in crown architecture appears to be crucial in determining mixture performance. The extent is to be examined to which down-scaling approaches trace structural stand-level features back to tree species allometries and organ morphologies (e.g. at the branch level). Given the scarcity of respective databases, generalization and potential for theory development remain to be clarified. It appears to be conducive to hypothesize that stress gradients are regulators of the effectiveness of competition *versus* facilitation, with the transition being related to the morphological rather than physiological plant differentiation.

Callaway (2013, this volume) questions that species interdependence in plant communities is determined individualistically, i.e. according to Darwin, as a consequence of independent individual adaptation. In such a case, a maximum degree of competition should prevail amongst individuals of different species. However, the observation that facilitation does exist is in conflict with purely individualistic foundations of plant communities. Rather competition/facilitation balances prevail in community-level interactions, determined by the impact of environmental gradients (see above, Fig. 1). If such balances characterize community dynamics rather than individualistic principles, facilitation may have been underestimated in its potential for community evolution and as a driver of biodiversity across spatio-temporal scales. Is facilitation rather than competition the actual ecological core of community development? If so, this would foster differential niching of species in ecosystems, providing an extended quality of species fitness by enhancing reproduction chances rather than accelerating extinction. And if so, would Darwin's view then on evolution be too narrow (cf. Gould 2002), by underrating the holobiontic principle, as reflected by facilitation, in community-level evolution? Is life history on Earth an outcome even of an overall "global" co-evolution rather than of loosely related emergences of traits in individuals (under the influence of environmental cues in epigenetic regulation; see synthesis of Section IV, Lüttge 2013, this volume)?

In relativizing competition *versus* facilitation, CAHILL (2013, this volume, Tab. 1) explores trait-based approaches in understanding competitive interactions (Fig. 1). Competitiveness is recognized as the balance between the ability to suppress other plants (*competitive effect*) *versus* that to resist suppression (*competitive response*; see above), although both features are only weakly related to each other. As the competitive effect is mostly defined through plant size, inconsistency prevails in functionally explaining competitive response. The latter is to be conceived, perhaps, as a function of stress avoidance and suppression ability. Under the Darwinian perspective, closely related species which are similar in form and function might be expected to display intense competitive interaction, although such a presumption hardly has been tested. Evidence suggests, however, absence of relationships between species relatedness and competition intensity. Traits for competition are not highly similar among species which based on molecular cladograms (see reservations of HAMACHER 2013, this volume) are apparently closely related. Conversely, trait similarity does cause intense competition. Inconsistencies with established theoretical concepts will be challenging here for upcoming research. It is to be examined as to whether the competitive effect/response balance is explainable through the adjustment between competition *versus* facilitation traits.

4. Conclusions

A forward-strategy in research appears to be required towards refining and extending the Darwinian concept about the functional grounds of evolution in view of the mechanistic complexity in plant and community level interactions. Without doubt, DARWIN did lay the foundations for understanding evolution. However, the horizon needs to be widened towards evolution and development, where recently epigenetic regulation moves into focus (Evo-Devo Concept), and where, as a consequence, ecology also strongly comes into play (Evo-Devo-Eco Concept, see Synthesis of Section IV; LÜTTGE 2013, this volume). This is evidently determined by outcomes from predominantly co-evolutionary mechanisms, exemplified through the balance between facilitation and competition. In such terms, it is to be examined, if life history is driven by holobiontic principles and by selection at levels above that of individual organisms.

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Section III
The Scales: Spatio-Temporal Pattern Formation

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A Network View on Patterns of Gene Expression and Metabolic Activity

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With 7 Figures

Abstract

In this contribution, we briefly review the current status of systems biology approaches to plant science, starting from an early example exploring circadian oscillations. We emphasize two important challenges of systems biology: incorporating the spatiotemporal organization of biological systems and understanding dynamical processes in networks. Building up on this general framework we present several case studies from our own work: the prediction of self-organized patterns from cell properties, the interplay of gene deletions and environmental conditions in metabolic networks, and the analysis of different categories of control and metabolic consistency in gene expression patterns from *E. coli*.

Zusammenfassung

In diesem Beitrag fassen wir kurz den gegenwärtigen Stand systembiologischer Ansätze in den Pflanzenwissenschaften zusammen, ausgehend von einem frühen Beispiel über circadiane Rhythmen. Zwei besondere Herausforderungen der Systembiologie werden hervorgehoben: die Einbeziehung der raumzeitlichen Organisation biologischer Systeme und das Verständnis dynamischer Prozesse in Netzwerken. Auf diesen allgemeinen Grundlagen aufbauend, fassen wir eine Reihe von Fallstudien aus unseren eigenen Arbeiten zusammen: die Vorhersage selbstorganisierter Muster aus Zelleigenschaften, das Zusammenwirken von Gen-Essentialität und Umweltbedingungen in metabolischen Netzwerken und die Analyse verschiedener regulatorischer Kategorien und metabolischer Konsistenz in Genexpressionsmustern für *E. coli*.

1. Systems Biology as a Framework for Understanding Biological Processes

Systems biology is a melting pot of many disciplines, contributing to the understanding of the larger-scale of organization of living cells and their dynamic behavior in response to external and internal stimuli, including disease development (see e.g., Kitano 2002, 2004, Klipp et al. 2005, Alon 2006, Kholodenko 2006).

Among various other features, systems biology is also about the relationship between mathematics and biology. This relationship has been summarized in a striking and concise fashion in the title of Joel Cohen's review article (Cohen 2004): "Mathematics is biology's next microscope – only better." The second half of the title emphasizes how reciprocal the relationship between these two disciplines actually is: "Biology is mathematics' next physics – only better." It is fairly obvious that the rich and remarkable inventory of complex phenomena biology has to offer provides outstanding challenges, many of which will actually require new mathematics.

Talking about the complexity of biological phenomena, it is important to emphasize the deep and fundamental difference between the complex-systems perspective on complexity and the genuine features of biological complexity. As Hiroaki KITANO described in his seminal paper (KITANO 2002): "A popular notion of complex systems is of very large numbers of simple and identical elements interacting to produce 'complex' behaviors. [In biology ...] large numbers of functionally diverse, and frequently multifunctional, sets of elements interact selectively and nonlinearly to produce coherent rather than complex behaviors."

The selective interaction of the components is obviously bringing the network aspect into systems biology: As nowadays discussed in the broader framework of "network biology" (BARABASI and OLTVAI 2004), abstracting cellular processes into networks or into a language of small regulatory templates (ALON 2007, BRANDMAN et al. 2005, BRANDMAN and MEYER 2008) can help to identify deviations from randomness and contribute to an understanding of how such cellular systems function. In the biomedical sciences there are many familiar examples including biochemical reaction networks in metabolism (e.g., JEONG et al. 2000) with pools and flows of metabolites, and gene regulatory networks (e.g., SHEN-ORR et al. 2002).

More globally, systems biology is navigating the space between these two remarkably insightful quotations: the one by COHEN, linking mathematics and biology, and the one by KITANO, emphasizing the distinction between an abstract notion of complexity and the features of true biological complexity. Systems biology thus has to find a suitable balance between the two types of complexity. At the same time, systems biology is required to be an iterative process between mathematical modeling and experimental efforts. Quite naturally, systems biology has two important branches on the theoretical side. One is the analysis of high-throughput data and the other is the mathematical modeling of biological processes.

2. Systems Biology and Plant Science

2.1 Current Status

Although still rather under-represented in plant science in general, over the last two years systems biology has at a rapid pace moved towards practical applicability in plant biochemistry and plant ecology.

In 2009 the first genome-scale metabolic model for *Arabidopsis* was published (POOLMAN et al. 2009), containing approximately 1200 metabolites and 1400 biochemical reactions. In particular, the authors have been able to demonstrate that the model reproduces growth patterns under different environmental conditions in a biologically plausible fashion.

In 2011 a tissue-specific model reconstruction for *Arabidopsis* has been proposed (MINTZ-ORON et al. 2011), building up on the model from 2009. In this model, tissue-specific gene expression patterns are employed to project the former 'template model' onto sub-models for tissues (leaves, flowers, roots, siliques, seeds).

2.2 An Early Example of a Plant Systems Biology Investigation

The idea of employing mathematical modeling for understanding biochemical phenomena in plant science is by no means new. In this section I would like to briefly summarize an investigation of a well-known metabolic adaptation of a plant, crassulacean acid metabolism (CAM). At the core of this investigation has been an intense and highly iterative dialogue between theory and experiment. In fact, this investigation, which stretched out over the 1990s and early 2000s can be considered as an early example of a plant systems biology investigation.

In experiments performed with the CAM plant *Kalanchoë daigremontiana* (see Fig. 1*D*), it has been observed that the CO_2 exchange with the environment can display a pronounced circadian oscillation under constant illumination and constant temperature. On the grounds of this endogenous rhythm, CAM can be considered a plant model system of a biological clock (see e.g. Lüttge and Beck 1992, Lüttge 2000).

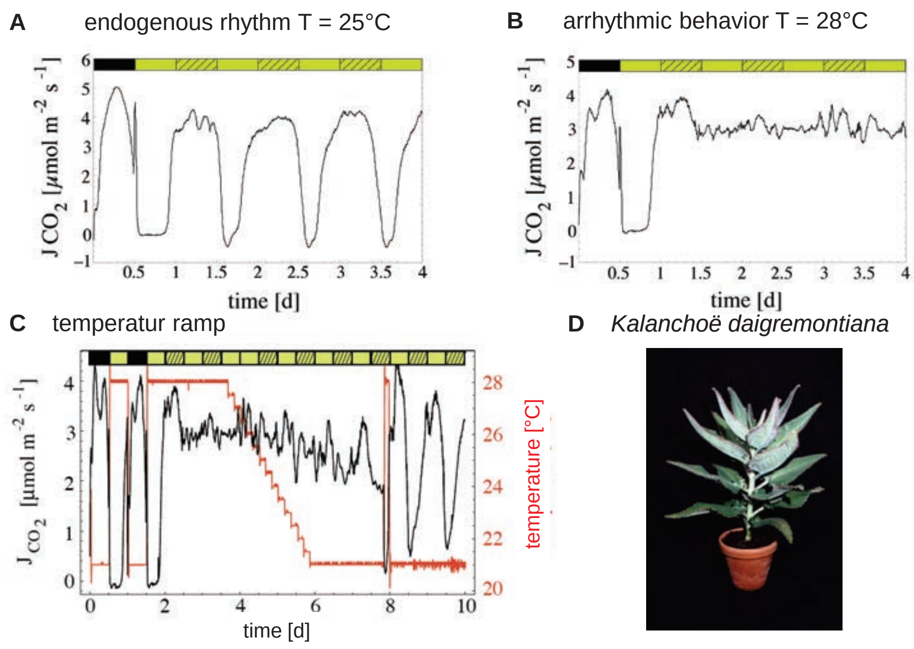

Fig. 1 Brief summary of experimental evidences for the dynamic properties of CAM circadian oscillations: (*A*) endogenous rhythm, (*B*) arrhythmic behavior, (*C*) response to a gradual temperature decrease, (*D*) CAM plant *Kalanchoë daigremontiana*. In (*A–C*), the experimentally observed CO_2 exchange is shown. The black bar on top of each plot indicates the last normal dark period, the hatched bars give the times of subjective dark periods in continuous light. Plots (*A*) and (*B*) have been adapted from Lüttge (2000), (*C*) has been adapted from Rascher et al. (1998); see also Hütt and Lüttge (2001); (*D*) was provided by Uwe Rascher (Jülich).

Remarkably, a small temperature increase (e.g., from 25 °C to 28 °C) lets the rhythm break down, and the CO_2 exchange curve shows an arrhythmic fluctuating pattern (see Fig. 1*A*, *B*). As soon as these key features had been established experimentally, mathematical modeling has been employed on two levels (representing in fact the two core pillars of systems biology work mentioned in the previous Section: data analysis and modeling): First, methods from nonlinear time series analysis have been applied to the CO_2 exchange data (in particular delay embedding and attractor reconstruction), in order to understand, whether the fluctuating arrhythmic behavior is generated by a low-dimensional nonlinear system and consequently needs to be considered as deterministic chaos.[1] Second, a mathematical model consisting of coupled ordinary differential equations (ODEs) has been formulated (with internal CO_2 and cytoplasmic and vacuolar malate concentrations as dynamic; see BLASIUS et al. 1997, 1999 for details).

This model helped classify the observations into the formal language of nonlinear dynamics, making the endogenous rhythm plausible as a limit cycle emerging from the bistability of the tonoplast membrane. The arrhythmic mode was considered a noise-driven fixed point in close proximity to the limit cycle. Furthermore, light, temperature and external CO_2 concentration, were the natural control parameters of the model.

The next striking experimental observation was the finding that, when the system is in the arrhythmic regime, a gradual decrease of the temperature will allow the arrhythmic behavior to persist even in the temperature regime associated with a rhythmic CO_2 exchange pattern (RASCHER et al. 1998). A short temperature pulse, however, would then restore the strong pronounced rhythmic behavior (see Fig. 1*C*). It was immediately clear that the simple coupled ODE model is not able to explaining this phenomenon, as unique dynamical behaviors are associated with individual points in parameter space.

One hypothesis formulated jointly on the grounds of the experimental findings and the theoretical attempts of explaining these findings was to consider the plant leaf as an ensemble of spatially coupled oscillators that can be in various synchronization states, and in this way considering spatial desynchronization as an important contribution to the transition from rhythmic to arrhythmic behavior.

The picture emerging from this early example of a plant systems biology investigation was that of the CAM circadian clock as a spatiotemporal phenomenon (RASCHER et al. 2001). Figure 2 summarizes the intense dialog between theory and experiment behind this investigation. Some additional details of this dialog and general aspects of modeling are discussed by HÜTT and LÜTTGE (2002).

In the following I would like to describe two research directions that have their seeds in this early study.

One is the importance of understanding spatiotemporal pattern formation and including the spatial organization of biological systems into dynamical analyses. In Section 3 I will provide an example of a novel view on classical spatiotemporal patterns that may serve as a starting point for a "next-generation pattern analysis" fitting the needs of systems biology. The key idea is to predict the emerging spatiotemporal patterns from an initially known distribution of cell properties.

The second research direction put forward by the early investigation is to consider patterns not only in the classical spatiotemporal sense, but also in a more abstract form adapted to the key role of networks in systems biology: patterns of dynamical processes on graphs. In

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[1] Irregular behaviors in time series are difficult to classify. A key distinction is between stochastic (i.e. random) and deterministic irregularity. While the former is considered as noise, the latter is associated with (deterministic) chaos. It should be noted that for the considerations presented here, the main difference between noise and deterministic chaos is the dimension of the generating system. Two small additional comments: (*i*) From a modeling perspective, noise is the summary of all dynamics on very fast time scales (compared to the time scale under consideration). (*ii*) For generating deterministic chaos, a system of nonlinear coupled ODEs needs to be at least three-dimensional; one- and two-dimensional systems can maximally display fixed-point dynamics or oscillations, respectively.

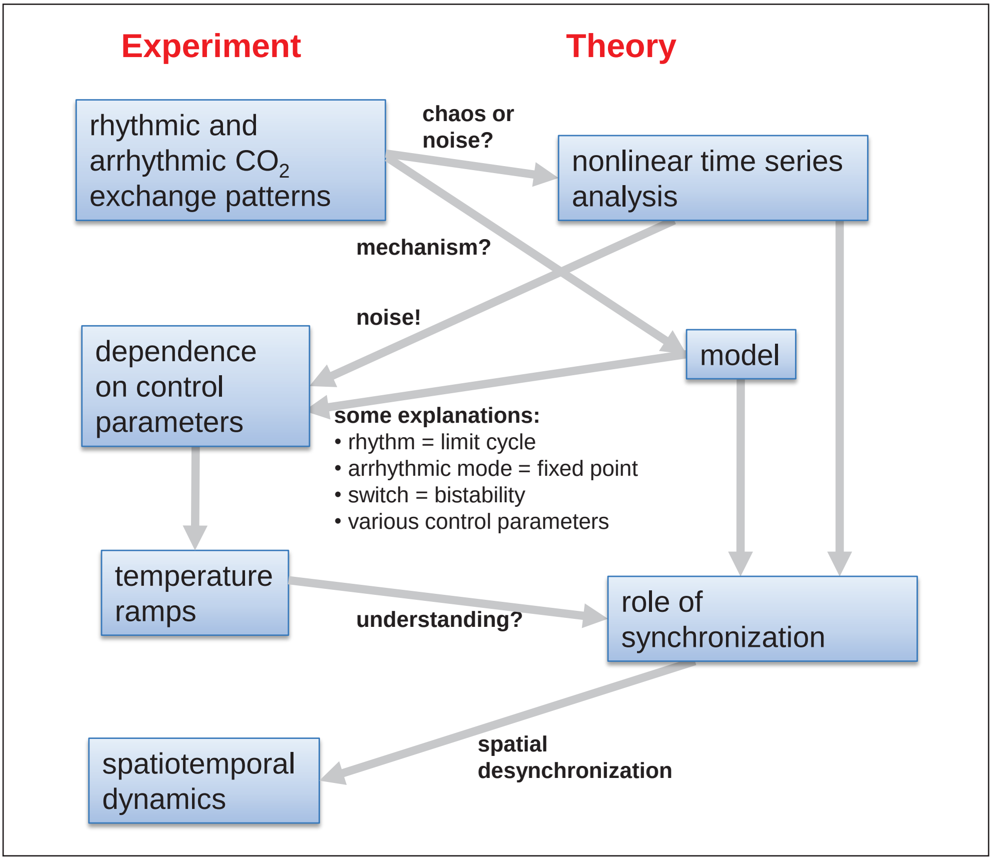

Fig. 2 Dialog between theory and experiment towards a spatiotemporal interpretation of CAM dynamics.

Section 4 I will describe various attempts of network-based analysis for high-throughput data and metabolic flux patterns.

3. A Modern View on Spatiotemporal Pattern Formation

A prototypical example of functionally important self-organized spatiotemporal patterns in biology is the chemotactic organization of *Dictyostelium discoideum* cells with their characteristic spiral wave patterns followed by aggregation streams (see Fig. 3*A*). Partitioning a plane into regions giving each cell a clear, unambiguous signal to which aggregation center to move, is a remarkable example of collective problem-solving and self-organized decisions based only on local information.

At the same time, there is evidence that the genetic feedback loop underlying the processing of the chemotactic signal (cAMP) by each cell is optimized (in its feedback strength) to achieve a particular size of these self-organized regions defined by the arrangement of spiral waves (Sawai et al. 2005).

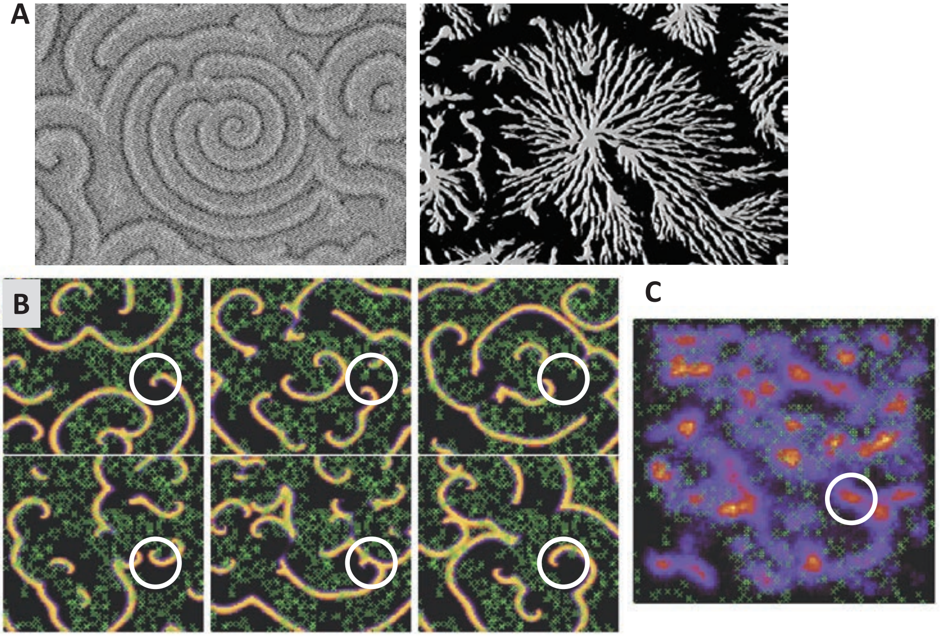

Fig. 3 Spiral wave data (*A*) and simulations (*B*, *C*). In (*A*) an example of a spiral wave pattern from *Dictyostelium discoideum* is shown (*left*), together with a snapshot of later-stage aggregation streams (*right*). In (*B*) simulations of spiral-wave patterns are shown at a fixed distribution of "pacemaker" cells. Those cells have the ability to spontaneously emit a chemotactic signal. All cells, which have this property are highlighted as green crosses, and a single snapshot of the established spiral wave pattern is shown per simulation run, which emerges eventually from the random firing of the pacemaker cells. In (*C*) the average of the spiral tip occupancy of 1000 numerical runs, for the pacemaker distribution from (*B*) is shown. The white circle in (*B*) and (*C*) is to guide the eye. The data for (*A*) were provided by Christiane Hilgardt (Magdeburg); see Geberth et al. (2009) for details; (*B*) and (*C*) are based on Geberth and Hütt (2008).

From our perspective, this optimization of an individual cell property for achieving a specific collective state, points to one of the striking differences between self-organized patterns in biology compared to similar patterns in chemistry and physics. In biological systems, these patterns often serve a systemic purpose and are, therefore, under evolutionary selection. It is thus highly plausible that the individual element in the system exerts a certain amount of control on the specific details of the self-organized pattern. Conversely, in physics and chemistry the details of the self-organized patterns rather emerge from small (e.g., density) fluctuations in large populations of (identical) elements.

Over the last few years we have tried to better understand these forms of control, cells have (*via* their individual properties) over the large-scale self-organized patterns (Hilgardt et al. 2007, Geberth and Hütt 2008, 2009, 2010, Geberth et al. 2009).

Specifically, we have asked, how the distribution of cell properties is translated into such patterns for an established model of *Dictyostelium* pattern formation (Levine et al. 1996). Figure 3*B* shows spatial snapshots from repeated simulations of spiral wave patterns, starting from random initial conditions. Common to all these simulations was the spatial distribution of pacemaker cells. These are cells with the capacity to spontaneously emit cAMP. If one focuses, e.g., on the circled region, one sees a surprisingly large number of spiral tips compared to other region. An overlay of spiral tip distributions over 1000 simulation runs (Fig. 3*C*) confirms this impression of a strong spatial heterogeneity in spiral wave distribution at a fixed distribution of cell properties (which in this case corresponds to the distribution of pacemaker cells. Details about this numerical experiment are given in GEBERTH and HÜTT (2008).

We have shown that as a dominant contribution to translating cell properties into self-organized patterns, a strong and pronounced anti-correlation between pacemaker cells and spiral wave tips emerges (GEBERTH and HÜTT 2008, 2009).

We believe that the search for such correlations in experimental data can be highly informative about the specific mechanisms of self-organization, signal processing and cell-cell interaction at work in given system. Reverse-engineering properties of signal processing and regulation from such correlations in spatiotemporal patterns could very well constitute a novel framework for studying biological pattern formation. This is what in the previous Section we somewhat boldly paraphrased as “next-generation pattern analysis”.

When for a given system the various shaping influences cells (*via* their individual properties) exert on the spatiotemporal patterns have been understood, one can even employ this knowledge for a cell-property-driven “design” of such patterns (see examples in GEBERTH and HÜTT 2008, GEBERTH et al. 2009).

4. Dynamical Processes on Networks

First I would like to describe a simple example of a dynamical process on a graph, showing that network topology (the global “architecture” of the network at hand) systematically and non-trivially shapes the properties of the dynamical process. As a next step, I briefly emphasize the importance of networks and an understanding of dynamical processes in networks for systems biology. In the last part of this Section, I will use this general framework to analyze high-throughput data in a biological system.

4.1 Patterns on Graphs

Figure 4 summarizes the dynamical process I would like to analyze on a graph as a first illustrative example. A node in the system can be in one of three discrete states: excitable/quiescent (Q), excited (E; as soon as an excitation occurs in the neighborhood), refractory (R; for a certain period of time).

These dynamics, on a lattice, can be seen as a stylized example of the chemotactic signaling discussed in previous Section for *Dictyostelium*. In fact, the letters Q, E and R introduced above can now be used almost in a telegram style to describe the dynamics of the slime mold: Sensing cAMP (which is, e.g., produced by one of its neighbors) the amoeba changes from Q to E (i.e., it emits cAMP itself), enters a rest phase R and starts to migrate to the aggregation center. After a short period (about five minutes) the amoeba is capable of emitting cAMP again, i.e., it again enters the stage Q. The existence of a refractory period, together with the communication (signal detection and emission) with neighbors results in the formation of characteristic excitable-media patterns, namely concentric rings and spiral waves (see e.g. WINFREE 1972).

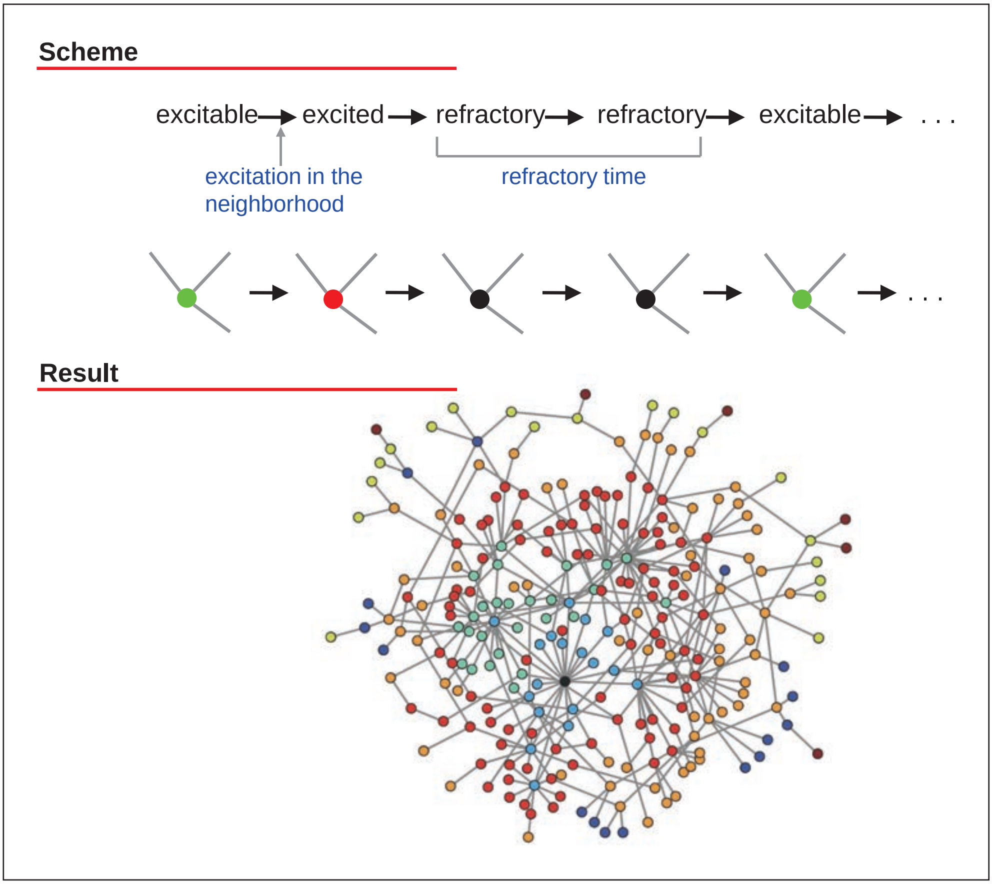

Fig. 4 Excitable dynamics on graphs. Upper part ("Scheme"): update rules underlying the dynamics of each node in the network, together with a possible state sequence of a single node in the network (green: excitable, red: excited, black: refractory). Lower part ("Result"): Summary of a simulation run, where two nodes in the network have the same color, when they have frequently been simultaneously excited (i.e., colors are derived from a clustering tree based on the co-activation matrix); see Müller-Linow et al. (2008) for details.

Due to the simple neighborhood condition, this dynamical process from Figure 4 can immediately be simulated on a general graph (see Müller-Linow et al. 2006, 2008 for details). A node will be excited in the next time step *t*+1, when at time *t* one of the neighbors is in the excited state.

In this stylized form the dynamics are one of the most generic categories of processes in biology: As discussed above, as a spatiotemporal pattern, these dynamics resemble the chemotactic signal propagating through a colony of *Dictyostelium* cells. On a graph we can consider these dynamics as a minimal model of coupled neurons. On a curved surface (resembling the geometry of the heart) we can consider the process as a symbolic encoding of the electrical excitations propagating through cardiac tissue.

In all these cases, it is clear that only much richer models can account for the manifold biological details of each of these individual systems. However, it is also clear that some features of the dynamics will be universal to all excitable systems, including the stylized three-state cellular automaton-like representation.

One of these features is wave propagation. Figure 4 (bottom) shows the result of simulating this dynamical process on a graph. The graph has been obtained by the preferential attachment algorithm described in BARABASI and ALBERT (1999). It thus has a broad degree distribution, i.e., many nodes have very few neighbors (low degree), while some nodes have a much larger number of neighbors (high degree). The latter are the "hubs" of the system. The results of the dynamical process are displayed in the colors of the nodes: Two nodes have the same color, when they frequently have been simultaneously active (i.e., groups of nodes with the same color have been obtained from performing a clustering on the coactivation matrix).[2]

Strikingly, in the graph layout chosen for Figure 4 these colors are organized as pronounced rings around a hub (displayed in black in Fig. 4). This is a remarkable example, where direct links between nodes are less important for understanding the collective behavior of the system, than some higher topological features: Two nodes tend to display synchronous activity, when they have the same distance from the hub. The network architecture thus influences how dynamics organize on the graph.

The pattern presented in Figure 4 is the network equivalent of classical target wave patterns known well from many self-organized spatiotemporal pattern formation processes.

4.2 Relevance for Systems Biology

Already in Section 1, citing KITANO, I emphasized the importance of networks for systems biology. Figure 5 illustrates this point by representing two layers of regulatory organization, genome structure and the transcriptional regulatory network (TRN), acting upon another network, the network of biochemical reactions (using data from the model organism *Escherichia coli*). The 3D structure of the chromosome of *E. coli* is one key component of its gene regulatory machinery (illustrated by the coiled circular chromosome in the center of Figure 5). This type of regulation mediated by topological transitions of the chromosomal DNA can be thought of as an *analog control*, complementing *digital control*, i.e. the network of regulation mediated by dedicated transcription factors, as shown in the upper part of Figure 5.

For both networks, dynamic processes are particularly interesting for the biological function: The gene regulatory network contributes to cellular function *via* gene expression levels. The distribution of metabolic fluxes, which is shaped by the underlying metabolic network, determines cellular growth and cellular performance under different environmental conditions.

In the following, I would like to explore, how the 'nestedness' of the system and the network-like organization in several layers reveal itself, both in gene expression data and in predictions of active metabolic fluxes.

First, I would like to briefly summarize, how genome-wide metabolic networks can formally be described using a simple linear steady-state model. The starting point is provided by a system of linear, coupled ordinary differential equations (ODEs):

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[2] See MÜLLER-LINOW et al. 2009 for details; see the supplementary information to MÜLLER-LINOW et al. 2009 for movies of this dynamical process on different graphs.

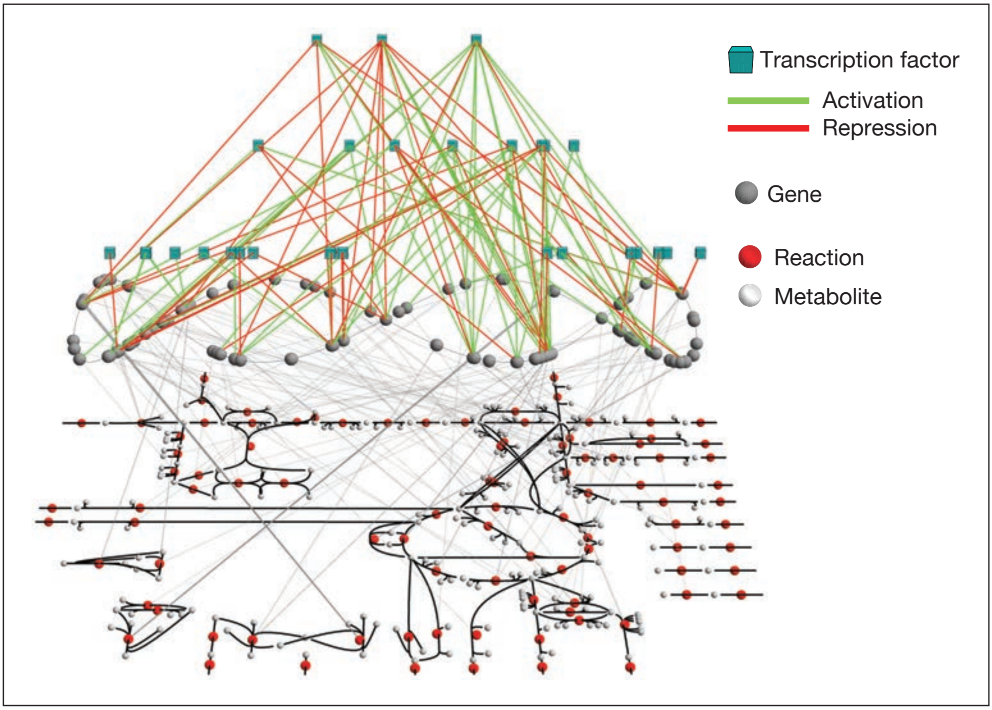

Fig. 5 Illustration of the different components involved in transcriptional regulation, transcription and metabolism for a biological model organism, the bacterium *Escherichia coli*, represented as three interconnected systems of cellular organization. Only a subset of the overall network elements is shown for the sake of clarity, i.e. only nodes and links involved in *E. coli* central metabolism are depicted. Upper part: the transcriptional regulatory network: transcription factors (*green cubes*) control the expression of metabolic genes (*gray spheres*) either by activation (*green links*) or repression (*red links*); middle part: genome organization; DNA topology is affected globally by supercoiling and locally by the binding of proteins; lower part: depiction of *E. coli* central metabolism (as described by Orth et al. 2009). Metabolic genes on the chromosome are connected to reactions (red spheres) according to their enzyme-reaction relationships. Figure adapted from Sonnenschein et al. (2011).

$$d\mathbf{S}/dt = \mathbf{N}\ \mathbf{v}, \qquad [1]$$

where **S** is the vector of metabolite concentrations, **v** is the vector of metabolic fluxes, and N is the stoichiometric matrix indicating, which metabolic flux (and to which proportion) contributes to the time change of a metabolite concentration.

This system is now studied in its steady state,

$$d\mathbf{S}/dt = \mathbf{N}\ \mathbf{v} = 0, \qquad [2]$$

translating the ODE system into a system of linear equations, for which a solution space can be computed. Additional constraints, like minimal and maximal flux sizes, the reversibility or irreversibility of reactions, and some objective function (e.g., biomass maximization) yield a finite solution space and a single (with a few caveats; see Price et al. 2004) optimal flux composition maximizing the objective function at hand. This is the key idea of flux-balance analysis and other forms of constraint-based modeling in the study of metabolic systems.

One of the remarkable consequences of this approach is that it implies a hierarchical, layered view on metabolic systems. The metabolic system consists of an input layer (given by the set of uptake reactions), a "processing layer" and, specified by the choice of the objective function (in this case: biomass), an output layer, comprising all components of the biomass vector. In Figure 6 this perspective on the metabolic network is summarized.

Strictly speaking, a graph representation of metabolism consists of two types of nodes, metabolites and enzymes (or reactions). Mathematically, this is called a bipartite graph. From this graph, two projections to single types of nodes are frequently used: the metabolite-centric graph and the enzyme-centric graph. In the first representation, metabolites are the nodes and a link denotes the involvement of any two (or more) metabolites used by a given enzyme reaction. Distinguishing between substrates and products of an enzyme reaction can provide directed links. In the second representation, enzymes are the nodes and edges indicate shared metabolites between their individual reactions.

All three representations (the bipartite graph, the metabolite-centric projection and enzyme-centric projection) can have very highly connected nodes. A given enzyme can, of course, be involved with more than one substrate or more than one product metabolite or currency metabolite. The illustration in Figure 6 shows a bipartite representation of a (fictitious) metabolic sub-network, together with its projections.

Additionally, Figure 6 qualitatively illustrates, how the enzyme-centric representation of a metabolic network is situated in the genome (*via* enzyme-encoding genes), thus providing a starting point for a formal comparison of gene expression levels and network structure.[3]

One of the implications of this layered structure is that the input of gene deletions can (and in many cases will) depend on the environment (metabolic "plasticity").[4]

In this way, the framework of flux-balance analysis becomes an ideal tool for statistically exploring the interdependences of gene deletions, cellular objective functions and environments, including evolutionary aspects on a very large scale.[5]

4.3 Application to the Analysis of High-throughput Data

After this brief summary of how systems biology employs modeling and linear optimization methods to understand the large-scale organization of metabolic fluxes, I now turn to the last topic of this methodological review: how to devise strategies for the analysis of high-throughput data on the grounds of our understanding of dynamical processes in networks.

A starting point of this exploration is the general relationship between network topology and simple dynamical processes (as discussed in Section 4.1, see also Marr and Hütt 2005, 2006, 2009, Müller-Linow et al. 2006, 2008, Hütt and Lesne 2009). In previous studies we have analyzed the match between the metabolic network and metabolomics data during the diurnal cycle of *Arabidopsis thaliana* (Müller-Linow et al. 2007). Specifically, we computed metabolic correlation networks derived from the covariance of metabolites in replicates of metabolomics experiments. They constitute an interesting intermediate between topology (i.e. the system's architecture defined by the set of reactions between metabolites) and dynamics (i.e. the metabolic concentrations observed as fluctuations around steady-state

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[3] See Section 4.3; see also Sonnenschein et al. 2011.

[4] See Harrison et al. 2007.

[5] See, e.g., Behre et al. 2008, Motter et al. 2008, Nam et al. 2011.

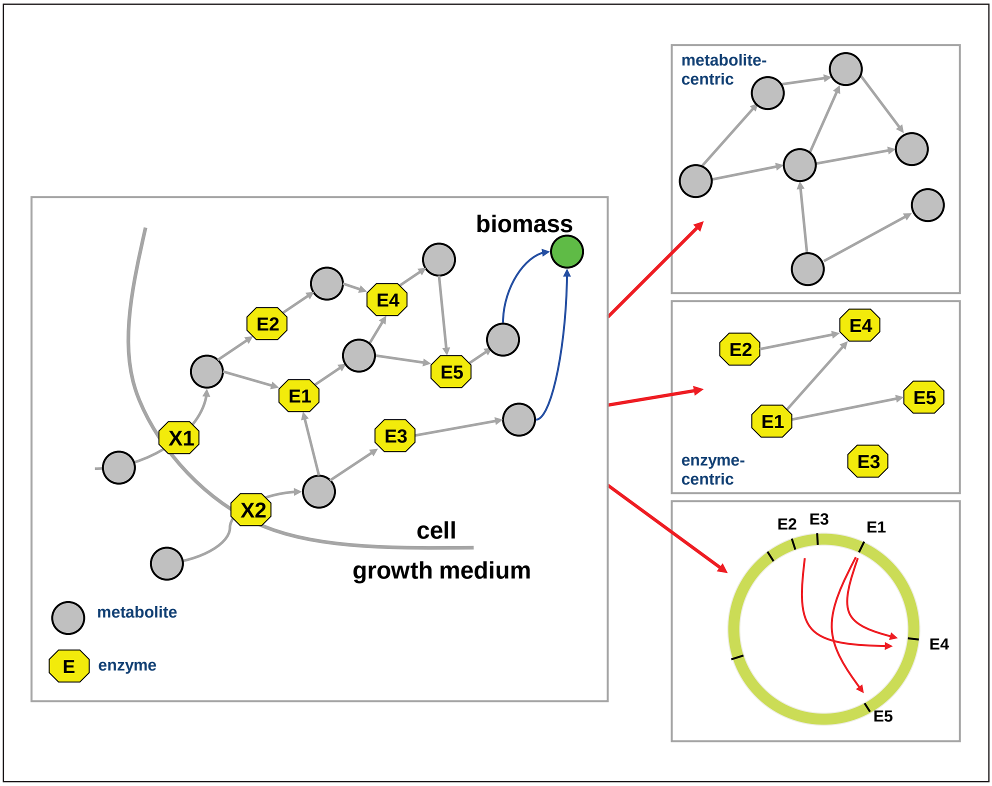

Fig. 6 Network representation of cellular metabolism (schematic view), together with the projection of the bipartite representation of metabolism (*left*) to a metabolite-centric graph (*right*; *top*) and to an enzyme-centric graph (*right*; *center*). An advantage of the enzyme-centric representation is that it can be discussed in its regulatory context. Here, the enzyme-centric metabolic network is shown inserted in the (circular) genome (*right*; *bottom*), where the enzymatic genes can be affected by the regulatory action from other genes, leading to higher or lower enzymatic activities and thus higher or lower flow in the metabolic network. Figure is taken from Smith and Hütt (2010).

values in the metabolic network). We find that network similarity indeed decreases with an increasing time difference between these networks during a day/night course and, counter-intuitively, that proximity of metabolites in the correlation network is no indicator of proximity of the metabolites in the metabolic network.

More recently, we studied the interplay of patterns in gene expression data consistent with genome structure (*analog control*) and those patterns consistent with the transcriptional regulatory network (*digital control*). In order to quantify these two contributions to gene regulation, we investigated gene expression patterns in *E. coli* comparative microarray experiments (see Marr et al. 2008). We compared the regulation mediated by gene-gene interactions through dedicated transcription factors on the one hand, and the continuous regulation of neighboring genes on the chromosome by alteration of the superhelical density, on the other (see Fig. 7*A* for a schematic representation of the two types of control). We find that the wild type cell uses both types of regulation and moreover, that the loss of functional capacity in one type of control is compensated by the other. In particular, loss of the hubs FIS and H-NS leads to compensatory increase of the impact of digital control.

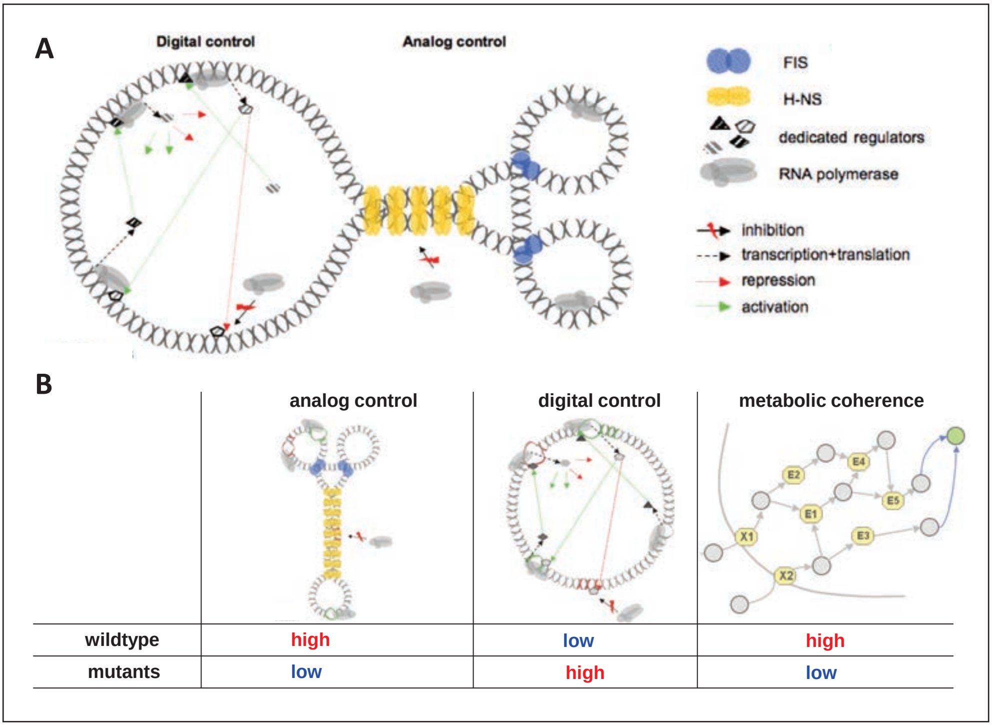

Fig. 7 Schematic representation of digital and analog regulation (*A*) and summary of gene expression analysis (*B*). In (*A*): Digital control – Dedicated regulators independently recruit RNA polymerase to distantly located genes to either activate (*green arrows*) or repress (*red arrows*) their activity. Analog control – Abundant DNA architectural proteins (only FIS and H-NS are shown for simplicity) form topological domains, thus rendering the distant genes under independent digital control similarly accessible to the RNA polymerase. The activation of transcription is indicated by the RNA polymerase associated with DNA, repression of transcription by "red-flashed" arrows. In (*B*) the results for digital and analog control (MARR et al. 2008), as well as on metabolic coherence (SONNENSCHEIN et al. 2011) are qualitatively summarized. In our experimental setup, the transcript profiles of three *E. coli* strains (wild-type, fis mutant, hns mutant) have been compared under low and high superhelicity and also with each other. Figure components partially adapted from MARR et al. 2008.

Using methods from point process statistics, we have been able to further support the distinction between digital and analog control by analyzing gene distributions (SONNENSCHEIN et al. 2009). Distinguishing between genes that do not participate in the transcriptional regulatory network (i.e. that are according to current knowledge not producing transcription factors and do not possess binding sites for transcription factors in their regulatory region), and genes that *via* transcription factors either are regulated by or regulate other genes, we find that the two types of genes ("isolated" and "regulatory" genes) show a clear statistical repulsion and have different ranges of correlations. In particular we find that isolated genes have a preference for shorter distances.

Using the method of control strengths derived from effective networks (MARR et al. 2008), we also studied the agreement of active metabolic networks (as predicted by flux-balance analysis; see Section 5.2) and gene expression data (SONNENSCHEIN et al. 2011). We find a significantly higher correspondence between gene expression and metabolism for the wild type expression changes compared to mutants in nucleoid associated proteins (NAPs), indicating that supercoiling induces meaningful metabolic adjustments. As soon as the underlying regulatory machinery is impeded (as for the NAP mutants), this coherence between expression changes and the metabolic network is substantially reduced. Furthermore, the regulatory control exhibited by DNA supercoiling is not mediated by the transcriptional regulatory network (TRN), as the consistency of the expression changes with the TRN logic of activation and suppression is strongly reduced in the wild type in comparison to the mutants. Again this observation emphasizes the importance of analog control. Figure 7 summarizes our results on the interplay of digital and analog control, as well as on metabolic coherence.

A hot topic of network research is the functional role of small subgraphs (like feedforward loops, branching points, small cycles and feedback loops). Statistically over-represented subgraphs (compared to randomized graphs), termed 'network motifs', have been hypothesized to be device-like building blocks capable of explaining some of the functional properties of complex networks (Alon 2007). In order to better understand the validity of the motif perspective for transcriptional regulatory networks, we explored the interplay between feed-forward loops and larger-scale structures (subsets formed by all nodes topologically down-stream of a reference node) in gene regulatory networks (Marr et al. 2010). The rationale of our analysis has been to explore the internal logic of gene regulation by looking at the interplay of two scales within the transcriptional regulatory network of *E. coli*. We observe that when one scale dominates (high subnet usage) few regulatory devices on the smaller scale are found (low feed-forward loop occurrence).

5. Conclusion

With this methodological review I wanted to describe possible strategies for addressing two major challenges of systems biology: understanding the spatiotemporal organization of systems and interpreting dynamical processes in networks. In particular I reviewed our contributions to a novel perspective on spatiotemporal pattern formation (the "predictability" of patterns from cell properties) and summarized our methods for analyzing high-throughput data derived from our theoretical understanding of dynamical processes on graphs.

Starting out with the current dialog between systems biology and plant science, I described, how an early case study of 'plant systems biology' already emphasized the need of addressing these two topics (spatiotemporal pattern formation and dynamics on graphs), which in fact turn out to be surprisingly deeply linked – by interpreting dynamics on graphs as 'patterns'.

In our network-guided interpretations of high-throughput data, metabolic models are an ideal mathematical framework for predicting the interplay between essentiality and environment. They also can serve as network models used to analyze gene expression levels.

The studies discussed in Section 4, from our perspective, provide reliable evidence that the abstraction of biological systems in terms of mathematical graphs has the capacity to explain features of large-scale, high-throughput data.

In our future work we want to understand the network properties behind medium-dependent essentiality and reverse-engineering cellular objective functions and likely cellular environments from the consistency of gene expression patterns with predicted metabolic fluxes. Regarding classical spatiotemporal patterns, we want to establish universal links between

regulatory mechanisms and the predictability of patterns. In this way we want to contribute to the general theory of spatiotemporal patterns, linking patterns to regulatory devices.
Systems biology can over the next years become an important partner discipline of plant science, contributing to an understanding of, e.g., metabolic strategies and resource allocation to growth and defense in plants. Some of the methods summarized here may help providing a basis for this interdisciplinary endeavor.

Acknowledgments

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Auf die „Grenzen des Wachstums“ hatte bereits 1972 der *Club of Rome* aufmerksam gemacht. Seitdem sind die gesellschaftlichen Dimensionen und Folgen eines ungebremsten wirtschaftlichen Wachstums national und international ein zentrales Thema öffentlicher Diskussionen, aber auch politischen Handelns. Auf der Gaterslebener Begegnung 2011 wurde dem quantitativen Parameter „Wachstum“ das qualitative Pendant „Reifung“ zur Seite gestellt und die Thematik in zwei Problemkomplexen „Wachstum in der unbelebten und belebten Natur“ und „Quantitatives und qualitatives Wachstum in der Gesellschaft“ behandelt. Am Anfang stehen „Kritische Anfragen“ an Wissenschaftler, gefolgt von einer sozialwissenschaftlich orientierten Darstellung zu „Wachstum und Nachhaltigkeit“. Die an Natur- und Geisteswissenschaftler, Publizisten, politisch Verantwortliche und interessierte Laien gleichermaßen gerichteten Beiträge umfassen Wachstum und Reifung als grundlegende Eigenschaften organismischen Lebens, aber auch in der Bevölkerungsentwicklung, in der Ökonomie („Wachstum in einer globalisierten Welt“, „Wirtschaftswachstum und Klimawandel“) und sogar im Weltall einerseits sowie in Literatur und Kunst andererseits.

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Exploration and Exploitation Strategies of Ectomycorrhizal Fungi

Reinhard Agerer (München)

With 6 Figures and 2 Tables

Abstract

Ectomycorrhizae can differ tremendously in their construction, particularly regarding amount, organization, and range of their extramatrical mycelium that extends into the soil. Based on its features we have been able to distinguish so-called exploration types that indicate the different capabilities of ectomycorrhizae to occupy and explore the soil. The exploration of the soil by the mycelia is functionally connected to soil exploitation through uptake and transport of water and nutrients, the basics needed for tree growth. Here, with a few examples the importance of the space area occupied by ectomycorrhizae is shown and it is focused on some features that point out the putative explorative and exploitative function.

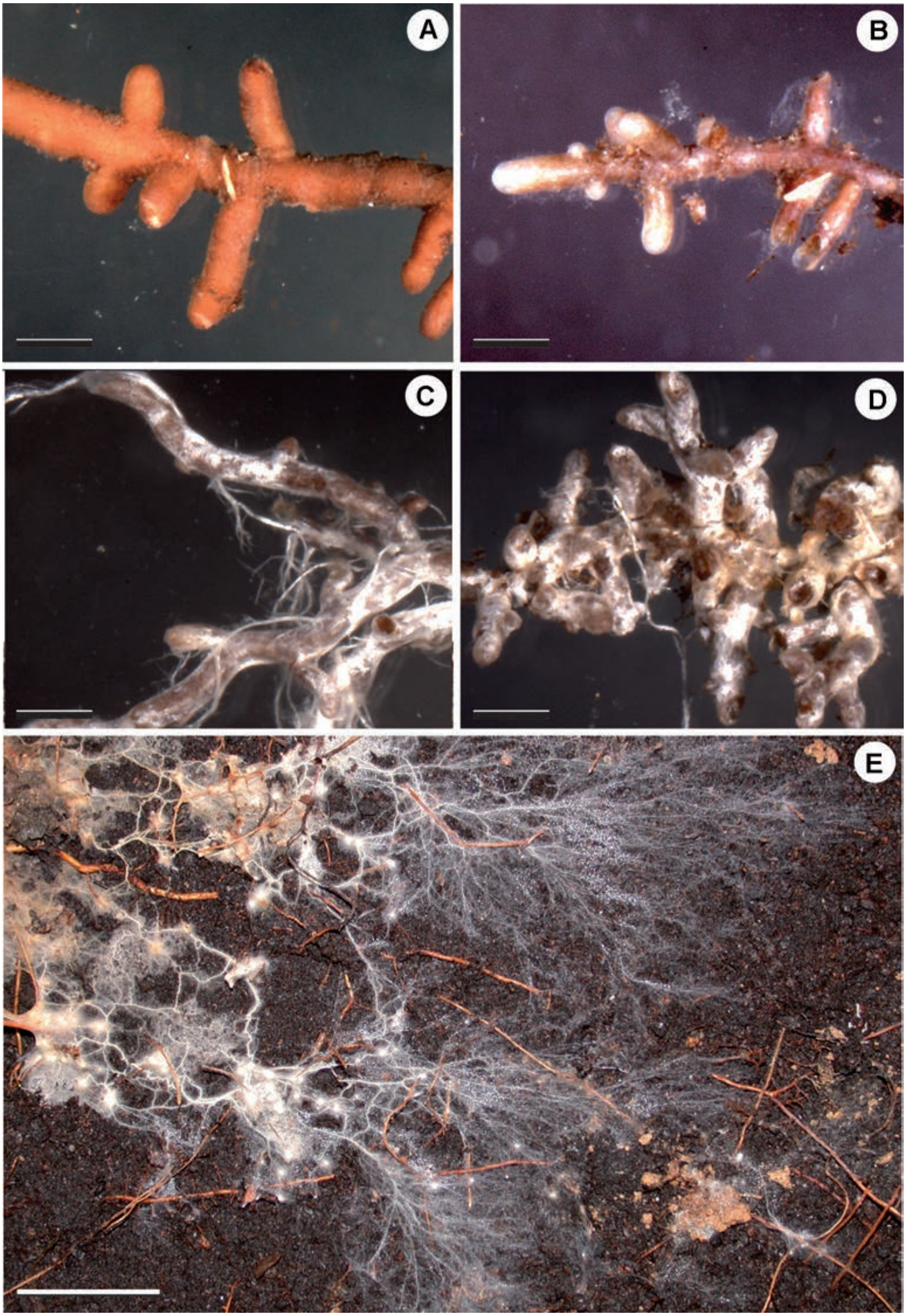
Zusammenfassung

Ektomykorrhizen können sich hinsichtlich ihres Baus enorm unterscheiden. Dies betrifft besonders Menge, Organisation und Reichweite ihres in den Boden hineinreichenden extramatrikalen Mycels. Auf diesen Merkmalen gegründet, konnten wir sogenannte Explorationstypen unterscheiden, die unterschiedliche Fähigkeiten der Ektomykorrhizen widerspiegeln, den Bodenraum zu besetzen und ihn zu erkunden. Die Erkundung des Bodens durch das Mycel ist funktionell mit der Ausbeutung des Bodens durch Aufnahme und Transport von Wasser und Nährstoffen verbunden. Diese Eigenschaften bilden damit eine Grundlage für das Baumwachstum. Im Folgenden zeigen wir mit einigen Beispielen die hohe Bedeutung des durch Ektomykorrhizen besetzten Bodenraumes und konzentrieren uns auf ein paar Merkmale, die ihre erkundende und potentiell ausbeutende Funktion zeigen.

1. Introduction

Ectomycorrhizae (ECM) are the main component of symbiotic organisms in temperate and boreal forests and responsible for tree growth (Smith and Read 2008). The crucial feature of ECM is the physical, but not physiological cut off of the root from the surrounding soil by the fungal tissue (Agerer 2009). A hyphal mantle covers the root and a net of hyphae, the so-called Hartig net, grows within the root cell walls (Smith and Read 2008). Frequently, hyphae are emanating from the mantle either solitarily or they are connected to often complex rhizomorphs (Agerer 1987–2008, 1993, Agerer and Rambold 2004–2011). Water and nutrients and sugars have to be transported hence through the extended fungal tissue (Smith and Read 2008). Mantle, emanating hyphae and rhizomorphs are the means to explore the substrate (Agerer 2009). They are the components to occupy and exploit the soil (Agerer 2001, 2007).

Different types of connection to the soil environment can be revealed when ECM grown in natural soil are uncovered. Amount, range and organization of their extramatrical myce-


A
B
C
D
E

lium (EMM), the mycelium that emanates from the mantle, have been used to categorize the ECM due to their putative ecological role (Agerer 2001, 2007, 2009) into different exploration types (ETs). Smooth ECM without visible emanating hyphae, and contacting the soil almost exclusively by their mantle surface, are regarded as belonging to the *Contact ET* (Fig. 1*A*); if they possess a remarkable hyphal envelope of solitary hyphae they are assigned to the *Short Distance ET* (Fig. 1*B*). Many ECM form extended rhizomorphs in addition to emanating hyphae and grow to a wider range into the soil; they are designated as *Medium Distance ET*. This ET has been further divided into three subgroups with respect to the shape of the rhizomorph margin and the relation to water: the fringe ($\mathrm{MD_f}$-ET, Fig. 1*C*), mat ($\mathrm{MD_m}$-ET), and smooth ($\mathrm{MD_s}$-ET) subtype (Agerer 2001, 2007). *Long Distance ET* (Fig. 1*D*) ECM possess only few rhizomorphs, but they bridge a wide distance within the soil, often more than half a meter (Schramm 1966). They are internally highly differentiated (Agerer 1991a, Agerer and Rambold 2004–2011) to facilitate transport (Duddridge et al. 1980, Kammerbauer et al. 1989). Not uncommonly, different ETs are naturally associated (Agerer et al. 2002, 2013).

An additional functionally important feature regards the relation of the ECM to water (Unestam 1991, Unestam and Sun 1995). C- and SD-ETs are mostly hydrophilic (Fig. 1*A*, *B*), whereas ECM of the $\mathrm{MD_m}$-, $\mathrm{MD_f}$- (Fig. 1*C*), and LD-ET (Fig. 1*D*) are hydrophobic (Agerer 2007). The latter two ETs enclose, due to this character, air between their hyphae preventing easy water contact at their mantle and rhizomorph surface. Although the mantle and the proximal areas of the EMM are hydrophobic, the distal portions of the hyphae and of the rhizomorphs are consistently hydrophilic (Raidl 1997) to allow there easy contact with soil particles, distinctly glued onto the hyphal surface, enabling uptake of water and dissolved nutrients. From an ontogenetical point of view even hydrophobic ECM, being over a large area hydrophobic in later stages, have in earlier ontogenetical phases their hydrophilic hyphal front rather close to the mantle surface (Fig. 1*E*). This is due to the continuous growth of the mycelial front away from the mantle into the substrate. Thus the exploring and exploiting hydrophilic front shifts to the periphery, after having had the opportunity to exploit the more proximal areas, i.e. closer to the ECM mantle.

For estimating the density and range of the EMM of different ETs, Agerer and Raidl (2004) established a method to quantify, based on synthesized ECM, the cover of the dark background of the peat substrate by light colored EMM (compare Fig. 1*E*). For estimating the mycelial density, areas between defined distances from the ECM mantle have been chosen (Tab. 1). Series of photographs and image analyses revealed an ET-specific density and range of the mycelium of the SD-ET and the $\mathrm{MD_f}$-ET. These results had been supported by

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Fig. 1 Exploration types of ECM: (*A*) Contact ET, hydrophilic (*Tomentella* sp.); emanating hyphae and rhizomorphs lacking. – (*B*) Short Distance ET, hydrophilic (*Tylospora* sp.); emanating hyphae as a rather dense envelope, rhizomorphs lacking. – (*C*) Medium Distance ET, fringe subtype, hydrophobic (*Cortinarius* sp.); many rhizomorphs and emanating hyphae, rhizomorphs with hairy margin. – (*D*) Long Distance ET, hydrophobic (*Xerocomus* sp.); emanating hyphae lacking, rhizomorphs restricted in number (here only one) growing a considerable distance into the soil. – (*E*) Long Distance ET, hydrophobic (*Rhizopogon roseolus*); synthesized ECM on *Pinus silvestris* on peat substrate; rhizomorphs extend from dense aggregations of ECM to form at their periphery fans of solitary hyphae being their hydophilic whereas more proximal portions and the ECM present hydrophoby. (bars *A*–*C* = 1 mm, *E* = 10 mm; *A*: RA15255; *B*: RA15239; *C*: RA15264; *D*: RA15268; *A*–*D*: from Nationalpark Bayerischer Wald, Germany, leg. 18. 8. 2011; *E*: SR1146, photo S. Raidl, with permission).

further studies and been complemented for the LD-ET (Weigt et al. 2011b). The density of the EMM of the SD-ET decreases quickly with increasing distance from the mantle, showing approximately 20 % cover of the black background at a distance between 4 and 6 mm and could not be detected further away than 12 mm from the ECM surface (Tab. 1); the MD_f-ET EMM declines less rapidly and reaches about 20 % cover of the black background in an area between 9 and 12 mm (Tab. 1); whereas the LD-ET mycelium density diminishes even more rapidly than that of the SD-ET at an area up to 1 mm distance, but had an almost constant density until the last analyzed distance of up to 96 mm (Tab. 1) (Weigt et al. 2011b). Although these data originated from synthesized ECM not exposed to saprotrophic and/or symbiotic fungal competitors, these data can be applied to reveal at least the potential influence of the ECM on the surrounding soil and their possible exploration and exploitation capacity by averaging the density and range of the EMM around the ECM. These data could be successfully used to compare Norway spruce fumigated with ozone or carbon dioxide with their controls, regarding the potential space occupancy of ECM (Weigt et al. 2011b, Agerer et al. 2013).

The range and density properties of the mycelia will be applied in the following to micromaps (McMps) of ECM that had earlier been drawn (Agerer et al. 2002) and that depict ECM communities in their natural distribution, quantity, dimensions and species affiliation in an area of 50 × 50 mm of an approximately 5 mm thick natural Of-layer of a mature *Picea abies* stand. Agerer et al. (2013) highlight the potential space occupancy and competition scenario of a *Picea abies* ECM community (McMp 0002) consisting of the C-ET *Russula ochroleuca* (Pers.) Fr., the SD-ET *Tylospora fibrillosa* (Bondord.) Donk, the MD_s-ET *Piceirhiza internicrassihyphis* (Agerer 2002), and the MD_f-ET *Cortinarius obtusus* (Fr.) Fr. Three further differently complex ECM communities will be evaluated in the following with respect to potential space occupancy and in addition regarding their potential space exploitation with respect to phosphate (PO_4), organically bound nitrogen (N_{org}), ammonium (NH_4), and nitrate (NO_3).

2. Material and Method

The McMps have been obtained as described and published by Agerer et al. (2002) under the designations McMp 0032, McMp 0043, and McMp 0050. For depicting the potential space occupancy of the different ECM species, the data published by Weigt et al. (2011b) are used in the following manner (Tab. 1). C-ET ECM are regarded as having very limited space occupancy due to the apparently lacking EMM, and therefore, the space they occupy is only represented by the mantle surface and no remarkable space is covered beyond the ECM itself. For the SD-ET, MD_f-ET, and LD-ET, only 12 mm, 19 mm, and 29 mm, respectively, beyond the mantle surface are depicted. This of course is a considerable under-representation of the range of the EMM of the LD-ET (see above, and Tab. 1). But, for better comparison reasons of the different strategies in one and the same small area of 25 cm² this was an appropriate distance for the Long Distance ET.

For depicting the potential space occupancy area and contemporarily the different mycelial densities the following procedure was chosen (Tab. 1). Applying photoshop 6.0 (Adobe), the envisaged distances expressed in pixels (1 mm = 25 px) around the mycorrhizal surface, as shown in Table 1, were obtained by the system's enlargement function, and then the proximal area towards the ECM was filled by the color of the treated ECM in the percent density as indicated in Table 1 (“Operation for designing …”). For instance, for the SD-ET, a line of 25 px (= 1 mm) was drawn around the ECM and its proximal area was filled with the color density of 20 % (photoshop feature). The next step increased the distance again by 25 px (= 1 mm), and the whole proximal area (0–50 px = 0–2 mm) was filled with 5 % color density. After the last enlargement of 75 px, color of 5 % density was added again, finally all zones between 0 and 12 mm revealed a distance-dependent color density as shown in Table 1 (“Approximal mycelial cover …”), indicating the potential mycelial range and cover density around the exploration type in focus. This was repeated for all ECM of the same ET, using always the species-specific colors as indicated in the McMps. If more ETs were present in the McMps, all of them were treated in the same way, considering the ET-specific range and densities (Tab. 1). C-ET were left untreated as, by definition, their mycelial influence is almost completely restricted to the ECM surface.

Tab. 1 Distance-related mycelial density of three different exploration types according to Weigt et al. (2011b)

Distance from mantle surface according to Weigt et al. (2011b)										
0–1 mm	1–2 mm	2–4 mm	4–6 mm	6–9 mm	9–12 mm	12–15 mm	15–19 mm	19–24 mm	24–29 mm	29–35 mm
Distances as represented by pixel (25 px equals 1 mm)										
25 px	+25 px	+50 px	+50 px	+75 px	+75 px	+75 px	+100 px	+125 px	+125 px	+150 px
Short Distance ET										
Approximal mycelial cover according to Weigt et al. (2011b)										
50 %	30 %	25 %	20 %	10 %	5 %[1]					
Operation for designing the Figs. (addition of color density at the actual distance towards mantle surface)										
20 %	+5 %	+5 %	10 %	5 %	+5 %[1]					
Medium Distance (fringe) ET										
Approximal mycelial cover according to Weigt et al. (2011b)										
75 %	55 %	40 %	35 %	30 %	20 %	10 %	5 %[1]			
Operation for designing the Figs. (addition of color density at the actual distance towards mantle surface)										
20 %	+15 %	+5 %	+5 %	+10 %	+10 %	+5 %	+5 %[1]			
Long Distance ET										
Approximal mycelial cover according to Weigt et al. (2011b)										
35 %	25 %	25 %	20 %	20 %	20 %	20 %	20 %	20 %	20 %[2]	
Operation for designing the Figs. (addition of color density at the actual distance towards mantle surface)										
10 %	+0 %	+5 %	+0 %	+0 %	+0 %	+0 %	+0 %	+0 %	+20 %[2]	

[1] The whole range as depicted by Weigt et al. (2011b).

[2] Only to this distance the mycelial cover has been depicted in the figs. although the mycelium could be detected up to 96 mm distant from the mantle. This restriction was applied to clearly arrange the different exploration types in one and the same fig. and due to the fact that in early ontogenetical stages the mycelia are more evenly distributed up to this distance than in later stages.

To present the potential zone of space exploitation, three different issues had to be regarded. First, the mycelial cover is composed by individual hyphae with their special nutrient depleting influence around their surface. It was therefore important to calculate the mean distance between the hyphae of the potentially occupied area. Weigt et al. (2011a, b) calculated for the MD_f-ET an average hyphal distance of < 0.05 mm. It was suggested that this applies also for the SD-ET and for the LD-ET at least for younger ontogenetical stages (Agerer et al. 2013). Second, the influence of the nutrient uptaking surface is dependent upon the addressed nutrient. The depletion area, the zone where a surface can take up more rapidly nutrients than can dissolve in more distant regions and diffuse into this zone again, differs with PO_4, NH_4, NO_3, and N_{org}, approximately with 0.2 mm (Nye and Tinker 1977), 1–2 mm (Chapin et al. 2002), 6–10 mm (Chapin et al. 2002), and 0.002 mm, respectively (Agerer et al. 2013). The latter width was concluded from Liese (1964, 1970) and Schmid and Liese (1964), showing an approximately 2 µm erosion zone around wood saprotrophic hyphae. Figures 4–6 have therefore been constructed with depletion zones for PO_4, NH_4, NO_3, and N_{org} with 0.2, 2, 10, and 0.002 mm, respectively. With the exception of the latter measure, the potential space exploitation area, e.g. of 0.2 mm regarding PO_4, meets very likely every point between the hyphae revealing a mean distance of 0.05 mm. Therefore, the complete space occupied by the mycelium disregarding the space between the hyphae can be considered as the potential area of space exploitation for PO_4, NH_4, and NO_3. Third, the uptake capability differs between hydrophobic and hydrophilic ECM. It can be concluded that hydrophilic ECM and their EMM can contact water with dissolved nutrients over their whole surface. Hydrophobic ECM have this capability only at the periphery of the EMM and lack it in their more proximal hydrophobic portions (Raidl 1997). Therefore, during ontogeny of hydrophobic ECM, the hydrophilic periphery of the EMM migrates continuously away from the ECM surface, indicating a possibly remarkable influence of the ontogenetical stage on nutrient exploitation (Fig. 2*A–D*). As it is not possible in the figures to consider in addition the influence of ontogeny, the potential space exploitation will refer to the maximal space occupancy only, as indicated in Table 1. For construction of the potential space exploitation areas compare Table 2.

The present study tries to offer a method to fill the gap that Erland and Taylor (2002) pointed out: “[…] no studies have examined the microspatial distribution of individual ECM fungal species in relation to the chemico-physical soil environment […]”

3. Results

3.1 Potential Space Occupancy and Potential Space Exploration

The three McMps (Fig. 3) were selected because of their great variability regarding abundance and distribution of ECM and with respect to their species composition. McMp 0050 (Fig. 3*A*) reveals a very high density of predominantly ECM of *Lactarius decipiens*, intermixed with only three tips of *Russula ochroleuca*. All belong to the C-ET and are hydrophilic (Fig. 3*B*). The space occupancy is actually only accomplished by the ECM proper (i.e. root tip enveloped by the mantle). Their density, total length and frequent ramifications (Fig. 3*B*) indicate that apparently a rather large portion of the soil is occupied, although no EMM is evident, as is typical for the C-ET. Three different ECM are shown in McMp 0032 (Fig. 3*C*). The MD_f-ETs *Piceirhiza cinnbadiosimilis* and *Dermocybe cinnamomea* are rather distant from

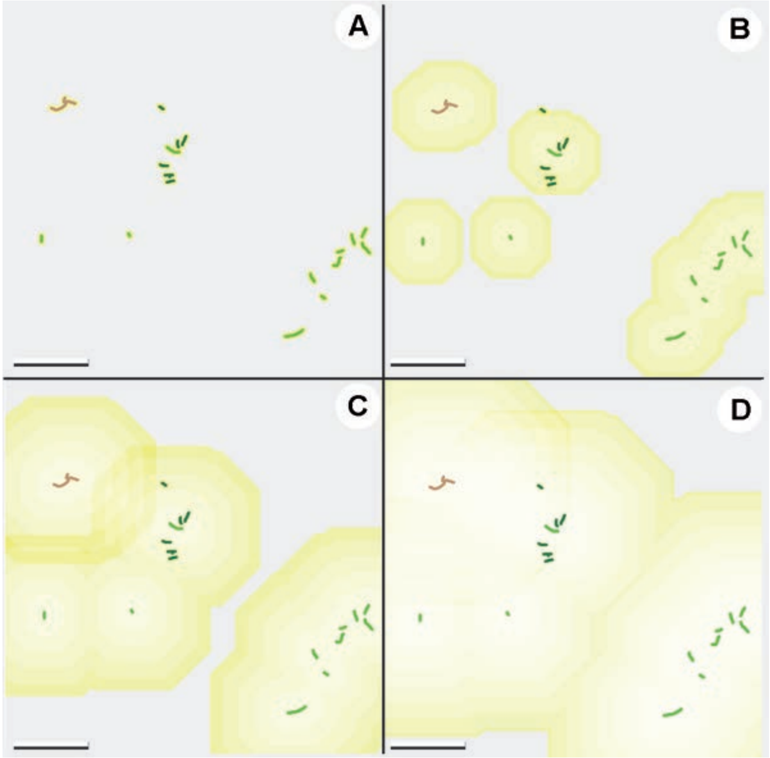

Fig. 2 Mycelial ontogeny (from *A* to *D*) of two different MS_f-ET (ochre: *Piceirhiza cinnbadiosimilis*; light green: *Dermocybe cinnamomea*) indicating the migration of the hydrophilic zone of the EMM during aging. – Dark yellow = hydrophilic front; light yellow to white = increasingly hydrophobic area proximally. The originally almost completely hydrophilic area around the ECM (*A*, *B*) becomes later (*C*, *D*) hydrophobic. (bar = 10 mm; McMp 0032, adapted from Agerer et al. 2002)

another, the C-ET *Russula ochroleuca* lays just in between (Fig. 3*C*). Only the C-ET is hydrophilic, the others are hydrophobic (Fig. 3*D*). Although *P. cinnbadiosimilis* consists only of two tips it potentially covers with its mycelium a large area, surpassing the space occupancy of the C-ET considerably. *Dermocybe cinnamomea* with many more ECM, potentially occupies an even larger area. Particularly the latter species claims the same area as *R. ochroleuca*. Although the density of the EMM of both MD_f -ETs decreases with increasing distance from the ECM proper, their periphery extends considerably. Both MD_f-ETs potentially occupy together almost the complete area (Fig. 3*D*). The third example comprises four different ECM. *Xerocomus* cf. *chrysenteron*, a hydrophobic LD-ET is represented exclusively by one ECM with a short side branch. *Tylospora fibrillosa*, a SD-ET, reveals a single tip and is hydrophilic. The *Lactarius decipiens* and *Russula ochroleuca* ECM are both of the C-ET and hydrophilic (Fig. 3*E*, *F*). Both, *Xerocomus* cf. *chrysenteron* and *Tylospora fibrillosa*, occupy in this style of representation with their EMM rather large areas. A potential overlap is evident. Both the latter putatively grow their mycelia often at places where the C-ET ECM are located, whereas the two different C-ETs are apparently arranged with little overlap. Their space occupancy is restricted to their immediate surroundings. Although *X.* cf. *chrysenteron* formed only one ECM with a short side branch, its potential space occupancy by its apparently not very dense mycelium comprises almost the complete area, even at places were the SD-ET und the C-ET are located (Fig. 3*F*).

3.2 Potential Space Exploitation

The three delineated ECM communities differ greatly regarding their potential impact on PO_4 exploitation (Fig. 4*A*–*C*). The depletion zone of PO_4 around the C-ET *Lactarius decipiens* and *Russula ochroleuca* is restricted to their immediate neighborhood (Fig. 4*A*–*C*). McMp0050 comprises only C-ETs (Fig. 4*A*), and, therefore, abundance, distribution and total lengths of the ECM mirror the area of influence. In contrast, in McMp 0032 almost the whole potential exploitation space is occupied by the mycelium of the two MD_f-ETs (Fig. 4*B*). The hydrophobic periphery of the EMM is, dependent upon the depicted ontogenetical stage, already rather distant from the ECM proper. The more proximal hydrophobic areas of the *P. cinnbadiosimilis* and *D. cinnamomea* mycelium appear at places where the C-ET *R. ochroleuca* resides (Fig. 4*B*). Both, the potential uptake area of the SD-ET *Tylospora fibrillosa* and of the C-ETs *L. decipiens* and *R. ochroleuca* are laying within the area of influence of the EMM of *X.* cf. *chrysenteron* (Fig. 4*C*). It depends upon the sequence of the depicted levels as offered by photoshop whether the potential space exploitation areas of *T. fibrillosa* and that of the C-ETs or that of *X.* cf. *chrysenteron* appear predominant (compare discussion and Fig. 6*A*, *B*).

As the potential area of influence on NH_4 of the SD-, MD_f- and LD-ETs is identical with the expansion of the mycelium (Tab. 2), as shown already above for PO_4, a stronger influence on the potential NH_4 exploitation in comparison to PO_4 owns to C-ETs. McMp 0050 indicates that already the ECM of *L. decipiens* and *R. ochroleuca* would be sufficient to potentially exploit the whole area. Only a few free spaces remain. The zone of influence of *R. ochroleuca* partially intersects with that of *L. decipiens* but lays mostly exactly within. As indicated in Figures 4*E* and *F*, the influential zone of the C-ETs reside mostly within that of the MD_f-ET (Fig. 4*E*) and LD-ET (Fig. 4*F*). Only in a very restricted region one *L. decipiens* ECM increases the potential uptake area for NH_4 of this ECM community (Fig. 4*F*).

With increasing depletion zone dimensions, from 2 mm for NH_4 to 10 mm for NO_3, the exploitive area of the C-ETs becomes almost dominant in comparison to that of those ETs with more or less extended EMM. Figure 5*A* shows for the community in McMp 0050 that the whole area can be potentially exploited already by the ECM of *L. decipiens*. Those of *R. ochroleuca* reside within the latter. This is not so evident in Figure 5*B* and *C*. But this is exclusively due to the sequence of the depicted levels (compare discussion and Fig. 6*C*, *D*). Below the area occupied by the MD_f -ET (Fig. 5*E*) and LD-ET (Fig. 5*F*), the whole area owns also to *L. decipiens* (Fig. 6*C*, *D*).

The depletion zone for N_{org} can be neglected in comparison to the diameter of the ECM that reveals about 0.3 mm. The potential N_{org} depletion is even considerably less than that for PO_4 (Tab. 2). C-ETs residing within the mycelium of SD-, MD_f -, and LD-ETs (Fig. 5*E*, *F*) have only 2 µm around the ECM for N_{org} exploitation, but their depletion zone does not completely overlap with that of those ECM with extended mycelium, even if the EMM covers the same area, since the hyphal mean distance of approximately 50 µm leave in between

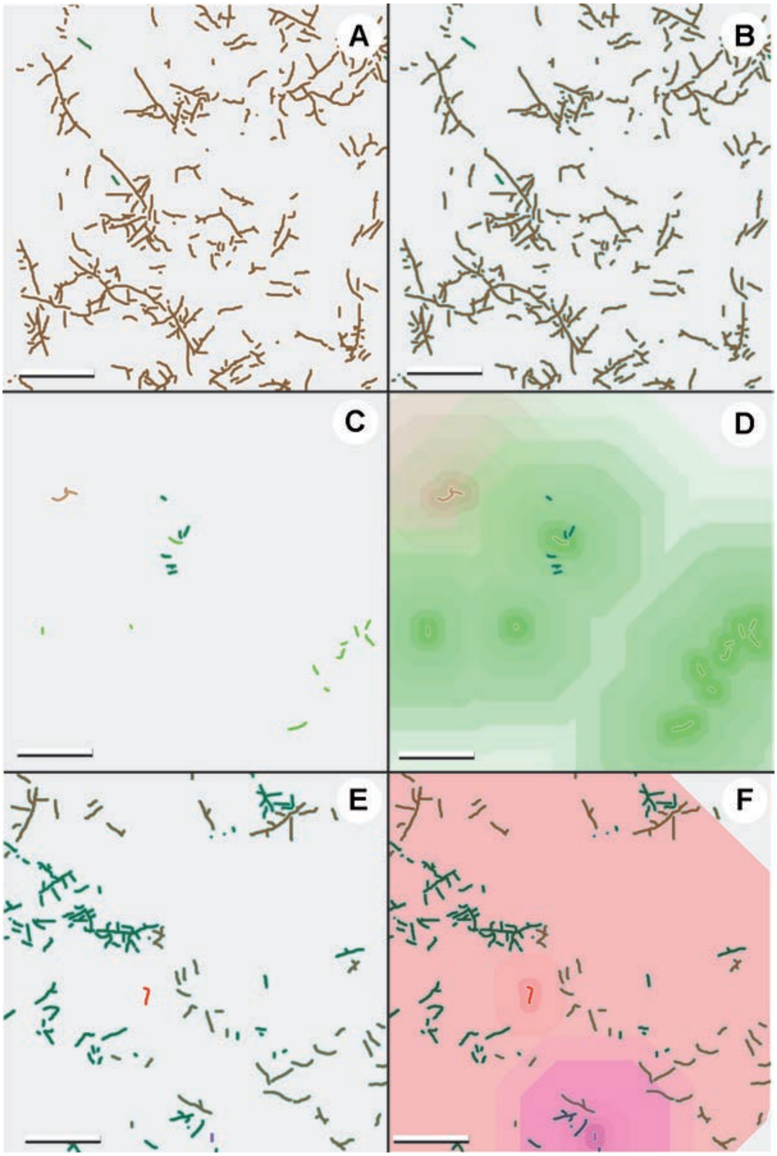

Fig. 3 Natural distribution, dimensions, and diversity of ECM (*A*, *C*, *E*), their potential space occupancy and hydrophoby properties (*B*, *D*, *F*; white outline of the ECM for hydrophoby, bluish outline for hydrophily). Dark green: *Russula ochroleuca* (C-ET, hydrophilic); light green: *Dermocybe cinnamomea* (MD_f-ET, hydrophobic); ochre: *Piceirhiza cinnbadiosimilis* (MD_f-ET, hydrophobic); brown: *Lactarius decipiens* (C-ET, hydrophilic); pink: *Tylospora fibrillosa* (SD-ET, hydrophilic); red: *Xerocomus* cf. *chrysenteron*. (LD-ET, hydrophobic). (*A–B*: McMp 0050; *C–D*: McMp0032; *E–F*: McMp0043; bar = 10 mm; adapted from Agerer et al. 2002)

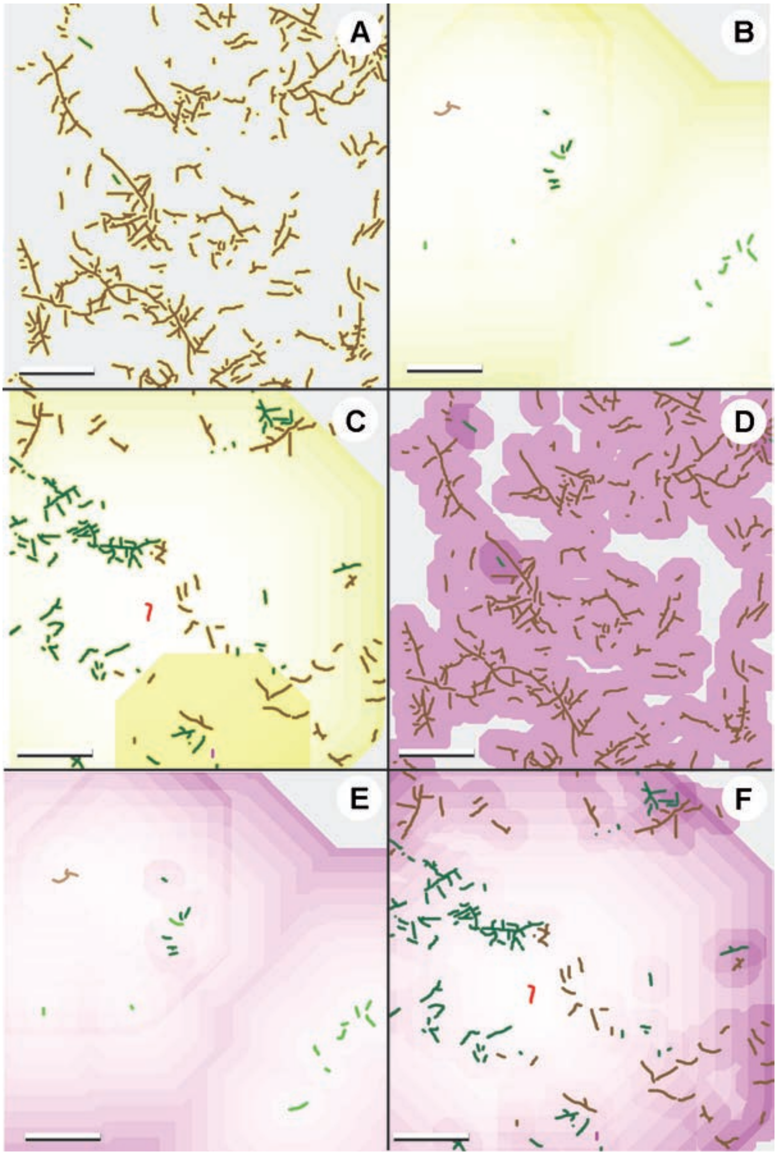

Fig. 4 Potential space exploitation of ECM communities. *A–C*: PO_4 (yellow); *D–F*: NH_4 (magenta). Dark green: *Russula ochroleuca* (C-ET, hydrophilic); light green: *Dermocybe cinnamomea* (MD_f-ET, hydrophobic); ochre: *Piceirhiza cinnbadiosimilis* (MD_f-ET, hydrophobic); brown: *Lactarius decipiens* (C-ET, hydrophilic); pink: *Tylospora fibrillosa* (SD-ET, hydrophilic); red: *Xerocomus* cf. *chrysenteron*. (LD-ET, hydrophobic). (*A*, *D*: McMp 0050; *B*, *E*: McMp0032; *C*, *F*: McMp0043; bar = 10 mm; adapted from Agerer et al. 2002)

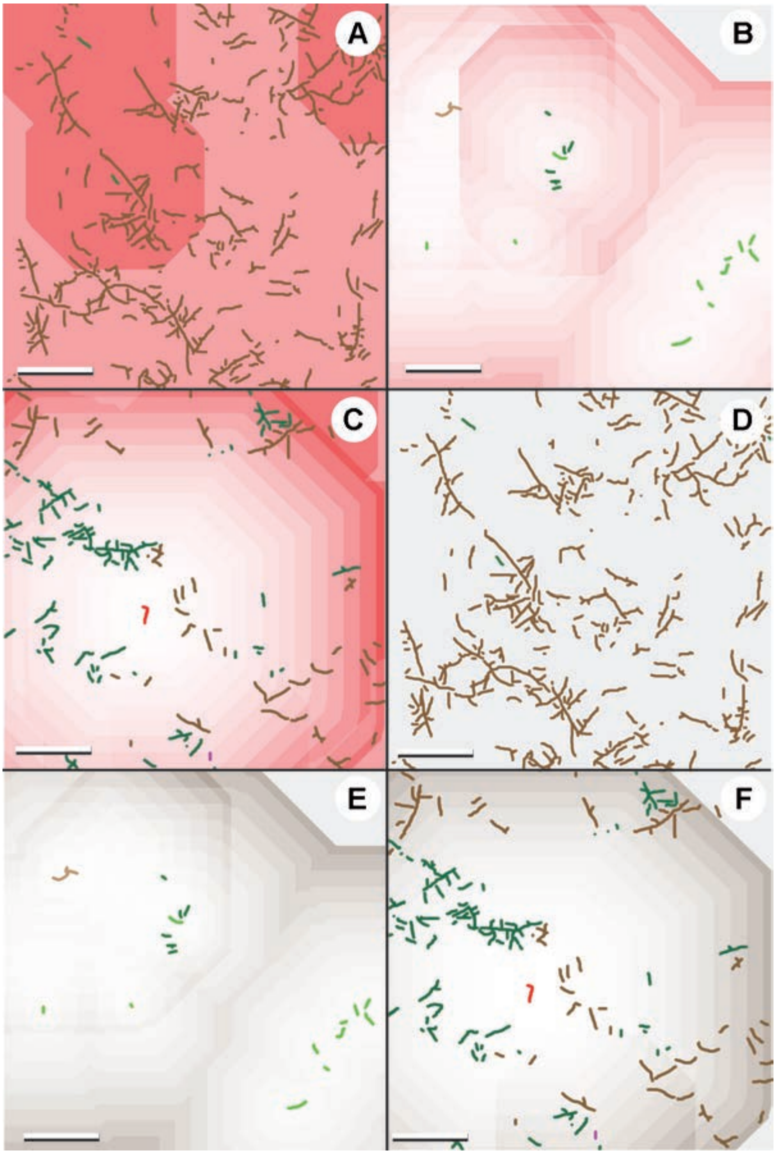

Fig. 5 Potential space exploitation of ECM communities. *A–C*: NO_3 (RGB red); *D–F*: N_{org} (dark brown, warm). Dark green: *Russula ochroleuca* (C-ET, hydrophilic); light green: *Dermocybe cinnamomea* (MD_f-ET, hydrophobic); ochre: *Piceirhiza cinnbadiosimilis* (MD_f-ET, hydrophobic); brown: *Lactarius decipiens* (C-ET, hydrophilic); pink: *Tylospora fibrillosa* (SD-ET, hydrophilic); red: *Xerocomus* cf. *chrysenteron*. (LD-ET, hydrophobic). (*A*, *D*: McMp 0050; *B*, *E*: McMp0032; *C*, *F*: McMp0043; bar = 10 mm; adapted from Agerer et al. 2002)

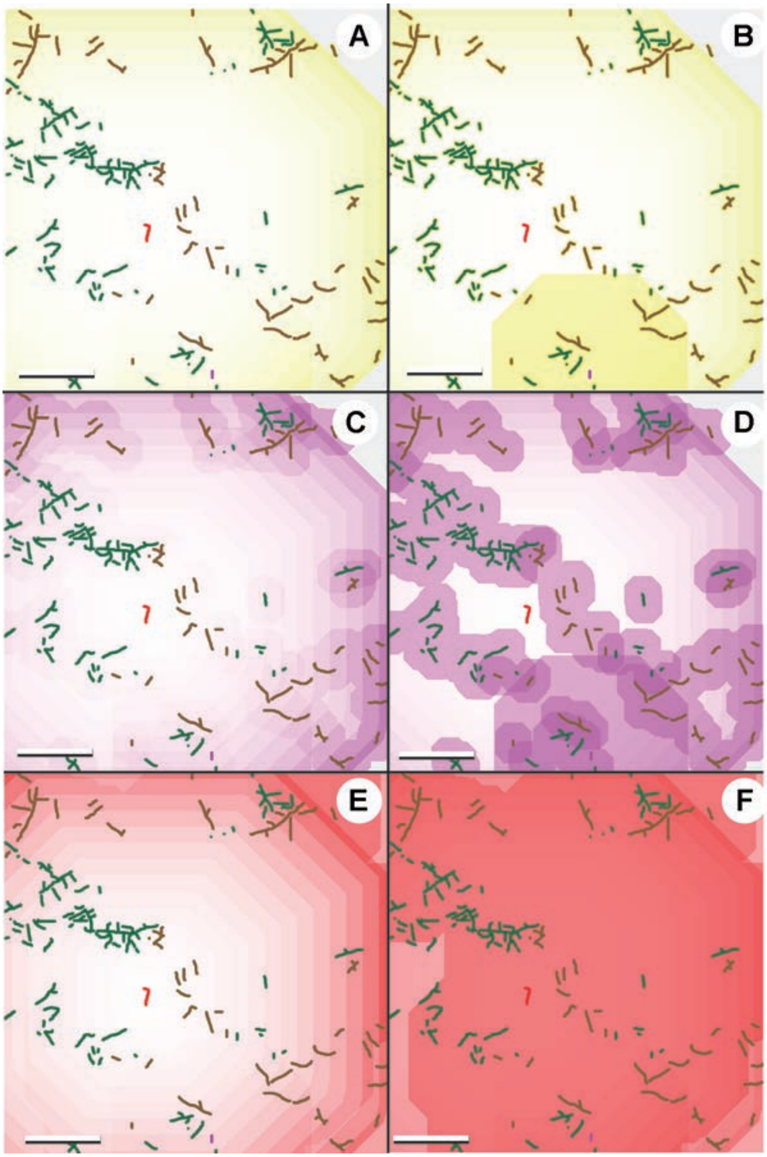

Fig. 6 Potential space exploitation areas of one diverse ECM community as arranged in two different manners. *A*, *C*, *E*: *Xerocomus* cf. *chrysenteron* arranged at upper level: *B*, *D*, *F*: *X.* cf. *chrysenteron* arranged at lower level. *A–B*: PO_4 (yellow); *C–D*: NH_4 (magenta); *E–F* : NO_3 (RGB red). Dark green: *Russula ochroleuca* (C-ET, hydrophilic); brown: *Lactarius decipiens* (C-ET, hydrophilic); pink: *Tylospora fibrillosa* (SD-ET, hydrophilic); red: *X.* cf. *chrysenteron* (LD-ET, hydrophobic). (*A–F*: McMp0043; bar = 10 mm; adapted from Agerer et al. 2002)

46 µm potentially unexploited. Figure 5*E*, *F* dramatically overestimate therefore the potential exploitation area when only the general space occupancy of the EMM as a whole – as it was justified for the other nutrients – is taken into consideration (Tab. 2). The rather limited individual depletion zones around the hyphae have to be in focus.

Tab. 2 Basic information on the method to depict the potential space exploitation areas (depletion zones) around the ECM regarding PO_4, NH_4, NO_3, N_{org}, in dependence of exploration types (ET) and their hydrophilic (hi) or hydrophobic (ho) properties.

Nutrient	Nutrient color in fig.	Color density (%)	ET/ hydrophilic, hydrophobic[1]	EMM maximal extension (px/mm)	Depletion zone around ECM (px/mm)
PO_4	yellow	30	C-ET/hi	0/0	5/**0.2**
			SD-ET/hi	300/12	300/12
			MDf-ET/ho	475/19	475/19
			LD-ET/ho	725/29	725/29
NH_4	magenta	30	C-ET/hi	0/0	50/**2**
			SD-ET/hi	300/12	300/12
			MDf-ET/ho	475/19	475/19
			LD-ET/ho	725/29	725/29
NO_3	RGB red	30	C-ET/hi	0/0	250/**10**
			SD-ET/hi	300/12	300/12[2]
			MDf-ET/ho	475/19	475/19[2]
			LD-ET/ho	725/29	725/29[2]
N_{org}	dark brown warm	30	C-ET/hi	0/0	0/**0.002**[3]
			SD-ET/hi	300/12	300/12[4]
			MDf-ET/ho	475/19	475/19[4]
			LD-ET/ho	725/29	725/29[4]

[1] The nutrient color got an overlay by white of 25 % density towards ECM of all zones (including previous ones) obtained in 50px steps until maximal extension of the EMM, with the last zone remaining free of an overlay. This is performed only for hydrophobic ECM (MD_f-ET and LD-ET), indicating the increasing hydrophobicity of the EMM towards the mantle.

[2] As also the hyphal tips should have a distal depletion zone of 10 mm, the actual influence possibly extends beyond these estimated and applied distances.

[3] The depletion zone of 2 µm for N_{org} cannot be depicted.

[4] This is a dramatic overestimation due to the mean distance of appr. 50 µm between the individual hyphae. As 2 µm are only a fraction of the hyphal interspaces, the estimated 46 µm between the hyphae remain uninfluenced by N_{org} degrading enzymes. Therefore the exploitation area is rather similar to the hyphal dimensions. But this cannot be depicted in the presented figures.

4. Discussion

The importance of EMM, and particularly of rhizomorphs, for uptake and transport of water and nutrients has been repeatedly pointed out since the first clear statement regarding its paramount ecological role (Read 1992, Leake et al. 2004, Smith and Read 1997, 2008). The amount of nutrients removed from the soil by the ECM is species dependent (Agerer and

Göttlein 2003, Bending and Read 1995, Perez-Moreno and Read 2000) and correlates to the hyphae occupying the substrate (Ekblad et al. 1995, Rosling and Rodenstock 2008, Rousseau et al. 1994, Wallander et al. 2003), with hyphal length being a significant factor at least for phosphate nutrition (Ekblad et al. 1995, Rousseau et al. 1994). In addition, ECM deficient of EMM have the capability of nutrient uptake, too (Harley and McCready 1952). A simple calculation may highlight the impact of EMM to increase surface area for uptake. Given an ECM is a cylinder of, e.g., 10 × 0.3 mm (Figs. 1*A–D*, 3*A*, *C*, *E*), the volume results to approximately 0.7 mm³ with a surface area of roughly 9.5 mm². This volume is equalled by 99 m hyphae of approximately 3 µm diameter (compare Agerer and Rambold 2004–2011), with a total surface area of ca. 930 mm². The roughly 100-fold surface area of the hyphae with the same volume as the ECM proper shows the significance of the EMM to increase nutrient acquiring surface areas.

Efficient transport of water and PO_4 through rhizomorphs underpin the crucial meaning of the soil mycelium (Duddridge et al. 1980, Kammerbauer et al. 1989) since rhizomorphs are able to develop to a length of many decimeters into the soil (Raidl 1997, Schramm 1966). Therefore, several attempts have been made to quantify and to locate the mycelium of ECM in natural soil (e.g. Genney et al. 2006, Gordon and Apple 2011, Hedh et al. 2008, Hortal et al. 2008, Wallander et al. 2001). Quantification of EMM of ECM is hence a target to better understanding functional ecology of ECM as it is also a strong sink for tree derived carbohydrates (Simard et al. 2002, Weigt et al. 2011a). Since ECM differ significantly in amount, elaboration, and range of the mycelium and its relation to water (Agerer 2001, 2006, Agerer and Rambold 2004–2011), a general, non-specific quantification of EMM in the soil is not enough for getting ecologically relevant data regarding nutrient uptake and carbon sinks of ECM communities. The actually presented method tries to evaluate the differences *via* ET-specific estimation of space occupancy, an indicator of potential space exploration and of potential space exploitation. The basis for this was, firstly, to accurately delineate ECM communities in natural soil with respect to their abundance, density, and species affiliation (Agerer et al. 2002) and, secondly, to quantify the EMM exploration type specific (Agerer 2001, 2007) according to density, distribution and range (Agerer and Raidl 2004, Weigt et al. 2011b). Here, it is tried to unite these types of analyses to gain ecologically and functionally relevant data.

Natural communities of ECM vary considerably in composition, species richness, abundance and density (Agerer et al. 2002, Baier et al. 2006, Di Marino et al. 2009, Horton and Bruns 2001) (Fig. 3*A*, *C*, *E*). The connection to the surrounding soil is often mediated by the mycelium that extends from the hyphal mantle or is simply operated by the mantle itself (Fig. *1A–D*). These features had been used to classify ECM into so-called exploration types indicating their putative ecological role (Agerer 2001, 2006, 2007). Therefore, space occupancy of the mycelium is of paramount importance for space exploitation, particularly for those nutrients that have a restricted mobility as known for organically bound nitrogen, ammonium and phosphate (Chapin et al. 2002, Nye and Tinker 1977).

Species- and community-dependent space occupancy strategies of ECM have been selected and provided in Figure 3*A*, *D*, *F*. Simple-structured communities consisting exclusively of hydrophilic, smooth ECM (C-ET) of one or a few species, have to be densely aggregated (Fig. 3*B*) to occupy as much space as ever possible, to increase the contact surface area with the soil by increasing their total length within a given space. This is especially important for the rather immobile nutrients N_{org}, PO_4, and NH_4, with depletion zones of 0.002, 0.2 and

2 mm width, respectively (Agerer et al. 2013, Chapin et al. 2002, Nye and Tinker 1977). This becomes evident when the depletion zones for these nutrients around the ECM of McMp 0050 are considered (Figs. 4*A*, *D*, 5*D*). But a considerably less density of these ECM is sufficient to completely explore and exploit the same substrate area for NO_3 (Fig. 5*A*). Nitrification has its optimum at soil pH > 5.5 (Scheffer and Schachtschabel 2010). Thus NO_3 with its high mobility and therefore with larger depletion zones around up-taking surfaces is often more abundant and more easily available in higher pH soils, whereas in lower pH soils the less mobile NH_4 bound nitrogen is rather dominant. Therefore, for NH_4 acquisition, smooth hydrophilic ECM, if present in a high amount and dense aggregation, could be of advantage in soils of low pH and also in thick organic layers rich in NH_4. Less densities, however, would be sufficient for NO_3 acquisition.

PO_4 and organically bound nitrogen are less mobile nutrients and an extension of the surface area by ECM is certainly of great advantage. The difference of potentially exploited areas of C-ET and those with extended EMM becomes hence for PO_4 much more significant than is the case with NH_4 and NO_3. The mycelium is able to influence the soil at any space between the hyphae, leaving at random due to the low distance between the hyphae (< 50 µm) and the 200 µm wide depletion zone of PO_4, putatively no space unexploited. In contrast, because of the very limited surface-dependent depletion area, the C-ETs do not contribute much to PO_4 acquisition. For N_{org}, the importance of the active surface area of smooth ECM (C-ET) might be in comparison to ECM with extended mycelium relatively high, as large areas between the randomly <50 µm distant hyphae remain scarcely exploited due to the very narrow 2 µm depletion zone for N_{org} (Agerer et al. 2013). Therefore, organically bound nitrogen will still be well available for smooth ECM even if they have been overgrown by active hydrophilic, mycelium. Under such conditions, a dense mixture of smooth ECM with those forming extended EMM would even be of great advantage for the mutualistic system and an invaluable complementation within the ECM community.

Admittedly, the presentations of the potential space occupancy provided here, proceed from a homogeneous distribution of the EMM around the ECM. But this is certainly not the case in nature, chiefly when older ontogenetical stages and mainly those of the LD-ET are considered (Fig. 1*E*). But a more homogeneous distribution can be suggested for earlier ontogenetical stages (Raidl 1997). This has been taken into account, when delineating the EMM of the LD-ET, only within the closest 29 mm of a total range of 96 mm. In spite of these assumptions, rhizomorphs and hyphal fans may make headway in different directions, what could be hypothesized for *X.* cf. *chrysenteron* in Figure 3D, indicating gaps between the C-ET colonies of *L. decipiens* and *R. ochroleuca*. As a consequence, it has to be kept in mind that, at least in later ontogenetical stages, the EMM might advance to positions not occupied yet by any competitor and, perhaps preferably within an inhomogeneous soil environment, into nutrient rich sites (Agerer et al. 2002, Agerer and Göttlein 2003). Nevertheless, this method of presentation enables to consider possible conflicts resulting from competition for space and nutrients in space and time. But the delineated potential space occupancy can only rely on average distributions and can exclusively refer to established ECM communities without a possibility to follow successions.

A partial or even a complete spatial overlapping may occur between EMM of different ETs and also with those having no evident EMM (Fig. 3*D*, *F*). This becomes especially obvious, when hyphal systems are followed in nature or under semi-natural conditions (Agerer 1991b, 2002, Wu et al. 1999). When an overlapping occurs a functionally important feature of the EMM is its relation to water. The SD-ET apparently holds the possibility to immediately contact water and nutrients through the whole mycelial system. In contrast, the continuously migrating hydrophilic periphery of the mycelium of the MD_f- and LD-ETs (Raidl 1997) forages for new nutrient rich sites, while the more proximal substrates could have already been impoverished (shown by the lighter and finally whitish colors in Figs. 4*B*, *C*, *E*, *F*, 5*B*, *C*, *E*, *F*). They remain impoverished unless new nutrients are dissolved. These newly available nutrients could be a second chance for the hydrophilic C- and SD-ETs after they having been previously overgrown by the hydrophilic front of the competitor's mycelium. Therefore, a beforehand competition between hydrophilic ECM and the hydrophilic mycelial front of hydrophobic ECM (Figs. 4*B*, *C*, *E*, *F*, 5*B*, *C*, *E*, *F*) might later be cured and further on avoided due to passed migration of the hydrophilic front. A previous overlap of this foraging EMM with C-ETs appears with respect to PO_4 in comparison to the situation for NH_4 and NO_3 in the point of view of ECM with extended hydrophobic EMM less dramatic, as the latter resources are more mobile and can be taken up in later ontogenetical phases from more distant and from larger areas. Particularly the catchment area for NO_3 extends with ca. 10 mm far beyond the ultimate mycelial front. Changes in competition attitudes are not only ontogenetically driven but also by time of ECM establishment. The question arises, who established first? This addresses Figure 6, when the superimposed potential space exploitation areas of the competitors are changed regarding their sequence. Figure 6*A*, *C*, *E* depict for PO_4, NH_4 and NO_3, respectively, the exploitation area of the hydrophobic *X.* cf. *chrysenteron* EMM at the upper (front) level with its proximally increasing hydrophoby, whereas in Figure 6*B*, *D*, *F* it appears in the background and the hydrophilic *L. decipiens*, *R. ochroleuca* and *T. fibrillosa* ECM are at the front. When the hydrophilic ECM establish first, they take advantage of their "early birth" and exploit the substrate likely leaving little for the migrating hydrophilic front of the generally hydrophobic *X.* cf. *chrysentron* and *vice versa*. Therefore, timing appears a relevant factor of ECM communities. The applied method, however, can neither unveil within a community the influence of a chronosequence nor of ECM ontogeny. But it becomes evident that abundance, proportion and competition of different exploration types within an ECM community can potentially depend on the nutrient distribution and availability (Agerer and Göttlein 2003) and might crucially influence nutrient acquisition and source sink relations of the mutualistic system (Weigt et al. 2011b).

Species- and possibly ET-specific abundance and distribution of ECM in a heterogeneous soil environment with respect to nutrient distribution is highly probable (Agerer and Göttlein 2003, Cavender-Bares et al. 2009, Kranabetter et al. 2009, Lilleskov et al. 2002). By studying soil micro-niches at distances of roughly 1.5 cm, Agerer and Göttlein (2003) found that the hydrophilic C-ET *Lactarius decipiens* was significantly positively correlated to NH_4, K, Na, Mg, and Fe+Mn concentrations, while negatively to the pH. In contrast, the hydrophobic MD_f-ET *Cortinarius obtusus* appeared only significantly positively correlated to NH_4 and Mg. The different behavior of the two species might be species-specific. But it could also be based upon their ETs and their deviating relation to water. The calculated correlation included only the ECM proper and not the mycelium of *C. obtusus*. Very likely it forages at more distant places than the ECM proper, because water and nutrient uptake might be limited at proximal sites due to the hydrophobic feature. Since the active foraging mycelium is rather close to the mycelial front, the lower and less significant correlation of *C. obtusus* ECM (proper) abundance with nutrient quantities is evidently in line with the features of the MD_f -ET and contrasts therefore reasonably well to the hydrophilic C-ET *L. decipiens*. This is an example how different niches can be used even when ECM, but of different ETs, inhabit the same site close together (Agerer et al. 2002).

Although additional studies indicate community changes in dependence of nitrogen availability (e.g. Kranabetter et al. 2009, Lilleskov 2002), and regarding hydrologic gradients (Cavender-Bares et al. 2009), unraveling exploitation and competition strategies in nature is only possible at micro-scales.

5. Conclusions

The combination of exploration type-specific density and range of extramatrical mycelium with the location-specific, and distribution-, abundance-, and length-true mapping of ectomycorrhizae are an opportunity to estimate and depict potential explorative space occupancy and potential space exploitation within a natural ectomycorrhizal community. The application of nutrient-specific depletion zones may help to interpret competition of ectomycorrhizae regarding space and nutrients. The preliminary data give evidence that a higher amount of those ectomycorrhizae with less mycelium is necessary to effectively exploit the soil of a given space in comparison to such with more extended mycelium. This regards at least less dissolvable and less diffusible nutrients. As nutrient acquisition of the mycelium is often also dependent on its space-related changing affinity to water, its hydrophily/hydrophoby properties have to be considered, too. Although ontogeny- and seasonal-related community changes could not be addressed, it can be hypothesized that, depending on proportions and abundance of exploration types, "most efficient communities", i.e. communities that have the potential to maximally exploit the soil, should exist within a given soil space. Further studies have to address this supposition.

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Computermodelle in der Wissenschaft – zwischen Analyse, Vorhersage und Suggestion

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Nicht nur die Technik- und Naturwissenschaften, sondern auch die Lebens-, die Sozial- und Kognitionswissenschaften, sogar Kunst und Archäologie sind immer stärker durch eine Informatisierung gekennzeichnet, die einige Disziplinen sogar revolutioniert. Standen früher Wissenschaften auf den fundamentalen Säulen Theoriebildung und Experiment, so hat sich in den letzten Jahrzehnten eine dritte gleichberechtigte Komponente herausgebildet – die der digitalen Modellierung, Simulation und Visualisierung von Strukturen und Prozessen. Der Band behandelt mathematische Grundlagen der Computertechnik, Möglichkeiten und Grenzen der Computermodellierung in den Lebenswissenschaften (Biochemie, Proteomanalyse, Systembiologie) und der Medizin (Tumorchirurgie, Hirnforschung, Rehabilitation), Computer als Dialogpartner (Spracherkennung, Schnittstelle zwischen Gehirn und Maschine), Klimamodelle, Computermodellierungen in Physik und Chemie, aber auch Probleme aus Philosophie (Simulation und Erkenntnis, Implikationen der Hirnforschung) und Ökonomie (Konsequenzen der Alterung der Gesellschaft). Die Beiträge bieten sowohl Laien als auch Experten überraschende Einblicke in eine faszinierende Forschungswelt.

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Moving Towards Measuring Multifunctionality in Ecosystems: FieldScreen – A Mobile Positioning System for Non-Invasive Measurement of Plant Traits in Field Experiments

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With 4 Figures and 1 Table

Abstract

In the face of rapidly declining diversity interest in how plant diversity and ecosystem functioning interrelate and how this relationship may differ across various systems is high. We know that grasslands with more species and functional traits interacting can positively affect ecosystem functioning such as productivity or nutrient cycling. These findings usually relate to highly managed experiments, however, and we still know little of how diversity and ecosystem function relate in more natural systems subjected to invasion. Latest findings also point to the need to focus on more than a few ecosystem functions (multifunctionality), and hence also a suite of traits of species, at the same time to better understand how diversity and ecosystem properties are connected. Ecosystems are subjected to dynamic changes at many different spatial and time scales. There are short-term variabilities, rhythms over days or years, and changes and interaction happening on longer time scales. These dynamic changes in nature can lead to alteration of ecosystem functions over time. To describe these changes and the multifunctionality of ecosystems, spatial and temporal analyses at various scales are essential and new approaches are necessary to complement traditional ecological measurements.

Here we present a combined approach linking community assembly and physiological research with an automated non-invasive positioning system for measuring multiple traits of vegetation in the field. The “FieldScreen” is set up over the “Habitat Garden” Experiment, a grassland assembly experiment. The FieldScreen can accurately position a set of sensors enabling automated measurements of the plants and soil surface below by means of high-resolution photos, hyper-spectral reflectance or sun-induced fluorescence measurements. The Habitat Garden Experiment addresses how priority effects of species that arrive first in a system may affect both productivity and diversity (assembly) over time.

The first 3 years of observation showed that with the FieldScreen it is possible to non-invasively follow changes of species turnover and selected plant traits over time. With the photos taken with a camera mounted on the FieldScreen trolley we can clearly distinguish that sowing initially different diversity levels has an abiding influence on the further development of the plant communities, the spatial spread of species and the overall vegetation cover. These time series have the potential to address research questions on the dynamic nature of ecosystem functioning. This could include measuring several traits of plants at the same time and hence helping to address the need to measure multifunctionality in natural systems if we are to better understand how diversity and ecosystem functioning are linked in natural systems subjected to many disturbances and drivers.

Zusammenfassung

Angesichts des weltweiten Artenverlustes ist das Interesse, die Zusammenhänge zwischen Pflanzenvielfalt und den Funktionen von Ökosystemen und deren Veränderung zu verstehen, hoch. Graslandgesellschaften mit einer hohen Artenvielfalt und dazugehörigen funktionellen Merkmalen haben positive Auswirkungen auf verschiedene Ökosystemfunktionen wie z. B. Produktivität und Stickstoffhaushalt. Diese Zusammenhänge wurden jedoch in kontrollierten Experimenten gefunden, und es ist bisher noch unklar, inwieweit Zusammenhänge zwischen Artenvielfalt und Ökosystemfunktion in natürlichen Systemen bestehen, die durch Einwanderung neuer Arten charakterisiert sind. Um die Zusammenhänge zwischen Diversität und Ökosystemeigenschaften besser zu verstehen, ist es nötig, Beobachtungen nicht nur auf ausgewählte Ökosystemfunktionen zu fokussieren, sondern ein Ökosystem als Gesamtgefüge zu betrachten und gleichzeitig die Merkmale von Arten einzubeziehen. Ökosysteme stehen ständig in Wechselbeziehung mit anderen Organismen und der Umwelt und verändern sich dynamisch auf unterschiedlichen Zeitskalen. Diese Veränderungen können kurzzeitliche Änderungen sein oder auch Rhythmen über Tage, Jahre oder sogar auch Veränderungen und Interaktionen auf längeren Zeitskalen. Diese dynamischen Änderungen in der Natur (z. B. Veränderung von Artenzusammensetzungen und Einwanderung von Arten) können zu Veränderungen in den Funktionen der Ökosysteme über die Zeit führen. Um Aussagen über die Änderungen von Ökosystemen machen zu können, bedarf es zeitlich und räumlich entsprechend aufgelöster Untersuchungen mittels neuer technischer Ansätze.

Wir präsentieren hier einen experimentellen Ansatz, bei dem Untersuchungen zu Pflanzengesellschaftsentwicklungen und physiologische Messungen verknüpft werden. Ein neu entwickeltes mobiles Positionierungssystem (‚FieldScreen‘) wurde über dem ‚Habitat Garten‘, einem Experiment zur Untersuchung der Graslandgesellschaftsentwicklung, errichtet. Der FieldScreen verfügt über eine bewegliche Traverse, mit der verschiedene Sensoren automatisch positioniert und zeitlich wiederholte Messungen durchgeführt werden können (zurzeit Kameraaufnahmen und Messungen der hyperspektralen Reflexion und sonneninduzierten Fluoreszenz).

In den ersten drei Jahren konnte gezeigt werden, dass es möglich ist, mit Hilfe des FieldScreen nicht-invasiv detaillierte Veränderungen des Artenumsatzes und ausgewählter Pflanzenmerkmale über die Zeit aufzunehmen. Die anfänglich unterschiedliche Artenzusammensetzungen der trockenen Grasgesellschaften hatten einen deutlichen Einfluss auf die weitere Entwicklung der Pflanzengesellschaften, und Unterschiede in der räumlichen Ausbreitung von Arten und des Bedeckungsgrades wurden sichtbar. Mit dem FieldScreen ist es nun möglich, ökologische und physiologische Informationen kontinuierlich über eine gesamte Vegetationsperiode zu erhalten. Solche Zeitserien haben das Potenzial, Informationen über Entwicklungsprozesse von Ökosystemen in ihrer Gesamtheit zu liefern und damit die Gesamtfunktionen in natürlichen Systemen zu beschreiben.

1. Introduction

Global change, including not only climate change but also the consequent alteration of ecosystems due to biodiversity loss, land use change, and invasion by exotic species, for example, are major challenges of our time (Chapin et al. 2000). Society and politicians are requesting practical solutions to the multiple current strains on ecosystems (Beck 1993). To get a grasp on how global change is affecting how ecosystems work and provide ecosystem services (Diaz et al. 2006, 2007), environmental and ecological science have to rise to the challenge to understand already highly complex and dynamic ecological systems within a changing environment (Harris et al. 2006). To follow changes in vegetation we will need a whole suite of skills ranging from traditional ecological expertise (knowledge of species and their interactions), through better global monitoring of various habitat types to high-tech measurement of dynamic changes at various scales - both temporal and spatial (e.g. deriving physiologically or ecologically significant information on plant performance through remote sensing of vegetation; Rascher and Pieruschka 2008, Ustin and Gamon 2010). Information on plant performance derived at one scale or under one set of controlled conditions, needs to be now linked and integrated with plant performance at larger scales and under more natural conditions (Körner 1995).

In recent decades a high decline of biodiversity has been observed worldwide (Loreau et al. 2001, Butchart et al. 2010, Isbell et al. 2011), which can have a negative impact on ecosystem functioning and services (Schulze and Mooney 1993, Balvanera et al. 2006). Major direct drivers of biodiversity loss are climate change, invasion of exotic, non-native species, habitat destruction and degradation or land use changes at local and regional scale (De Meester et al. 2010). Land use changes, specifically the conversion of natural ecosystems into agro-ecosystems have led to changes in the species composition and to a decrease of biodiversity (van der Putten et al. 2000).

Semi-natural grasslands are the vegetation type with the highest diversity of species per m^2 world-wide (Willems et al. 1993). They form a key part of the European culturally-formed landscape and are maintained either by grazing or mowing. Due to both intensification of land use, and land abandonment, the area covered by semi-natural grasslands has shrunk considerably over the past century such that many native grassland species are now endangered (Rusina and Kiehl 2010, Kirmer et al. 2011). As well as preserving existing species-rich grassland habitats, recreating grasslands (restoring) is becoming a common tool to counteract biodiversity loss, either on ex-arable land (Bullock et al. 2007) or on marginal land (land not fit for intensive agriculture due to poor nutrients in soils, e.g. former mining sites [Baasch et al. 2012]).

In ecological science many biodiversity experiments, where for example plant diversity is manipulated and its effect on ecosystem properties such as productivity or nutrient cycling is followed, have shown that both species richness and functional diversity (i.e. the functional traits of the species in a system) can have positive effects on ecosystem functioning, especially in grassland systems (Schläpfer and Schmid 1999, Schläpfer et al. 1999, Hooper et al. 2005, Balvanera et al. 2006, Díaz et al. 2006). More diverse grasslands in biodiversity-ecosystem experiments, in which plots were sown with highly diverse plant seed mixtures, were found to be more productive and to take up available resources more efficiently than less diverse mixtures, and this positive diversity effect even increased with time (Marquard et al. 2009). In particular, very recent biodiversity research has shown that over time in a grassland (Allan et al. 2011) the species interacting most positively and hence driving ecosystem properties (such as productivity) change over time, such that a whole suite of species are necessary to maintain function over a longer time span, refuting early possible implications of such diversity experiments that only a small number of species may be necessary to maintain certain ecosystem functions such as nutrient cycling.

It is now becoming clear that if more functions of an ecosystem are considered, then more species or functional traits are needed to maintain those functions (so-called multifunctionality), particularly in face of climate change (Zavaleta et al. 2010). To focus more on studies of multifunctionality in the future will require either more researchers working together in one system, looking at many of its different functions, or the use of technology that enables simultaneous measurement of a suite of properties of an ecosystem.

Biodiversity Ecosystem functioning experiments are somewhat unnatural, however, in that species are chosen at random from a total species pool, and species levels are maintained by weeding (i.e. are "closed" systems). This poses the question therefore as to how important such positive diversity effects may be in more natural systems where there is immigration of species into and out of the system ("open" systems) (Srivastava et al. 2004).

The positive relationship found in biodiversity experiments between diversity and functioning of an ecosystem should motivate restoration practitioners to include a larger number of species during grassland restoration plantings or sowing (see successful examples in Bullock et al. 2001, 2007). But it is not yet generally clear whether this positive relationship between diversity and functioning of ecosystems also holds true in restoration of different habitat types and over long time, because this relationship has been developed through several tests involving short-term and highly controlled experiments (Doherty et al. 2011). There is still no clear picture of how many species or how many species with specific functional traits are needed in an ecosystem to maintain specific functions (Isbell et al. 2011). We need to know more about how initial condition (including e.g. the diversity of plant species sown, so-called priority effects [Facelli and Facelli 1993, Fukami et al. 2005, Bullock et al. 2001, Grman and Suding 2009]) influence the further development of diversity and ecosystem functioning within natural and semi-natural systems such as grasslands. In short we need to investigate how dynamic community assembly (vegetation development) interacts with biodiversity effects.

Only a few research groups have actively tested whether positive biodiversity effects found in ecosystem-functioning experiments also occur in systems with natural assembly as well as in degraded systems or in ecosystems with extreme or highly fluctuating environments like dry lands (van der Putten 2000, Bezemer and van der Putten 2007, Lepŝ et al. 2007, Tischew and Kirmer 2007, Bullock et al. 2007, Kirmer et al. 2008, Baasch et al. 2009, Doherty et al. 2011).

Ecological systems going through natural assembly, and especially grasslands, are highly dynamic. No two years are the same in terms of plant species composition due to weather fluctuations and other random factors. Changes occur not only at different spatial but also at different time scales, ranging from minutes, through days, months, years, to changes and interactions needing more time (evolutionary processes) (von der Wiesche and Werner 1998). Research questions such as effects of biodiversity loss or differences in assembly and further development due to starting diversity are scale-dependent and outcomes of measurements will be highly spatially and temporally dependent.

Obtaining a relatively complete description of the spatial and temporal changes and interactions in an ecosystem requires very high costs with a high effort (costs, man power and time) and is in general not feasible. Therefore, researchers have to focus on a few aspects they want to analyze in their experiments, because of such limitations. This can lead to a loss of important information for understanding restoration and assembly under natural conditions. For this reason, we need to test the potential for using measurement of a number of plant or canopy traits at the same time (knowing that traits and species relate directly to ecosystem function, see above), and at wide range of time spans or scales than has been possible using usual workload.

We test new approaches using technology specifically developed to address research questions on multifunctionality, to complement ecological expertise on the ground, to assess changes in ecosystems over space and time and how they relate to the functioning of these systems. One new approach is a Mobile Field Positioning System called "FieldScreen", which accurately positions a sensor and enables automated and repeated non-invasive measurements of the plants and soil surface in the "Habitat Garden" (Fig. 1*A*). A sensor platform taking measurements for example involving high resolution photos, hyper-spectral reflectance or sun-induced fluorescence. In this manuscript we present the strengths and possibilities of this new approach in a grassland assembly experiment. We use the FieldScreen to follow changes both spatially and over time in different grassland habitats and their development over the course of several years. We want to test or to see what kind of ecological or physiological traits can be followed using the FieldScreen at community and species-level. We describe the experimental design, explain the construction and measurement concept of the Habitat Garden and the FieldScreen and present the first results of the FieldScreen from the first four years measurements of seasonal dynamics of vegetation development (assembly) in the experiment.

2. The "Habitat Garden": A Community Assembly Field Experiment to Quantify How Diversity and Ecosystem Function is Affected by Priority Effects

In December 2007 we started a grassland assembly experiment, the Habitat Garden, with two different grassland habitats (dry and mesic grassland). The dry grassland plots were initially sown with two different diversity levels (2 grass and 25 forbs *versus* 7 grass and 32 forbs) and the mesic plots were sown with the same diversity of species at the start. The experiment was designed to quantify changes in early successional grassland systems over time (e.g. diversity, productivity, spatial spread of species, phenology of plant species, specifical traits of species) depending on the different starting diversity (priority effects).

The ecological field plots were set up on an ex-arable soil in the area of the Forschungszentrum Jülich, located in West Germany (6° 22'0''E, 50° 56'0''N). 12 plots were prepared, each plot being 2 × 2 m in size and separated from each other by 1 m rows. The 12 plots were set-up as randomized and three different treatments were selected: a mesic grassland (M), a dry acidic grassland with medium species diversity (S2) and a dry acidic grassland with high species diversity (S7) (Fig. 1*B*). For each plot the original soil was removed to a depth of 40 cm, a geomembrane permeable to water and nutrient laid to avoid germination of seeds from the origin soil. For the mesic grasslands an ex-arable soil type of the region Heinsberg, Germany (Geilenkirchen 6° 7'0''E, 50° 58'0''N) was selected, for the dry grasslands washed sand (Quarzwerke Witterschlick GmbH, grain size 0.7–1.4 mm) mixed with 10 % potting soil (Einheitserde Werkverband e. V.) was used.

The plots were hand-sown in the first year only, with seed mixes from Rieger Hofmann GmbH, a company specializing in native grassland seed mixes of local provenance. The seeding rate for each plot was 4 g/ m^2. The mesic grassland with moderate soil nutrients availability (M in Fig. 1*B*) was sown with a seed mix containing 11 grass species and 23 forbs (including 2 legumes) (Tab. 1*A* and *B*) and removal experiments (removing specific species or functional groups of plants) were performed at later dates providing ecological experimental treatments (data not shown). The dry acidic grassland communities were established on sand mixed with one tenth potting soil with very low nutrient and water availability (S in Fig. 1*B*) and was sown with two biodiversity levels: a medium diversity seed mixture with either 2 starting grass species and 25 forb species (including 1 legume) (S2 in Fig. 1*B*) and a high diversity seed mixture with either 7 starting grass species and 32 forb species (including 4 legumes) (S7 in Fig. 1*B*) (Tab. 1*A* and *B*). One quarter of every plot was not sown and kept as control subplot (upper right corner of each plot in Fig. 1*B*). The whole area was fenced to reduce confounding factors such as grazing by deer or wild boar.

During the experiment invasion of new species could occur in three ways (in addition to establishment from the sown seeds): from the seed bank in soil, from neighboring plots or from vegetation outside of the Habitat Garden. The sites were not fertilized, but they were mown ones or twice a year depending on the grassland habitats (dry grasslands once a year in August, mesic grasslands twice a year in early June and late August according to typical mowing regimes for such grasslands in Central Europe). In the first year (2008) there was only one late hay cut in October for the mesic grasslands. In the next years there were two hay cuts in June and in August for the mesic grasslands and only one hay cut every year in the end of July beginning of August for the dry grasslands at the vegetation peak.

Since 2007 establishment of species and the dynamics of restoration was continuously monitored. Every year before mowing vegetation was assessed for the presence and absence

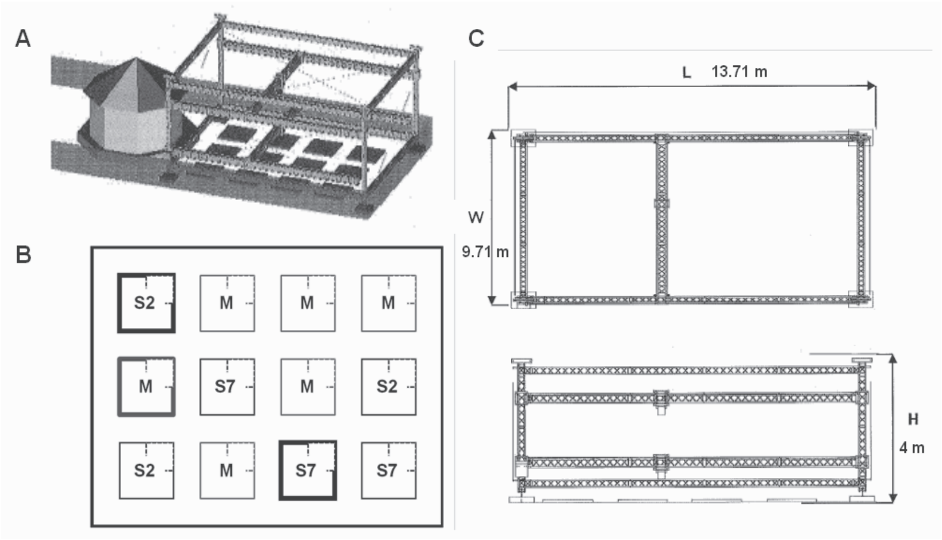

Fig. 1 (*A*) Schematic view of the FieldScreen set up over the plots in the Habitat Garden (© Manual FieldScreen, Visser International trade and engineering B. V. [‘s-Gravendeel, Netherlands]). (*B*) Layout of the field experiment showing the plots sown in December 2007 with mesic grassland species (M) or with dry grassland species (S), S2 is the species mixtures with 2 starting grass species and 25 forb species (including 1 legume) sown, S7 with 7 starting grass species and 32 forb species (including 4 legumes) sown. Three plots (one plot of each regime, identified by bold outline), were subject to further analysis (Fig. 2, 3, 4). In one corner of every plot the dotted line shows the control region where no seeds were sown. (*C*) Frame of the FieldScreen with the maximum size of a height of 4 m a width of 9.71 m, and a length of 13.71 m (© Manual FieldScreen, Visser International trade and engineering B. V. [‘s-Gravendeel, Netherlands]).

of each species. Additionally the cover of every species was visually estimated using a decimal scale (Londo 1976). In the first year (2008) two vegetation assessments for the mesic grassland and one for the dry grassland were done even though there was only one hay cut for the mesic grassland and non for the dry grassland. To calculate the invasion pressure the species number and their cover at the plot border was also estimated separately. Aboveground biomass production (dry matter yield) was measured in two 0.1 m^2 quadrates in every plot (one in the control area and one randomly in the plot) before mowing. Plant material was cut 3 cm above the soil surface and dry weight was taken (dried biomass is stored for potential later analyses). In 2010 and 2011 biomass was sorted according to three functional groups: legumes, forbs and grass species. In June and August 2011 the leaf area index (LAI) was measured in all grassland plots using a LAI-meter of Licor (LAI-2000, Plant canopy analyzer, Licor Biosciences, Lincoln, USA). Furthermore, every year soil samples were taken from the top soil layer (0–15 cm) and analyzed for pH, total P content, mineralized soil N, and total C and N content.

Since 2010 various functional traits have been recorded for *Lotus corniculatus*, *Plantago lanceolata*, *Trifolium* spec., *Ranunculus* spec. and *Hypochaeris radicata*. Photosynthetic light response curves were measured with a Fluorescence Yield Analyzer Mini-PAM (Heinz Walz GmbH, Effeltrich, Germany). Chlorophyll content for samples from 2–5 individual plants of each species was analyzed by an extraction method and a spectro-photometrical analysis (Lichtenthaler 1987, Lichtenthaler und Buschmann 2001). For a sample of 3–5 leaves from each species, the area was estimated with a leaf area meter (LI-3100C, Licor Biosciences, Lincoln, USA) and fresh and dry weight was taken to calculate leaf water content and specific leaf area. Nitrogen and carbon content was determined from dried leaf samples. Results on dynamic changes of functional traits during establishment are currently being analyzed and will be reported elsewhere.

Tab. 1 List of species sown in the Habitat Garden in 2007, in the mesic grassland plots or in the dry grassland plots, S2 is the species mixtures with 2 starting grass species and 25 forb species (including 1 legume) sown, S7 with 7 starting grass species and 32 forb species (including 4 legumes) sown. A List of grass species. B List of forb species including legumes which are shown in bold font.

A	Species	Dry grassland		Mesic grassland
		S2	S7	
Grass species	*Agrostis capillaris*		×	
	Alopecurus pratensis			×
	Anthoxanthum odoratum			×
	Arrhenatherum elatius			×
	Bromus erectus			×
	Corynephorus canescens	×	×	
	Cynosurus cristatus			×
	Dactylis glomerata			×
	Deschampsia flexuosa		×	
	Festuca guestfalica	×	×	
	Festuca nigrescens		×	×
	Festuca pratensis			×
	Helictotrichon pubescens			×
	Luzula campestris		×	
	Poa compressa		×	
	Poa pratensis			×
	Trisetum flavescens			×
B	Species	Dry grassland		Mesic grassland
		S2	S7	
Forb species	*Achillea millefolium*	×	×	×
	Anchusa officinalis	×		
	Anthemis arvensis		×	
	Anthriscus sylvestris			×
	Armeria maritime/elongata	×	×	
	Artemisa campestris	×		
	Berteroa incana		×	
	Calluna vulgaris	×		
	Campanula patula			×
	Campanula rotundifolia	×	×	
	Carum carvi			×
	Centaurea jacea			×
	Chrysanthemum segetum		×	

B	Species	Dry grassland		Mesic grassland
		S2	S7	
	Crepis biennis			×
	Daucus carota	×	×	×
	Dianthus carthusianorum	×		
	Dianthus deltoides	×	×	
	Echium vulgare	×	×	
	Galium album	×		×
	Galium verum		×	
	Genista tinctoria		×	
	Hieracium pilosella	×	×	
	Hypericum perforatum	×	×	
	Hypochaeris radicata	×	×	
	Jasione montana	×	×	
	Knautia arvensis			×
	Leontodon autumnalis	×		
	Leontodon hispidus	×	×	×
	Leucanthemum ircutianum			×
	Linaria vulgaris		×	
	Lotus corniculatus		×	×
	Oenothera biennis	×		
	Papaver argemone		×	
	Papaver rhoeas			×
	Petrorhagia prolifera	×		
	Pimpinella major			×
	Plantago lanceolata		×	×
	Potentilla argentea	×		
	Prunella vulgaris		×	×
	Ranunculus acris			×
	Reseda lutea		×	
	Reseda luteola		×	
	Rumex acetosa			×
	Rumex acetosella		×	
	Salvia pratenis			×
	Sedum acre	×	×	
	Silene flos-cuculi			×
	Silene latifolia ssp alba		×	
	Silene viscaria		×	
	Silene vulgaris	×		×
	Spergula arvensis		×	
	Thymus pulegioides		×	
	Tragopogon pratensis			×
	Trifolium arvense	×	×	
	Trifolium campestre		×	
	Trifolium pratense			×
	Verbascum thapsus	×		
	Verbascum densiflorum	×		
	Viola tricolor		×	

3. The "FieldScreen": A New Method to Non-invasively Track and Quantify Functional and Structural Traits of Plants During Assembly.

The FieldScreen was installed over the Habitat Garden in 2009 to follow the development and dynamic changes in this grassland community assembly over time (years and also during the course of days). Conventional ecological assessments (see above) can only be taken at selected time points during the course of the year and thus may miss multifunctionality during assembly. The FieldScreen provides a new approach to provide temporally high resolution data during an ecologically highly dynamic process. With the FieldScreen we aim to non-invasively observe plots, to characterize dominance of species, and to quantify their functional and structural traits during this assembly process.

The FieldScreen is a mobile scanner transport system that was built by Visser International trade and engineering B.V ('s-Gravendeel, Netherlands) (Fig. 2*A*). The whole system consists of 42 parts, none of which is longer than 5 m and heavier than 50 kg. The whole FieldScreen weighs 400 kg. The major part of the frame consists of standard aluminum truss modules with minor modifications. The single parts are connected by a robust plugging system allowing a rapid and easy assembly. The FieldScreen can thus be assembled in half a day with 4–6 persons, allowing to set up the system in fields not accessible to heavy machines. However, a 400 V electric power supply needs to be available to power the electric drives. The frame and electric parts are completely weather-resistant. However, the traverse may not be operated during frost and snow. The FieldScreen spans a total length of 13.71 m and a width of 9.71 m (Fig. 1*C*). The height is adjustable up to 4 m. On top of the upper frame is a movable traverse (Fig. 2*A*) which can be moved and exactly positioned by a PLC (programmable logic control) using a laser positioning system as a positioning feedback. A traveling trolley carrying up to 50 kg of measurement equipment is driven on this traverse and can be moved and positioned along the traverse. Traverse and trolley (sensor platform) can be positioned with an absolute accuracy better than 1 cm by the laser positioning system and an arbitrary series of measurement positions can be programmed. The velocity of the traverse and the slide is 5 and 10 cm/ s, respectively.

In our case we have fixed the traverse at 4 m height and have installed two different sensors. First, we installed a conventional programmable digital camera (Imperx Inc., Boca Raton, USA, IPX-11M5-G: Imperx 11 Mega pixel/ Nikon AF Nikkor objective 50 mm 1:1.4D) with a water protective casing (Stemmer Imaging GmbH, Puchheim, Germany). This camera is a high resolution, industrial grade, fully programmable CCD camera. The camera provides a 4000 × 2672 pixel resolution and can deliver up to 5 frames per second at full resolution. By inspection of the photos we are able to visually identify and characterize single species within the plots, including time of flowering. Additionally, the spreading of vegetation and the invasion of species was followed. In the future we aim for quantifying total vegetation cover (2D) automatically by applying suitable texture classification and image segmentation algorithms.

Secondly, a hyperspectral measuring unit was developed and installed. The unit consists of two HR4000 spectrometers (Ocean optics, Dunedin, USA) having different spectral ranges (200–1100 nm / 670–860 nm) and resolution (0.1 nm and 0.01 nm), housed in a water-proof and temperature controlled box. One spectrometer is used for computation of vegetation indices, the other to estimate sun-induced fluorescence (Fs) using the atmospheric O_2-absorption bands (Rossini et al. 2010a, b). Light is collected by two fibre-optics one looking upwards for incoming radiation, one looking downwards for reflected radiation. The openings of the fibers are covered by a custom made cosine receptor that was adapted from Rossini et al. (2010a, b). Light of the fiber optics is divided by an optical multiplexer allowing simultaneous measurement from the two spectrometers (Meroni et al. 2010, Rossini et al. 2010a, b). An optical multiplexer can switch between channels measuring the light of both fibre-optics (looking upwards and downwards) and a blind channel for dark current measurements (Meroni et al. 2010, Rossini et al. 2010a, b). The data of the spectrometers are currently analyzed and will allow calculation of hyperspectral canopy reflectance, a variety of vegetation indices, and sun-induced fluorescence (not shown) (Malenovsky et al. 2010, Meroni et al. 2009, Rascher et al. 2011).

Automation of the positioning system and the coordinated measurement of all sensors are ensured by a dedicated software program (Erler 2011). This software synchronizes imaging and hyperspectral measurements with the automation software for the positioning of the FieldScreen. A small computer inside the hyperspectral measuring box communicates with the PLC and further controls the hardware that is needed for the measurements, e.g. cameras, two hyperspectral sensors and an optical multiplexer to switch between incoming and reflected light measurements. The software can calculate the sun-induced chlorophyll fluorescence at wavelength of absorption bands on-the-fly. One of three implemented Fraunhofer Line Discrimination approaches (namely the standard FLD method, the Maier-Method and the improved FLD method, Meroni et al. 2009) can be used.

Since the establishment in 2009, FieldScreen measurements of every plot were performed from March to November every year if the weather conditions were favorable (i.e. clear skies). In 2009 data were recorded on 10 measurements days, in 2010 17 measurement days, and in 2011 33 measurements days.

4. Following Visual Changes in the Development of the Different Grassland Habitats Over 4 Years

Seasonal changes of the grassland were observed with high resolution cameras over 4 years and characteristic differences between the two diversity treatments (testing priority effects) of the dry grassland and the mesic grassland are detectable (Fig. 2).

In Figure *2B–D* time series showing vegetation development between 2008 (photos taken by hand with a camera, since 2009 photos taken with a camera mounted on the trolley of the FieldScreen) and 2011 in three representative plots: a dry grassland plot with high species diversity (S7) (Fig. 1*B* plot marked bold outline), a dry grassland plot with medium species diversity (S2) (Fig. 1*B* plot marked bold outline) and a mesic grassland plot (M) (Fig. 1*B* plot marked bold outline).

By visually comparing the photos (Fig. 2*B–C*) it is clear that the S7 plot had a faster canopy closure and also a higher biomass production. Also differences in species presence between the two diversity treatments occurred and are visible in the photos (Fig. 2*B–C*). In the S7 plot the dominant species was the nitrogen fixing *Lotus corniculatus* (yellow flowers in Fig. 2*B*). In photo 4 of Figure 2*B* (07/2010) the pink flowers of *Dianthus deltoides*, a forb species are visible between the yellow flowers of *Lotus corniculatus.* In photo 5 of Figure 2*B* one year later (07/2011) a white flowering species has appeared, which was identified as *Daucus carota*, a tall forb species. All these were species that had been sown at the beginning of experiment. For comparison in the S2 plot also a yellow flowering species could be identified using the photos but the anatomy of the flower was different to *Lotus corniculatus*. This yellow species was *Hieracium pilosella*, another forb species. In photo 4 Figure 2*C* (07/2010)

Dianthus deltoides with the pink flowers is also clearly visible in the S2 plot. Furthermore, in photo 5 of Figure 2*C* (07/2011) a white flowering species has appeared, this is *Achillea millefolium* a forb species. Clearly visible in the free spaces of the plots is the grass *Corynephorus canescens*, sown at the beginning of experiment as well as the other dominant species.

Figure 2*D* shows a time series (2008–2011) of one mesic grassland plot. Directly in the first year a high canopy closure has existed, but over the next years biomass increased, as all species have grown more compactly. In the first two photos (05/2008 and 07/2008) there are visible additional species that were not sown, mainly species from the seed bank of the soil. Starting 2009 the sown species become observable. Comparing mesic and dry grassland plots, fewer flowering species were growing (depends on the sown mixtures), but all growing species had a higher biomass production and vegetation cover in all years. The dominant flowering species were *Leucanthemum ircutianum* and *Crepis biennis*. *Rumex acetosa* had a dominant ground cover because of its big leaves.

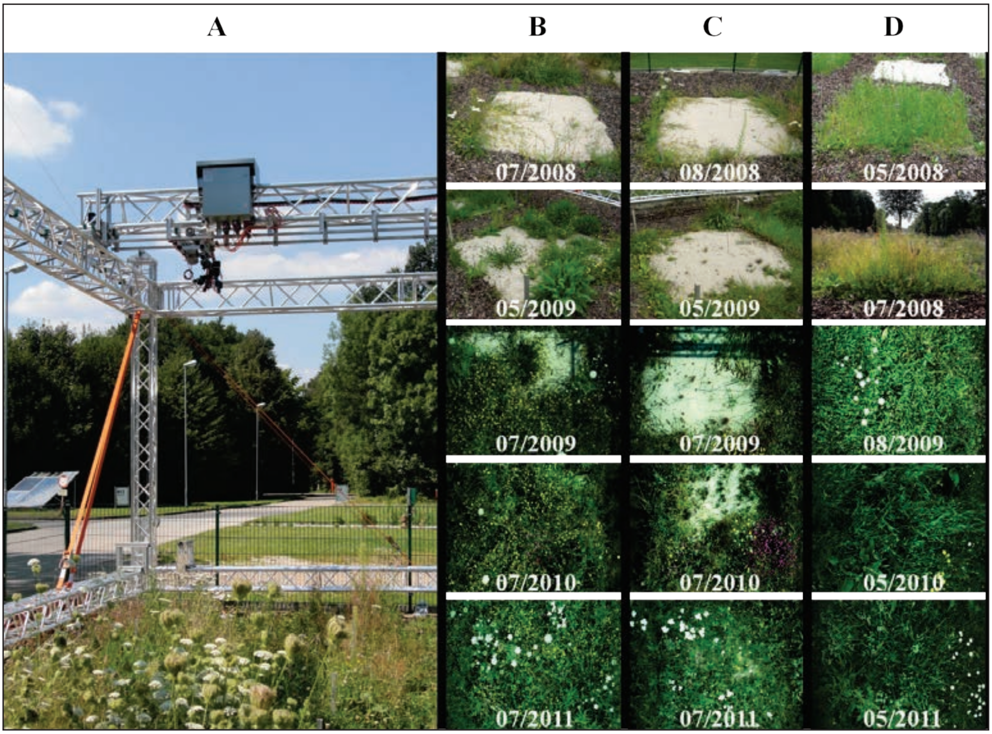

Fig. 2 (*A*) View of the FieldScreen set up over the Habitat Garden assembly experiment at Plant Sciences (IBG-2) Forschungszentrum Jülich. (*B*, *C*, *D*) time series showing vegetation development from 2008 over 4 years in three representative plots for the different grassland habitats. (*B*) Dry grassland sown with 7 starting grass species and 32 forb species (S7 plot marked bold in Fig. 1*B*) (*C*) Dry grassland sown with 2 starting grass species and 25 forb species (S2 plot marked bold in Fig. 1*B*) (*D*) Mesic grassland plots (M plot marked bold in Fig. 1*B*). The top two rows were taken by hand-held camera, whereas the last three rows of photos for each representative plot were taken using a camera mounted on the trolley on the FieldScreen and are currently being assessed for potential to follow phenology, invasion timing of specific species and canopy structure using image analysis tools.

In Figures 3 and 4 time series of both diversity levels within the dry grassland are shown in more detail, covering the dynamics of 3 seasonal cycles (2009–2011). In Figure 2 only one time point in the year is shown and one may assume that in 2009 the only dominant species in S7 plot was *Lotus corniculatus* (Fig. 2*B* 07/2009 and Fig. 3*A*). Looking in detail (Fig. 3) at the temporal changes it is clear that species dominance changed: early in vegetation period 2009 *Daucus carata* was growing strongly and became dominant (Fig. 3*B* and *C*), but after mowing *Lotus corniculatus* and *Plantago lanceolata* had the fastest regrowth (Fig. 3*D* and *E*). In 2010 *Daucus carota* was not dominant (Fig. 3*J*), but the cover of *Lotus corniculatus* remained high. *Dianthus deltoides* (Fig. 3*I*) was also growing strongly in 2010 and also more grass species appeared between the forbs (Fig. 3*J*). In Figure 3*N* and *O*, showing recovery after mowing, there was fast recovery of *Plantago lanceolata* and *Lotus corniculatus*. The first canopy closure occurred in July 2010 (Fig. 3*I*).

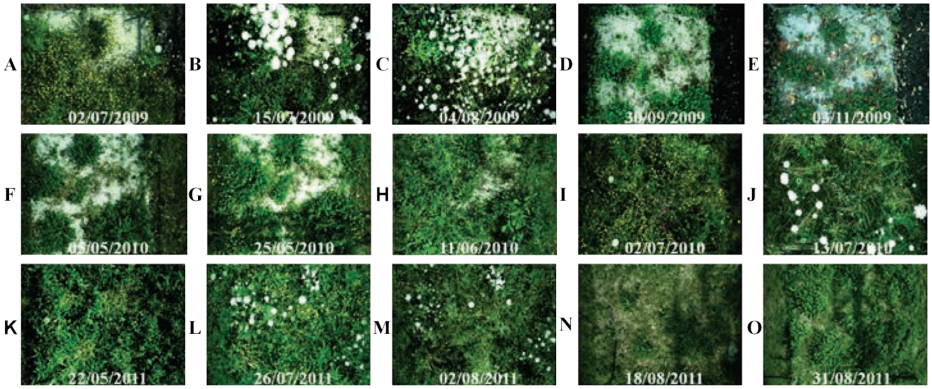

Fig. 3 A photographic time series over one example S7 dry grassland plot in more detail (7 starting grass species and 32 forb species sown, S7 plot marked bold in Fig. 1B). (*A–E*) July – November 2009, (*F–J*) May – July 2010, (*K–O*) May – End of August 2011.

For the S2 plot in Figure 4 the canopy closure has still not reached 100 % (*O*), and the dynamics of strong or dominant growing species is less compared to the S7 plot. By examining only one time point in the year (Fig. 2*C*) this interpretation is not possible. There, the dynamics of both systems look more similar and also the canopy closure (Fig. 2*C* 07/2011) seems to be 100 %. That this is not the fact is clear after mowing (Fig. 4*N* and *O*). But from all three years (Fig. 4) a higher canopy closure is visible since 2009. The less dynamics of dominant species is only visible through the detailed photo series (Fig. 4). The same dominant flowering species (Fig. 4*A*, *B*, *H* and *I*) *Dianthus deltoides* (pinc) and *Hieracium pilosella* (yellow) are also visible, as in the photos of Figure 2*C*, but because observation was frequent, we can be sure that that the cover for each species was always higher in 2010 than in 2009. Also *Achillea millefolium* (white) can be identified as more dominant in 2011 (Fig. 4*L* and *M*) compared to 2009 (Fig. 4*A–C*). For the year 2010 (Fig. 4*J*) also grass species can be identified, only by frequent observation, not be seen on one time point in the year (Fig. 2*C* 07/2010). The species *Cirsium arvense*, which had not been sown at the beginning of the experiment, invaded both grassland systems, and more so in 2011 (Fig. 3 and Fig. 4*K–O*) than in 2010 (Fig. 3 and Fig. 4*F–J*).

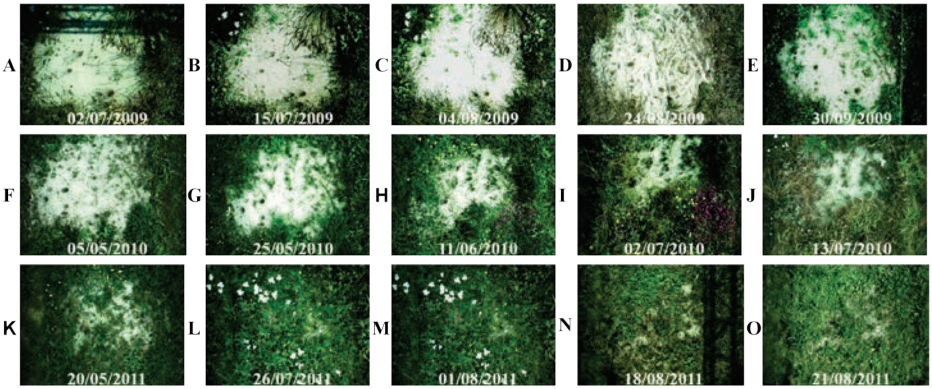

Fig. 4 A photographic time series over one example S2 dry grassland plot in more detail (2 starting grass species and 25 forb species sown, S2 plot marked bold in Fig. 1*B*). (*A–E*) July – November 2009, (*F–J*) May – July 2010, (*K–O*) May – August 2011.

5. Potential of the FieldScreen of Physiology and Ecology

The FieldScreen can follow changes in the development of plant communities over time. In the process it is possible to measure species and also vegetation traits with different kind of sensors. Using the photographs it is possible to gain information on: flowering phenology, species presence and absence, dominant species, vegetation spread and invasion of species and total vegetation cover (2D). In the future the data of the spectrometers will allow calculation of hyperspectral canopy reflectance, a variety of vegetation indices, and sun-induced fluorescence.

6. Conclusion

We have shown, that photos taken by the FieldScreen allow identification of single species, especially dominant species and plants traits, prevalent the phenology (flowering time) over time, but it was not possible to identify every single species or the cover of every species in this highly diverse system like conventional ecological assessments can. The total 2 dimension canopy cover can be estimated using the photos, but not the 3 dimensional cover. Nevertheless, for research on open ecological systems, FieldScreen is a useful tool to follow species spread and invasions.

In our grassland assembly experiment differences between the two treatments in the dry grassland that had different starting diversity levels (testing priority effects) were detectable using photos taken with the camera mounted on the FieldScreen trolley. It is clear that the

S7 plot had a faster canopy closure than the S2 plot. Additionally, species identity differed between the systems with the S7 plot having a higher cover of legumes than the S2 plot. With the photos of the FieldScreen one can clearly see that the priority effects of which species arrive first have an influence on the development of the dry grassland over time. By comparing the photos in Figure 2, showing only one time point in each year, and the detailed photos in Figure 3 and 4, taken at several times within each year, indicate that classical ecological assessments often only done once or twice a growing season (due to manpower limitations) may miss important dynamics within the vegetation cycle. Continuous ecological monitoring systems, such as the FieldScreen have the potential to reveal novel properties during assembly.

For the future we aim to relate hyperspectral measurements with functional traits such as biomass, canopy N or leaf area index. We expect that hyperspectral and fluorescence data will open new paths to characterize changes in the Habitat Garden as a whole. It has the potential to give more information on overall vegetation traits over time than conventional ecological measurements. Thus the FieldScreen can be expected to be valuable tool to provide high-quality ecological data, and to help vegetation assessment, without the need to invest so heavily in human resources to measure multifunctionality. Additionally, its capability for much higher observation frequencies than conventional techniques will facilitate our attempts to describe fast ecosystem dynamics that happen at time-scales of hours to days, such as shoot development, leaf angle and flower opening. Further, as the FieldScreen can be dismantled and reconstructed rapidly, it can therefore be used repeatedly at more than one site for intensive intermittent monitoring, for example to measure stress via fluorescence or hyperspectral measurements over different vegetation patches.

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Was ist Leben?

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Es gibt nur wenige Fragen, welche die Menschheit seit Beginn ihrer geistigen Auseinandersetzung mit sich selbst und der sie umgebenden Welt unaufhörlich begleitet haben. „Was ist Leben?“ zählt zu diesen Grundfragen des menschlichen Daseins. Angesichts der beeindruckenden Erfolge der empirischen Forschung an Lebewesen wird die Schwierigkeit immer offenkundiger, eine allgemein überzeugende Antwort auf die Frage nach den grundlegenden Eigenschaften derjenigen Systeme zu geben, die wir „lebendig“ nennen. Diese Rätselhaftigkeit fasziniert Natur-, Geistes- und Kulturwissenschaftler und bringt sie dazu, gemeinsam das Wesen des Lebens zu erkunden. In dem Band behandeln hervorragende Vertreter der unterschiedlichsten Wissenschaftsdisziplinen den noch nicht zufriedenstellend geklärten Ursprung des Lebens. Sie untersuchen das neue, umfassende Verständnis der molekularen Grundlagen von Lebensprozessen aus der Sicht der Genomforschung und beschreiben die Möglichkeiten, Leben in Analogie zu Maschinen zu modellieren. Sie behandeln die ungeheure Vielfalt des Lebens, die Beeinflussbarkeit des Lebens durch menschliche Eingriffe und die Grenzen des Lebens aus biologischer, psychologischer und philosophischer Sicht. Dabei wird deutlich, wie hartnäckig sich die Frage nach dem Leben durch die Wissenschaftsgeschichte, ja durch die ganze Geistesgeschichte zieht und welche Brisanz sie angesichts aktueller Forschungsergebnisse erhalten hat.

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Mixing Patterns of Tree Species and their Effects on Resource Allocation and Growth in Forest Stands

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With 9 Figures and 1 Table

Abstract

The focus of this study is on differently structured mixed forest stands and their influence on resource allocation and growth patterns. Virtual mixed forest stands with different spatial structures, i.e. with a random distribution of European beech and of Norway spruce, and a mixture with groups and clusters of beech, were used for simulations over a period of 10 years. The calculations were done by using BALANCE, an individual tree based growth model which includes the simulation of the total water- and carbon flows of a forest stand. Tree growth is simulated based on physiological processes which are driven by external conditions, e.g. weather and soil conditions. The presented scenario simulations delivered realistical results for mixed spruce beech stands with random, clustered and grouped mixture. The simulations result in different resource distributions (radiation, water) within the three mixed stands which along with different leaf area index values increased biomass increment and efficiency of the random mixed stand more than of the clustered and grouped mixed stand.

Zusammenfassung

Im Mittelpunkt dieser Untersuchung stand, den Einfluss von Mischbeständen mit verschiedenen Mischungsstrukturen auf die Ressourcenverteilung im Bestand und die Wachstumsmuster zu untersuchen. Virtuelle Mischbestände in verschiedenen räumlichen Anordnungen, d.h. mit zufällig verteilten Buchen und Fichten bzw. mit Einmischungen von Buchengruppen sowie -clustern bilden die Basis für die 10-jährigen Simulationen. Die Berechnungen wurden mit dem einzelbaumbasierten Wachstumsmodell BALANCE durchgeführt, das den gesamten Wasser- und den Kohlenstoffkreislauf eines Waldbestandes abbildet. Die Berechnung des Wachstums beruht dabei auf physiologischen Prozessen, die von externen Bedingungen wie der Witterung oder den Bodenbedingungen gesteuert werden. Die Szenariosimulationen lieferten realistische Ergebnisse für Mischbestände aus Fichte und Buche mit zufälligen, gruppenweisen und geclusterten Anordnungen. Sie ergaben unterschiedliche Ressourcenverteilungen (Strahlung, Wasser) in den drei Mischungen, die zusammen mit unterschiedlichen Blattflächenindizes den Biomassezuwachs und die Effizienz des Bestandes mit zufälliger Anordnung stärker ansteigen ließen als die des gruppierten bzw. geclusterten Bestandes.

1. Introduction

In the next decades our forests must be adapted to a changing climate, which means for Central Europe higher temperatures, changed precipitation patterns and a higher number of stronger and longer lasting extreme events (*IPCC* 2007, Beniston et al. 2007). Therefore, knowledge about climate effects on resource and growth allocations among trees is necessary to predict forest dynamics and to choose appropriate adaptation strategies. While allocation dynamics of tree individuals (e.g. Landsberg 1986, Niklas 1994) and growth dynamics at the average tree level is understood quite well (e.g. OLIVER and LARSON 1996; PRETZSCH 2009), there is a lack of knowledge about growth patterns and resource allocations between the trees of a stand and their dependency on site conditions (SCHWINNING and WEINER 1998, WEINER et al. 2001, PRETZSCH et al. 2012, 2013). Because of intense interactions among individuals, the responses of individual trees cannot account for stand growth. The distribution of small and big, young and old, dominated and dominant trees within a stand as well as the species combination of a stand can alter tree interactions such as facilitation, competition or compensation. Additionally, the supply of above and below ground resources strongly depend on growth and space occupation, hence they determine the acquired amount of resources (e.g. GRAMS et al 2002, GAYLER et al. 2006, RÖTZER et al. 2009). According to MATYSSEK et al. (2005) the reaction pattern of stands are more than just the sum of individual tree responses and, thus, cannot be derived from trees grown solitarily.

Furthermore, it is still unclear to what extent mixed stands produce more or less than pure stands. PRETZSCH et al. (2010) for example found in a transect study of 23 long-term plots of Norway spruce (*Picea abies* (L.) H. Karst.) and European beech (*Fagus sylvatica* L.) along an ecological gradient throughout Central Europe that dry mass growth of mixed stands varied between −46 % to 138 % based on the growth values of corresponding pure stands. The effects of mixture may be modified by e.g. the temporal and the spatial structure of the stands, the silvicultural treatment and the site conditions (HOLMGREN et al. 1997, KÖRNER 2006). But also the mixing pattern of forests, i.e. the formation of the single trees within the stand, can alter resource allocation and thus growth and productivity. In consideration of climate change it is questionable whether existing mixed forest stands will remain unaffected and balanced because mixing effects can also be explained by altered resource supply (e.g. VANDERMEER 1989, ROTHE and BINKLEY 2001, PRETZSCH and DIELER 2011).

To uncover such effects, i.e. resource allocation and reaction patterns of differently structured mixed forest stands, physiological growth models which include modules for resource allocation and their modification by species mixing can be proper tools. Physiological growth models simulate forest growth on the basis of generally accepted eco-physiological principles (e.g. MOHREN 1987, RUNNING and COUGHLAN 1988, SLOBODA and PFREUNDT 1989, KELLOMÄKI et al. 1993 or BOSSEL 1994). They can be used to study competition and facilitation effects of mixed forests under changing environmental conditions (e.g. RÖTZER et al. 2005, 2013). BALANCE (GROTE and PRETZSCH 2002, RÖTZER et al. 2009, 2010b) as a representative of this model type is a highly developed eco-physiological individual tree growth model adapted to simulate the growth of complex mixed species stands.

Consequently, to study the influence of different mixing patterns of tree species on resource allocation and growth patterns, scenario simulations for mixed Norway spruce (*Picea abies* (L.) H. Karst.) and European beech (*Fagus sylvatica* L.) stands were done and analyzed. The objectives of this study were in detail:

- to uncover resource allocation patterns within the stands, i.e. the distribution of light and water, as well as the spatial distribution of the production area;
- to analyze the effects of the mixing structure and of the resulting resource allocations on productivity and efficiency; and
- to compare and explain the relationships of resource allocation and growth patterns both for the two species of the mixture and the entire stand along with the influence of yearly variations.

2. Model BALANCE

BALANCE, the model used for the scenario simulations, is a physiological growth model that calculates the 3-dimensional development of trees and forest stands and estimates the consequences of environmental influences. BALANCE simulates growth responses on the individual tree level and enables an estimation of the influence of competition, stand structure, species mixture, and management impacts. This is achieved by describing tree development as a response to individual environmental conditions and environmental change of conditions with individual tree development. This way, additionally to the weather conditions, CO_2 concentration, soil condition, competition between individuals, and stress factors can be regarded. The initial biomass of an individual tree is calculated from the dimensional variables tree position, tree- and crown base height, diameter, and crown radii. The increase in biomass is the result of the interaction of physiological processes which depend on the physical and chemical micro-environment that is itself influenced by the spatial structure of the stand. It is calculated based on the carbon and nitrogen that is taken up from each segment in dependence on the local energy supply and resource availability. Stress conditions as for example droughts are considered by changing the specific uptake rates below- and aboveground.

Individual carbon-, water- and nutrient balances of the tree species beech, oak, spruce, pine and Douglas fir are the fundamental processes for the growth simulations. The spatial calculation levels range from stand level and individual trees over tree components (i.e. foliage, branches, stem, fine and coarse roots) to crown and root layers, which are vertically and horizontically divided into eight crown- and root segments.

BALANCE enables the representation of asymmetric crown shapes and a spatially explicit consideration of the environment. For each layer resp. each segment micro climate and water balance are calculated by using temperature, radiation, precipitation, humidity and wind speed. While the spatial variability for light- and water availability is both calculated on a daily basis, the physiological processes assimilation, respiration, nutrient uptake, growth, senescence and allocation are calculated in 10 day time steps from the aggregated driving variables. All tissues of a segment, i.e. foliage, branches or fine and coarse roots as well as the biomass of the stem are mechanistically related to each other according to the pipe model theory (Shinozaki et al. 1964, Chiba 1998).

To describe the relationships between environmental influences and growth, the development of the foliage in time and space must be known. With the beginning of bud burst foliage, biomass and leaf area as well as light availability and radiation absorption change. Thus, the date of foliage emergence of a tree determines its assimilation and respiration rate but also affects the environmental conditions of the trees in its vicinity. In BALANCE the beginning of bud burst is modeled by using a temperature sum model (Rötzer et al. 2004), while foliage senescence is estimated in dependence on the respiration sum (Rötzer et al. 2010b).

Photosynthesis is calculated by using the approach of Haxeltine and Prentice (1996) as a function of leaf surface, light, temperature and CO_2 concentration and is reduced by the lack of water and nutrients as well as changed by pollutants. The most prominent factor of this calculation is the radiation amount which a segment receives in a time step. To determine the radiation sum of a segment, a ‘competition-cone’ is placed over each segment and all segments within this cone are counted and weighted according to their leaf area. This calculation of the relative light consumption was derived from the competition algorithm of the growth simulator SILVA (Pretzsch 1992) and extended by a light extinction function of a Lambert-Beer type. In contrast to SILVA, in BALANCE the search cone for the competition estimation is not applied once for every individual tree, but separately for each single crown segment. Consequently, light intensity of each segment is obtained based on the global radiation, the extinction coefficient of foliage and the competition factor of the segment.

Respiration is estimated according to Thornley and Cannell (2000). It is separated into respiration for uptake and transport processes and compartment-specific growth respiration, as well as into respiration for maintenance purposes.

The Penman-Monteith equation (e.g. *DVWK* 1996), i.e. the calculation of the evapotranspiration, forms the base to estimate the water balance of each single tree. Hereby, the change in the soil water content is calculated from the daily values of precipitation, interception, percolation, and transpiration. A simple multi-layer bucket soil water model with a fixed layer width considers vertical water flows. In the horizontal extension, the model distinguishes between rooted and non-rooted fractions in each layer. The water within the rooted fractions can be used to fulfill the tree's transpiration requirements. At the end of each day the water content within the rooted and non-rooted fractions of a layer is aligned.

Via stomatal closure water balance is connected with the carbon and nitrogen cycle. The fixed carbon that is not needed for respiration is used for the distribution into the plant compartments foliage, branches, stem and roots depending on their relative sink strength (Grote 1998). It is computed according to functional balance (Mäkelä 1990) and pipe model principles (Shinozaki et al. 1964, Chiba 1998).

Dimensional tree growth is calculated once a year on the base of the biomass increase that has been accumulated during the year. The share that every crown resp. root segment gains is defined by its relative contribution to the net carbon and nitrogen increase. The volume expansion depends on the necessary amount of twigs and transport branches, resp. on the amount of coarse roots for the root segments. Therefore, crown development is preferred in the direction towards the best assimilation conditions during the previous year.

A detailed description of the model can be obtained in Grote and Pretzsch (2002), Rötzer et al. (2005), Rötzer et al. (2009) and Rötzer et al. (2010b).

3. Scenario Definition

For the scenario simulations the site conditions from the 'Kranzberger Forst' were used. This site – located in southern Bavaria (Germany), about 40 km northeast of Munich (48.420° N, 11.662° E, elevation 490 m a.s.l.) – was the intensive forest research plot of the SFB 607 (Matyssek et al. 2010) and formed the base for the parameterization of the model BALANCE.

3.1 Climate

On average of the 10 year simulation period from 2000 to 2009 the annual air temperature was 8.3 °C, and the annual precipitation sum was 854 mm. Thus, temperature surpassed the long term average which ranges between 7–8 °C for the period 1951–1980. Precipitation, on the other hand, was on the upper limit of the long term regional average with 750–850 mm for the period 1961 to 1990 (*BayForklim* 1996).

Within the 10 year period a substantial inter-annual variability of temperature and precipitation is obvious (Fig. 1). Extremes are the year 2003, which was very hot and the driest year, and the year 2001 with a low annual mean temperature and the highest precipitation sum.

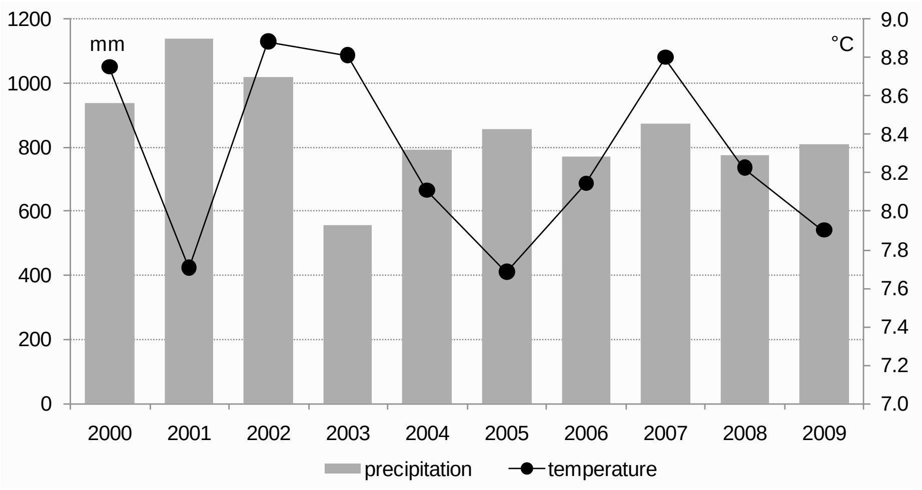

Fig. 1 Annual air temperature and precipitation sums for the simulation period from 2000 to 2009

3.2 Soil Conditions

The soil of the 'Kranzberger Forst' can be assigned as Luvisol, derived from loess over tertiary sediments, with good water and nutrient supply. The soil is composed of 60 % silt, 20 % clay and 20 % sand within the upper 100 cm, while below 100 cm the quantity of sand increases to 80 %. For the simulations the soil was classified in four layers (0–5 cm, 5–35 cm, 35–85 cm and 85–100 cm). Field capacity and wilting point for each soil layer were estimated with 49, 37, 37 and 37 vol % resp. 11, 8, 10 and 23 vol % based on the studies of SCHUHBÄCK (2004). The maximum rooting depth was assumed at 1.0 m.

3.3 Stand Description

Virtual mixed tree stands of adult Norway spruce (*Picea abies* (L.) Karst.), and European beech (*Fagus sylvatica*) form the base of the scenario simulations. As spatial structures a random distribution of beech and spruce trees as well as a mixture with groups and clusters of beech trees were assumed. In Figure 2 the crown maps of the three plots outline the spatial distributions.

The plot size was 30 m × 30 m for all stands. Because edge trees were not regarded in the analyses the stand area decreased to 456 m² for the random mixture *RM*, to 557 m² for the clustered mixture *CM* and to 625 m² for the group mixture *GM*. When creating the stand using

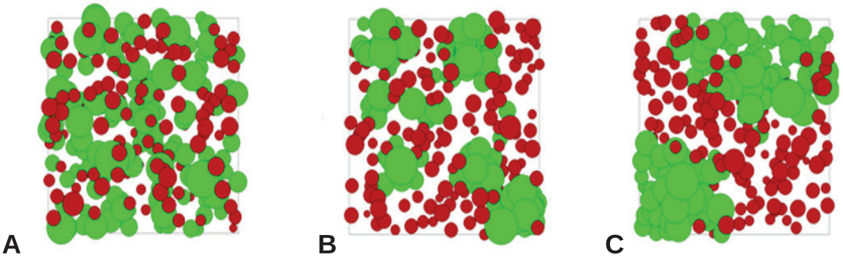

Fig. 2 Crown maps of the three virtual mixed stands: (*A*) random distribution, (*B*) cluster distribution, (*C*) group distribution (green circles denote beech trees, red circles spruce trees).

the STRUGEN simulator (Pretzsch 1997) we tried to maintain similar base stand parameters for the three stands. Consequently, the initial mean diameter in breast height *dbh* of all spruce trees for the analyzed plots was 14.7 cm for *RM*, 14.9 cm for *CM* and 14.3 cm for *GM*, while mean *dbh* of the beech trees was 9.2 cm, 9.5 cm and 9.5 cm, respectively. The average initial tree height ranged between 15.2 m and 15.3 m for the spruce trees and between 15.2 m and 14.7 m for the beech trees.

4. Results

4.1 Spatial Patterns of Resources and Leaf Area Index

In order to analyze the influence of resources on the growth of tree individuals and to upscale the increments on stand level, the spatial distribution of the resources must be known. Two crucial resources for growth are light and water. Their changes in space and time are mainly determined by canopy architecture, species composition, stand density but also by the annual biomass development.

The spatial distribution of the radiation within the three differently structured mixed forest stands of beech and spruce averaged over the 10 year period can be seen in Figure 3.

On the base of the virtual stands and influenced by the environmental conditions of the years 2000 to 2009 for the annual average radiation of the random mixed stand a maximum frequency of 26 % was calculated with radiation sums of 1630 MJ m^{-2}. The value range, however, for this mixture was narrow (1350–1750 MJ m^{-2}). On the other hand, broader ranges of radiation amounts were found for the grouped and clustered mixtures, with maximum frequencies of 19 % at 1700 MJ m^{-2} for *CM*, respectively 22 % at 1570 MJ m^{-2} for *GM*.

If the values of the individual trees are summed up over the analyzed stands, the mean radiation sum for the two different species as well as for the entire stand can be calculated (Fig. 4).

In all mixtures spruce trees received higher radiation sums than beech trees. In *CM* the difference was highest, while in *RM* there was no significant difference. For the entire stands the annual average radiation sum received by a tree was highest for *RM* with 1587 MJ m^{-2} and lowest for *GM* with 1499 MJ m^{-2}.

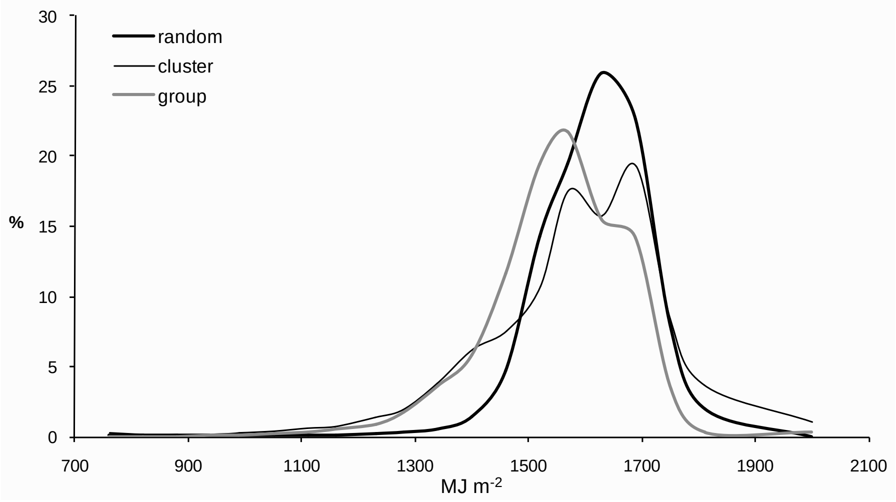

Fig. 3 Frequency distribution of the annual radiation sum a tree received in random, clustered and grouped mixtures of beech and spruce averaged over 10 years.

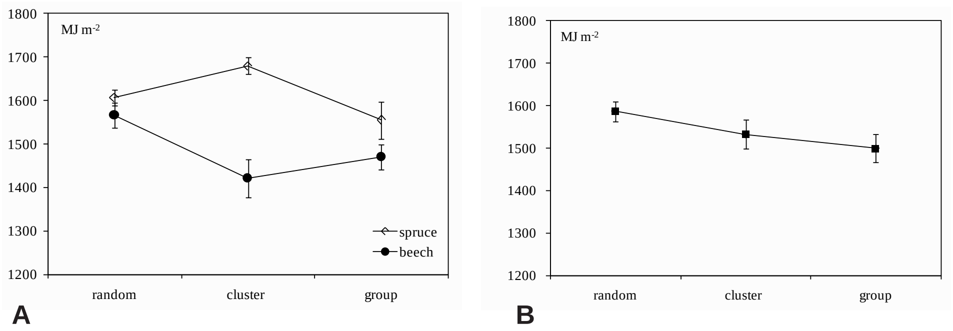

Fig. 4 Annual radiation sum received by a tree averaged over 10 years for the single species (*A*) and for the entire stand (*B*) in random, clustered and grouped mixtures.

The spatial distribution of the resource water is represented by the sum of the actual evapotranspiration in the vegetation period (May to September) eta_{VP}, which denotes the water supply of a tree very well. In Figure 5 the frequency distribution of the resource in random, clustered and grouped mixtures is given.

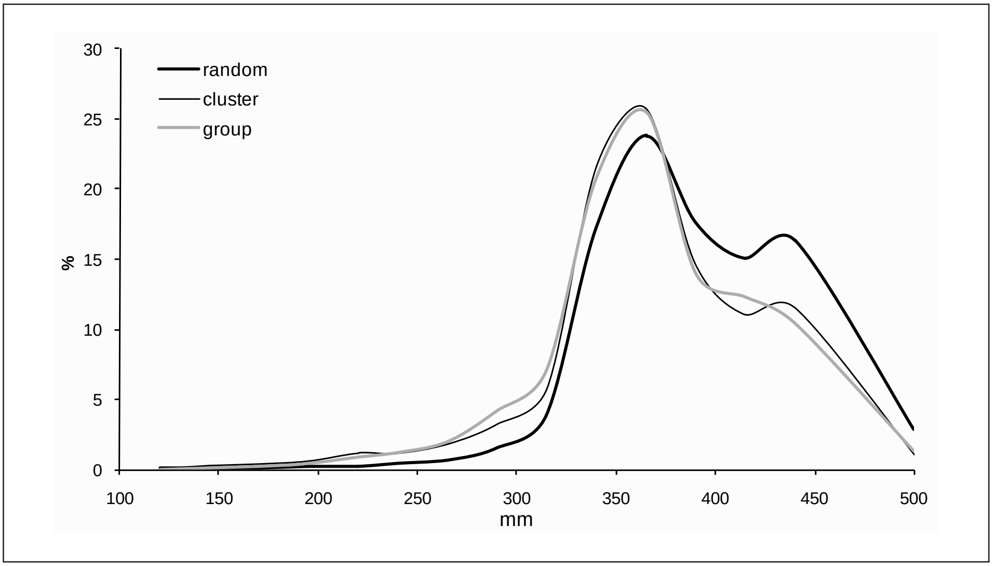

Fig. 5 Frequency distribution of the annual actual evapotranspiration in the vegetation period in random, clustered and grouped mixtures of beech and spruce averaged over 10 years.

In the random mixture the average actual evapotranspiration sum was higher than 400 mm in more than 50 % of all cases. For the grouped resp. the clustered distributions more than 60 % of all cases have eta$_{VP}$ values between 300 – 400 mm. If eta$_{VP}$ of the mixtures is calculated separately for the tree species (Fig. 6), the values for spruce trees were at 405 mm, the values for the beeches at 312 mm, both in the random mixture. While for the clustered mixture only a small difference between the two species can be seen (339 mm *versus* 344 mm), in the grouped mixed stand eta$_{VP}$ was higher for the beech trees (366 mm) compared to the spruce trees (304 mm).

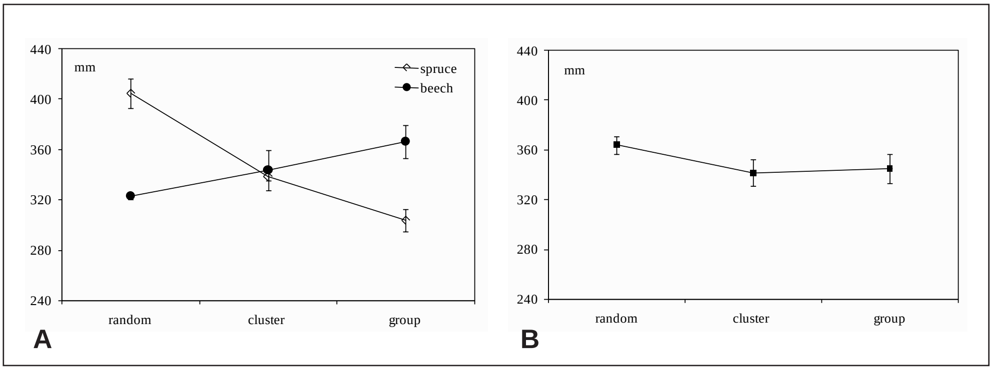

Fig. 6 Actual evapotranspiration sum in the vegetation period averaged over 10 years for the single species (*A*) and the entire stand (*B*) in random, clustered and grouped mixtures.

Summed up for the entire stands the actual evapotranspiration sum in the vegetation period reached the highest amount with 364 mm in the random mixture, but only 342 mm resp. 345 mm in the clustered resp. group mixture.

The same pattern as for the actual evapotranspiration of the three mixed stands can be found for the production area expressed by the leaf area index *LAI* (Fig. 7).

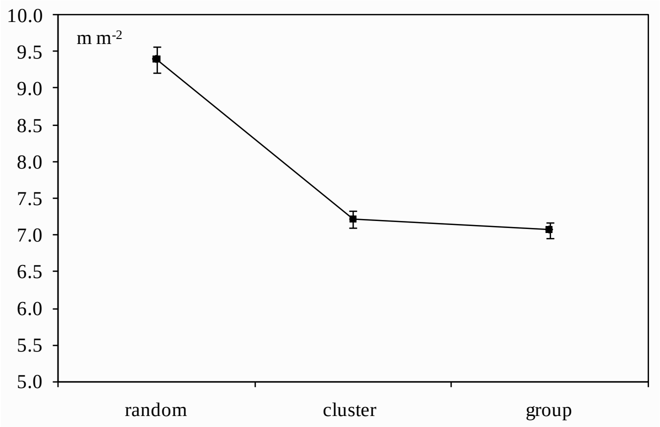

Fig. 7 Leaf area index within the period May to September (mean 2000–2009) averaged over 10 years in the random, the clustered and the grouped mixture.

Averaged over the 10 analyzed years a leaf area index of 9.4 $m^2\ m^{-2}$ was calculated for the random mixed stand for the vegetation period from May to September. With values of 7.2 $m^2\ m^{-2}$ for the clustered resp. 7.1 $m^2\ m^{-2}$ for grouped mixed stand the production area of the random mixed stand was significantly higher.

4.2 Growth and Efficiencies

Based on the spatial and temporal distribution of the resources and the production area within the three mixed stands, changes in their productivity could be expected, too. In Figure 8 the mean annual biomass increment both for the spruce and the beech trees in the different mixtures are presented. For all mixtures nearly no change in the biomass increment of spruce is detectable, the values vary from 7.8 t C $ha^{-1}\ yr^{-1}$ for the clustered mixture to 8.0 t C $ha^{-1}\ yr^{-1}$ for the group mixture. For beech trees clear but not significant differences are obvious: From 5.7 t C $ha^{-1}\ yr^{-1}$ in *CM* over 6.0 t C $ha^{-1}\ yr^{-1}$ in *GM* the annual biomass increment rose up to 6.2 t C $ha^{-1}\ yr^{-1}$ in *RM*. For the entire stands this denotes mean annual increments of 6.6 t C $ha^{-1}\ yr^{-1}$ for the clustered and grouped stand and 6.9 t C $ha^{-1}\ yr^{-1}$ for the randomly mixed stand.

The differences of 4 % between the randomly mixed stand of beech and spruce and the clustered resp. grouped mixed stands are based on the yearly variations of the biomass increments of the random mixed stand compared to clustered resp. grouped mixed stand (Fig. 9). While for the years 2000, 2001, 2002 and 2009 negative differences were calculated – i.e.

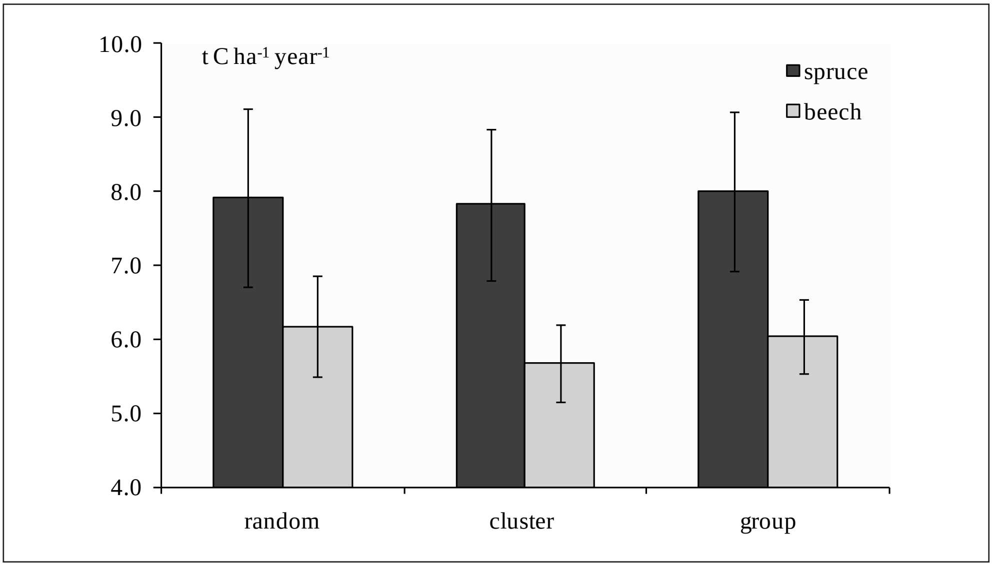

Fig. 8 Annual biomass increment of spruce and beech in random, clustered and grouped mixtures averaged over the years 2000–2009

higher growth rates for *RM* –, the period from 2003 to 2008 show higher increments for the clustered resp. grouped mixture – i.e. smaller growth rates for *RM* –. The highest differences can be seen for the years 2001 and 2003. In the year 2001, with a low mean annual temperature and the highest annual precipitation amount, the random distribution benefitted. In the dry and hot year 2003, on the other side, the clustered and grouped mixture gained.

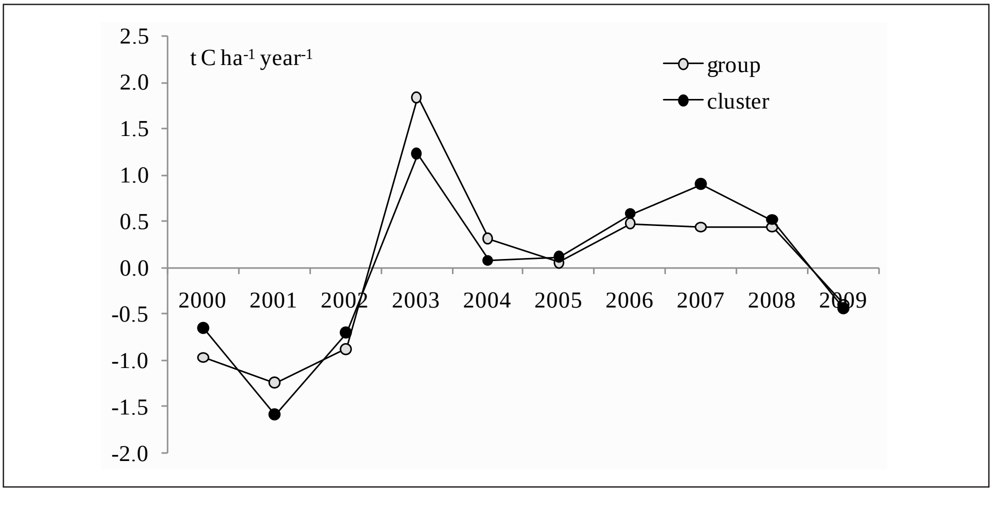

Fig. 9 Yearly differences between the annual biomass increment of the clustered resp. the grouped mixture and the random mixture for the years 2000–2009 (standardized on the annual mean)

From the net primary productivity and the actual evapotranspiration sum the water use efficiency *WUE* of spruce and beech trees in stands with random, clustered and grouped distribution can be calculated (Tab. 1). For spruce only very small differences in the efficiencies between 4.9 g kg^{-1} (*GM)* and 5.2 g kg^{-1} (*RM*) are obvious. Beech trees, however, were more efficient in the random mixture (3.9 g kg^{-1}) than in clustered or group mixture (3.3 g kg^{-1} resp. 3.5 g kg^{-1}).

Tab. 1 Water use efficiencies (g kg^{-1}) of the spruce and the beech trees as well as of the entire stands for random, clustered and grouped mixtures averaged over the years 2000–2009.

mixture	random	cluster	group
spruce	5.2	5.1	4.9
beech	3.9	3.3	3.5
entire stand	4.5	4.2	4.2

For the entire stands differences of 6 % resp. 7 % between the clustered resp. the grouped distribution and the random distribution were found.

5. Discussion and Conclusions

In the scenario simulations considerable spatial variations of the resources within the random, the clustered and the grouped mixtures were found both for the distribution of light and water. The growth responses were different for the two species beech and spruce. On base of the entire stand the higher light and water amounts which the random mixed stand received along with higher *LAI* values resulted in higher growth rates, all compared to the clustered and group mixed stand.

Baldocchi et al. (1984) or Ross et al. (1986) have already given examples of variations and changes in the radiation regime within an oak-hickory forest stand resp. in boreal forests. Crown structure and density as well as leaf properties influence the spatial distribution of radiation and result in different levels of sunny and shady areas (Canham 1994, Leuchner et al. 2012). When comparing beech and spruce Leuchner et al. (2012) found fundamental differences in the light regimes mainly due to the different shapes of the species: While the plane shape of the dense upper beech canopy absorbed most of the incoming radiation in the top layer, more radiation could penetrate into the spruce canopy due to larger gaps between the trees. This is especially true for high fractions of direct radiation. Thus, different structured mixed forest stands result in different radiation regimes. Because of the higher roughness the randomly mixed spruce beech stand received more radiation compared to a clustered or grouped distribution averaged over the 10 years.

On average, the actual evapotranspiration within the vegetation period – used here as an index for the spatial allocation of the resource water – was also higher in the randomly mixed stand than in the clustered respectively grouped mixed stand. Since rooting architecture varies to a great extent from tree species to tree species, different species can occupy different soil zones. While the rooting system of Norway spruce is rather shallow (e.g. Lyr et al. 1992, but see Puhe 2003), the roots of beech trees grow clearly deeper. For mixed spruce beech stands Schmid (2002) found higher fine biomass values in mixed stands compared to the corresponding pure stands. Compared to the pure stands the fine roots of the spruce trees were enriched in the upper soil layers (Schmid 2002), the fine roots of beech, however, in the deeper soil layers (Rothe and Binkley 2001, Schmid 2002). Other studies (Hendriks and Bianchi 1995, Bolte and Villanueva 2006) support these findings that beech trees in mixture concentrate their roots in deeper soil layers, while the roots of the second species dominate the upper layer. This way, trees within a mixed stand can obtain water from different fractions of the soil (Forrester et al. 2010). And an increased water uptake by the trees denotes higher transpiration rates. Comparing forest stands with different mixing patterns, a higher intermingling from the group mixture over clustered mixture to randomly mixture is obvious (Fig. 2). Hence, the stratification of the roots is improved from *GM* to *RM* resulting in higher water uptake rates and higher transpiration sums of the single trees (Fig. 5 and 6).

Following Zweifel et al. (2009) the complex physiological relationships between the water and the carbon cycle can be simplified in the way that more open stomata (induced by the improved water supply) provide higher carbon uptake rates and thus an increased biomass production (Forrester et al. 2010). This effect can also be seen for the stands with different mixing structures: while the mean annual biomass increment of the group resp. the clustered mixture was 6.6 t C ha^{-1} yr^{-1}, the increment of the randomly mixed stand was 6.9 t C ha^{-1} yr^{-1}. This denotes an increase of 4 %.

Elevated transpiration rates of a tree, on the other hand, can also be caused by the higher radiation amounts a tree receives. As stated above the different shapes of the tree species in a mixed forest stand allows radiation to penetrate deeper into the canopy. Since radiation is a main parameter for the evapotranspiration by providing energy for the process (e.g. *DVWK* 1996) the higher radiation regimes in a mixed forest stand may enhance evapotranspiration. Increasing radiation amounts of the entire stands from *GM* to *CM* to *RM* (Fig. 4) elevated the transpiration in the same direction (Fig. 6). Consequently, above as well as below ground competition is reduced and leads to higher growth rates, particularly within the beech trees. Pretzsch et al (2010) have already found that spruce in mixture with beech reduces the degree of intra-specific competition which is obvious in pure beech stands, and thus facilitates beech growth while the growth of spruce is shortened.

Because of the better occupation of space leaf biomass – expressed as *LAI* – is increased in mixed stands compared to pure stands (Forrester et al. 2010), but could also be enhanced in mixed stands with higher intermingling. This could be emphasized by the results of this study, i.e. by the increased *LAI* in the randomly mixed stand compared to the group or clustered mixture (Fig. 7).

As a consequence of a higher radiation regime and higher transpiration values along with higher *LAI* values total biomass increment was enhanced in the randomly mixed stand compared to the clustered and grouped mixed stands. The simulated mean annual total biomass increment was 7.9 t C ha^{-1} yr^{-1} for the spruce trees and 6.0 t C ha^{-1} yr^{-1} for the beech trees averaged over all mixing structures. Based on the data of Mund et al. (2002) the mean annual biomass increment of 35 to 43 year old, Central European spruce stands was at 8.2 t C ha^{-1} yr^{-1}. Under Central European conditions Rötzer et al. (2010a) calculated net biomass productivities for even-aged pure stands with up to 3.1 t C ha^{-1} yr^{-1} for spruce and up to 2.9 t C ha^{-1} yr^{-1} for beech. The higher values simulated within this study fit well with the estimated mean annual biomass increments Pretzsch et al. (2012, 2013) found in Southern Germany with 6.4 t C ha^{-1} yr^{-1} for spruce in pure stands and with 7.2 t C ha^{-1} yr^{-1} for spruce in mixed stands, respectively 5.8 t C ha^{-1} yr^{-1} for beech in pure stands and with 5.9 t C ha^{-1} yr^{-1} for beech in mixed stands.

However, climate extremes of different years altered biomass increments to a great extent. The differences of 4 % between the randomly mixed stand and the clustered resp. grouped mixed stands are based on the high year to year variability of the biomass increments (Fig. 9). It is remarkable that in the exceptionally dry and hot year 2003 the biomass increment of the clustered and grouped mixture was clearly higher than for the random mixture, while for the year 2001 with a low annual mean temperature and a high precipitation amount it was the other way round. A close intermingling of the species spruce and beech seems to be favorable under wet and cold conditions while a less close mixing copes better with dry and hot conditions.

The most efficient stand – based on the resource water – was the randomly mixed stand. Spruce trees as well as beech trees in this mixture show the highest *WUE* values with 5.2 g kg^{-1} resp. 3.9 g kg^{-1}. Cienciela et al. (1994) found an average *WUE* of 4.8 g kg^{-1} for spruces in Sweden. Polster (1950) and Pretzsch (2009) estimated the water use efficiency with values of 4.3 g kg^{-1} resp. 5.1 g kg^{-1} for spruce and of 5.9 g kg^{-1} resp. 4.4 g kg^{-1} for beech. Polster (1950), however, used aboveground biomass and transpiration for his calculations. From the measurements of Grünwald and Bernhofer (2007) a *WUE* for the Tharandt spruce forest of 7.8 g kg^{-1} on average can be derived, assuming net ecosystem productivity as numerator and evapotranspiration as denominator (hereby interception is estimated as 40 % of precipitation). Hence, the simulated water use efficiencies for the differently structured mixed forests are in line with the cited values.

Forrester et al. (2010) reported about higher *WUE* in mixed Eucalyptus and Acacia plantations compared to the corresponding pure stands which suggests structural differences such as increased biomass leaf area, sapwood area and root stratification additionally to physiological adjustments. The results presented here underline these findings for mixed stands with different mixing structures.

While the water use efficiency of spruce trees in mixture increased by 6 % from *GM* to *RM*, the efficiency of the beech trees increased by 18 % from *CM* to *RM* (Tab. 1). This implies that the beech trees profit from a closer intermingling (= random mixture) more than the spruce trees do. Despite of lower actual evapotranspiration rates of beech in *RM* compared to *GM* and *CM* (Fig. 6), but of higher radiation amounts (Fig. 5) biomass production and in consequence *WUE* was increased clearly. For spruce, however, higher actual evapotranspiration rates of *RM* compared to *GM* and *CM* (Fig. 6) and only small changes in the biomass production lead to only small increases in the water use efficiencies.

Generally, the presented scenario simulations delivered realistic results for differently structured mixed spruce beech forests. Physiological growth models – as e.g. BALANCE – can help to analyze the effects of structured mixed forest stands on resource allocation, productivity and efficiency. It could be revealed that the changes of the resource allocations, productivities, and efficiencies found in mixed stands compared to pure stands (Pretzsch und Dieler 2011, Pretzsch et al. 2012, 2013 Forrester et al. 2010) are also obvious in stands with different mixing structures. To quantify the influence of mixing structures more scenario simulations are helpful and more empirical analyses have to be done.

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Tree-internal Nutrient Distribution of Beech and Spruce at the Kranzberger Forst – Implications for Efficiency of Wood Production and for Nutrient Export with Different Harvest Intensities

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With 3 Figures and 4 Tables

Abstract

Amount and tree-internal distribution of nutrients have an influence on the efficiency of production and maintenance of biomass as well as on nutrient exports by harvest. At the Kranzberger Forst, a site with optimal nutrient supply, three trees each of beech and spruce, at the age of 54 resp. 61 years, were harvested and analyzed in detail. Because of the low proportion of leafs, the green biomass of beech has a high nutrient related efficiency of wood production as well as of above ground biomass maintenance. However, for beech with its high amount of nutrients in the wood the efficiency of wood formation is rather low. Because of the smaller crown projection area for spruce most calculated space related efficiencies for this species are more favorite. Calculating the space related efficiency of biomass maintenance, however, the two species have rather similar values. Whole tree harvest of spruce leads to clearly higher nutrient exports at the site Kranzberger Forst, due to higher tree density and the higher allocation of nutrients to the crown.

Zusammenfassung

Menge und bauminterne Verteilung von Nährstoffen beeinflussen sowohl die Effizienz von Biomassebildung und -unterhalt als auch den Export von Nährstoffen bei der Biomasseernte. Im Kranzberger Forst, einem Standort mit ausgeglichenem Nährstoffangebot, wurden je 3 Fichten und 3 Buchen im Alter von 54 bzw. 61 Jahren geerntet und einer detaillierten Beprobung unterzogen. Aufgrund des geringen Gewichtsanteils an Blättern zeigen Buchen eine hohe nährstoffbezogene Effizienz der grünen Biomasse sowohl in Bezug auf die Bildung von Holz als auch in Bezug auf die Unterhaltung der gesamten oberirdischen Biomasse. Die Buche hält jedoch mengenmäßig einen sehr hohen Anteil ihrer Nährelemente im Holz und ist somit relativ ineffizient in der direkten Holzbildung. Führt man bei den Effizienzbetrachtungen einen Flächenbezug ein, so schneidet die Fichte wegen ihrer geringeren Kronenschirmfläche deutlich günstiger ab. Bei der flächenbezogenen Effizienz des Biomasseunterhalts sind sich die Baumarten jedoch sehr ähnlich. Aufgrund der bauminternen Nährstoffverteilung und der höheren Stammzahl werden bei einer Vollbaumernte im Kranzberger Forst bei Fichte deutlich mehr Nährelemente exportiert als bei Buche.

1. Introduction

Although studies of nutrient concentrations in different tree organs and in foliage are available (e.g. Jacobsen et al. 2003, Rademacher 2005, Wyttenbach et al. 1995), detailed data for the comparison of beech and spruce with respect to nutrient allocation on the same site

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are rather scarce. For a better understanding of the differences between these two species, which may also reflect different ecological strategies, a comparative study of mature beech and spruce trees growing at the Kranzberger Forst (Bavaria, Germany), a site with nearly optimal nutrient supply was carried out. Nutrient concentrations of all plant compartments in combination with the respective biomass and volume increment were used to calculate the nutrient related efficiency of wood production as well as the implications of tree internal nutrient distribution for quantification of nutrient export for different harvest intensities.

2. Material and Methods

In 2004 at the experimental site "Kranzberger Forst" (Pretzsch et al. 1998), three representative trees each of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.) were chosen for total harvest and detailed nutrient analysis of above ground biomass. Tree ages were determined with 54 years for spruce and 61 years for beech. According to common practices for nutrient analysis (*BMELF* 1994) beech was harvested at the beginning of August (fully developed leaves before senescence) and spruce in November (vegetation dormancy).

For the three harvested trees of beech, three main branches each were chosen and representatively sampled for leaves in different distances from the top, as well as twigs and branches of different diameter classes. For branch samples > 1 cm diameter wood and bark were analyzed separately. For the three harvested trees of spruce, one representative branch each of the whorls 1, 4, 7, 10, 15 and 20 was chosen for analysis. For each branch representative samples from needles of each age class as well as twigs from each age class were taken. Samples from branches were grouped by diameter classes and analyzed separately for bark and wood. For beech and spruce stem discs were cut in different stem heights and separately analyzed for bark and wood. For more details with respect to the sampling scheme see Göttlein et al. (2013).

All samples were dried, ground to powder and analyzed for nutrient elements by acid digestion with HNO_3 and subsequent ICP-OES spectroscopy (for metals, P and S) as well as by combustion in an elemental analyzer (for N). For detailed description of the analytical methods used see *Gutachterausschuss Forstliche Analytik* (2005). Biomasses of the trees were calculated using the detailed measurements during harvest and the model Silva (Pretzsch et al. 2002). All data were specified resp. calculated on a dry weight basis. Growth increment of woody biomass of the selected trees was derived as an annual average from a five years period before harvest (1999–2004). The data given for the area of one hectare were calculated on the basis of all measured 482 beech trees respectively 553 spruce trees and subsequent extrapolation.

The soil at Kranzberger Forst is characterized as a luvisol derived from loess, layered above tertiary sediments and showing a tendency to stagnant moisture in the deeper mineral soil (according to the German soil classification: "im Unterboden schwach haftnässe-pseudovergleyte Parabraunerde aus Löß über tertiären Molassesedimenten"). The combination of low pH in the topsoil and high base saturation in the deeper soil enables an optimal nutrition for beech and spruce for macro and micro nutrients (Göttlein et al. 2013).

Although statistics with only three random samples per group are very critical, mean values of beech and spruce were compared using t-Test, supposing that there is an approximately normal distribution of the collectives and that their variances are homogeneous.

3. Results and Discussion

3.1 Tree Internal Nutrient Distribution of Beech and Spruce

For beech and spruce at Kranzberger Forst about three quarter of the biomass is allocated to trunk wood, in Figure 1 divided into the fractions stem and crown. The rest of the biomass is distributed between branches, twigs, bark and foliage, with differences emerging between species. Beech has a high proportion of branch biomass, with a considerable amount allocated to branches greater 7 cm in diameter which is the smallest diameter for merchantable wood. In contrast, spruce shows a higher amount of biomass allocated to needles and bark. Although the proportion of bole wood is similar for beech and spruce there are large differences in the proportion of nutrients stored in this compartment. Beech allocates much more nutrients into the wood than spruce, with highest differences found for K and Mg. With respect to branches (< 7 cm) and twigs the very high proportion of Ca allocated to these compartments by beech is striking. In contrast spruce allocates a much higher proportion of K, Mg and Ca into the bark. Due to its evergreen character spruce holds more biomass in its green parts and also incorporates there a higher proportion of nutrients (Tab. 1). It is well known since long time (Wolff 1880, Fiedler et al. 1973) that usually nutrient concentrations in plant tissues of beech are higher than in spruce. However, studies on nutrient partitioning within mature trees of beech and spruce are scarce especially with the focus to compare the two species on the same forest site. Weis and Göttlein (2002) compared nutrient storage in beech and spruce at the experimental site “Höglwald”, however, in the period of vegetation dormancy so that the green biomass of beech was not included in their study. For beech a study on nutrient partitioning was done in Brandenburg/Germany (Krauss and Heinsdorf 2008), resulting in a very similar biomass distribution as compared to the Kranzberger Forst. However, for the partitioning of nutrients there were marked differences with beech at Kranzberger Forst incorporating a lower proportion of P and K to stem wood, whereas the proportion of Ca in this compartment was considerably higher. These differences are on the one hand due to different site conditions, on the other hand genetic differences (provenance) may be of importance. Because it is long known, that nutrient contents in plant tissues vary dependent on soil chemical properties (Becker-Dillingen 1939) the detailed results obtained at Kranzberger Forst may only be extrapolated to comparable site conditions, cautioning generalization for beech or spruce. However, the obvious and general differences between the two species with respect to biomass and nutrient allocation can lead to universally valid conclusions about aspects of efficiency of wood production and nutrient exports by harvesting as described in the following chapters.

Tab. 1 Percentage of biomass and nutrients allocated to the green biomass of beech and spruce; lower values are printed in italics; significant differences between species are indicated by * for $p < 0.05$

	Biomass	N	P	K	Ca	Mg
Spruce needles	5.1*	22.0*	27.4*	20.8*	13.3*	14.7*
Beech leaves	*1.0*	*9.4*	*11.5*	*6.1*	*3.4*	*5.7*

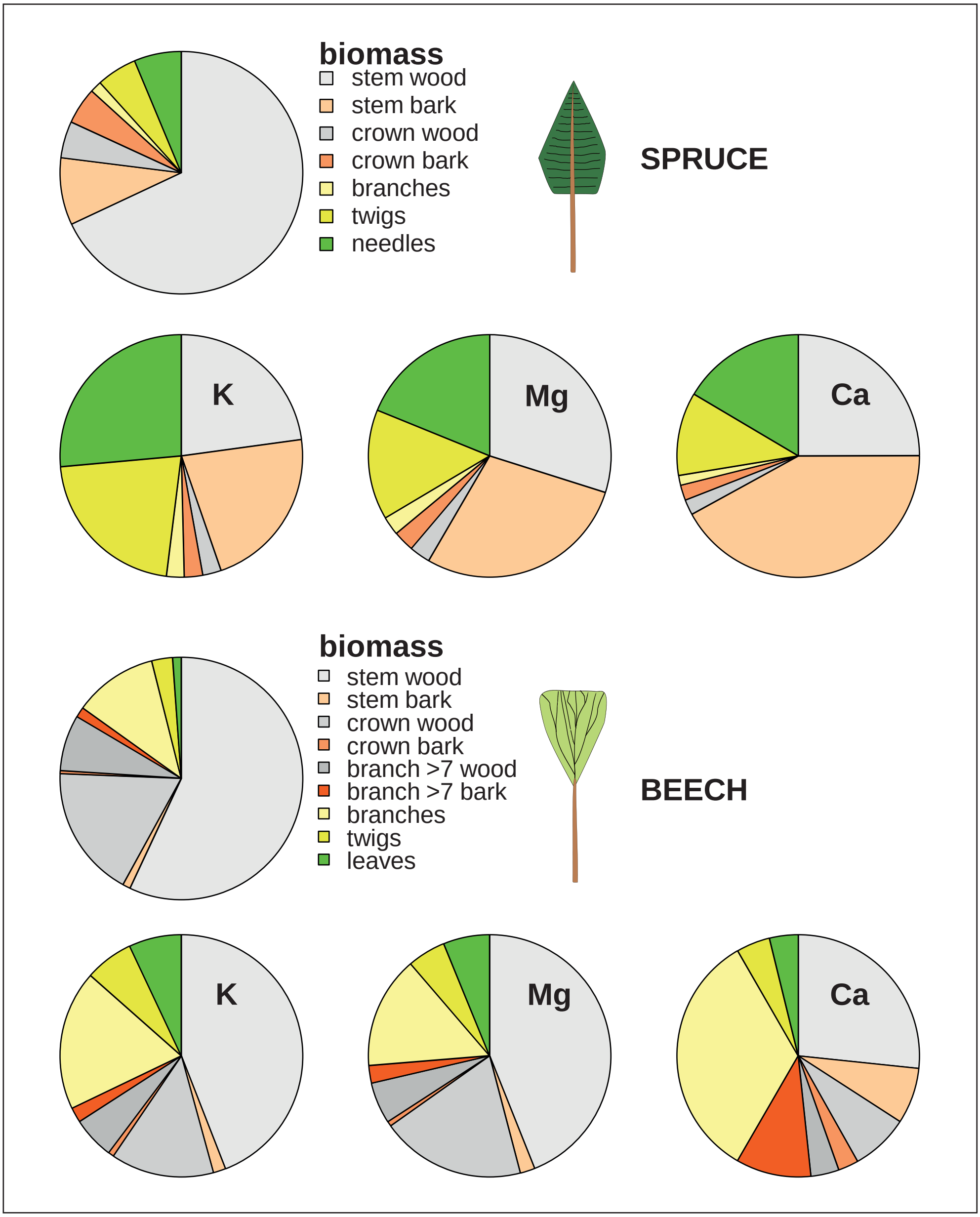

Fig. 1 Allocation of biomass and nutrients to different compartments of spruce and beech

3.2 Nutrient Related Efficiency Parameters of Wood Production

In Table 2 the nutrient related efficiency of wood production of single trees is calculated on three different principles. With respect to the amount of nutrients allocated to the green biomass for all elements beech is much more effective in wood production than spruce. This is valid for calculations based on volume (cm³ wood per g nutrient) as well as on weight (g wood per g nutrient). This differentiation is important because of the large difference in wood density of the two species. Especially for P the efficiency of beech is very high, leading to significant differences, although there are only three trees per species with considerable tree to tree variation. With respect to the efficiency of carbon incorporation (g wood per g nutrient) also N and K reach the range of significance. The clear advantage of beech against spruce for this calculation base is not surprising, because of the comparatively low amount of nutrients allocated to the green biomass (Tab. 1).

Tab. 2 Single tree efficiency of wood production of beech and spruce in relation to nutrients allocated to the green biomass, to the total aboveground biomass and to wood biomass; lower values are printed in italics; significant differences between species are printed in bold and indicated by ^{x} for $p < 0.10$ and * for $p < 0.05$

		Nutrient related efficiency of wood production basis: green biomass		Nutrient related efficiency of wood production basis: above ground biomass		Nutrient related efficiency of wood production basis: wood biomass	
		cm³ wood per g nutrient	g wood per g nutrient	cm³ wood per g nutrient	g wood per g nutrient	cm³ wood per g nutrient	g wood per g nutrient
Spruce	N	*63*	*32*	14	*7*	**1165***	*590*
	P	*820*	*415*	*226*	*115*	45269	22906
	K	*205*	*104*	**42**^{x}	21	**4842***	**2450***
	Ca	*170*	*86*	23	*11*	**2646***	*1339*
	Mg	*1025*	*518*	150	76	**14263***	**7217***
Beech	N	133	**92**^{x}	*13*	9	*924*	638
	P	**2577**^{x}	**1778**^{x}	302	208	*29181*	*20135*
	K	470	**324**^{x}	*28*	*19*	*1623*	*1120*
	Ca	630	435	*21*	14	*2054*	1417
	Mg	1741	1201	*104*	*71*	*5026*	*3468*

Regarding the efficiency of wood production in relation to the nutrients incorporated to the whole above ground biomass for N, Ca and Mg the differences between the two species are almost vanishing. For P there is still an advantage of beech, however, far away from getting significant. For K, however, only on a volume basis the efficiency of spruce is significantly higher. Although there are no big differences on the basis of individual trees as calculated in Table 2 there are still marked differences between beech and spruce on the basis of stand area. At our site there are 550 spruce trees per ha, as compared to 296 beech trees on the same area. This means, that for hypothetical pure stands of beech or spruce on an area basis spruce is more effective in wood production than beech (see also Tab. 3).

Regarding the efficiency of wood production based on the amount of nutrients invested to the wood biomass the results are completely changing, with spruce being much more effective on a volume basis than beech, significant for all elements except of P. On the weight basis, this clear advantage of spruce, however, disappears for the elements N, P and Ca. For K and Mg spruce still is about twice as effective as beech on the weight basis.

When calculating the efficiencies listed in Table 2 in relation to the crown projection area, space related efficiencies of wood production can be derived (Tab. 3). With respect to

Tab. 3 Space related efficiency of wood production of beech and spruce with relation to nutrients allocated to the green biomass, to the whole aboveground biomass and to wood biomass; space is defined as the crown projection area; lower values are printed in italics; significant differences between species are printed in bold and indicated by ^{x} for $p < 0.10$ and * for $p < 0.05$

		Nutrient related efficiency of wood production basis: green biomass		Nutrient related efficiency of wood production basis: above ground biomass		Nutrient related efficiency of wood production basis: wood biomass	
		cm³ wood per g nutrient	g wood per g nutrient	cm³ wood per g nutrient	g wood per g nutrient	cm³ wood per g nutrient	g wood per g nutrient
Spruce	N	5	3	**1.2***	**0.6**^{x}	**99***	**50***
	P	71	*36*	19.9	10.1	4082	2066
	K	18	9	**3.7***	**1.9***	**402***	**203***
	Ca	*15*	*7*	**1.9***	**1.0***	**224***	**113***
	Mg	89	45	**12.8***	**6.5***	**1171***	**593***
Beech	N	*3*	*2*	*0.3*	*0.2*	*23*	*16*
	P	*67*	46	*7.8*	*5.4*	*735*	*507*
	K	*12*	*8*	*0.7*	*0.5*	*41*	*28*
	Ca	17	11	*0.5*	*0.4*	*52*	*36*
	Mg	*45*	*31*	*2.7*	*1.9*	*129*	*89*

nutrients in the green biomass the efficiencies of beech and spruce were rather similar with no significant differences. However, area related efficiencies of spruce on the basis of nutrients in the total above ground biomass and on the basis of nutrients in wood were much higher, being significant for all elements, except of P.

Leaving the anthropocentric view of wood production, in Table 4 efficiencies of biomass maintenance (total aboveground biomass) by the green biomass were calculated. On the individual tree level, this efficiency is significantly higher for all elements for beech. However, because the crown area of beech (average 39.9 m²) is more than three times higher than that of spruce (average 12.2 m²), on an area basis this advantage of beech is vanishing and no significant differences were detected.

Beech is the naturally dominating tree species in Central Europe with a high physiological tolerance and competitiveness (Ellenberg 1996). The high efficiency of its green biomass to support biomass and growth is important for the competitiveness of beech trees. Because with relation to crown area this advantage is not given, the key of success of beech is its capability of space occupation (Pretzsch and Schütze 2005). Beech is very shade tolerant as seedling and also produces very dark stands as adult tree (Lyr et al. 1996). Furthermore, its plasticity of crown formation is high, and thus the potential of space sequestration and filling gaps in the forest canopy (Roloff 2001). Once the canopy space has been occupied the maintenance costs for the tree are relatively low. This advantage of beech is given in the range of its ecological optimum. At the edges of this range the high potential for space sequestration and space occupation is getting lower, and thus the advantage of low maintenance costs cannot be established.

The benefit or disadvantage of the relatively high proportion of nutrients incorporated in beech wood (stem and branches) remains unclear. Compared to other important central Eu-

Tab. 4 Efficiency of biomass maintenance (g total biomass per g nutrient in green biomass) of beech and spruce; lower values are printed in italics; significant differences between species are printed in bold and indicated by ^{x} for $p < 0.10$ and * for $p < 0.05$

		g total biomass	g total biomass
		per g nutrient	per g nutrient and m^2 crown
Spruce	N	*1453*	124
	P	*18878*	*1623*
	K	*4717*	403
	Ca	*3922*	*334*
	Mg	*23551*	2032
Beech	N	**4379***	*111*
	P	**83823***	2130
	K	**15363***	*390*
	Ca	**19844***	510
	Mg	**55755***	*1417*

ropean tree species (spruce, oak, pine, fir, maple) nutrient concentrations in wood are rather high (Jacobsen et al. 2003), which is the reason why in former times especially beech wood was burned for producing potash (Stinglwagner et al. 2005). The investment for acquiring relatively high nutrient contents in woody compartments, however, could also be a competitive strategy to withhold nutrients from potential competitors, to keep nutrients in the habitat and to slowly reallocate them for the next beech generation during decomposition. Such a strategy of beech may be of advantage in natural forests, even on nutrient poor sites. In managed forests, however, high nutrient contents in woody parts lead to higher nutrient export by wood harvest. Thus, forest management has to consider such differences in nutrient contents between beech and spruce in order to warrant nutrient sustainability for future tree generations, as shown in the following paragraph.

3.3 Implications of Tree Internal Nutrient Distribution on Nutrient Export by Harvesting

For biomass, as well as for all nutrients the proportion allocated to wood is higher for beech than for spruce (Fig. 2), especially for P, K and Mg for which the allocation to wood is more than doubled compared to spruce. Adding the proportions of wood and bark, which corresponds to the conventional harvesting, the difference between beech and spruce for biomass is vanishing. It should be noted that for this approach all woody biomass compartments of beech larger than 7 cm in diameter were included, with respect to fact that considerable fractions of thick branches being especially important for pulpwood or fire wood. Furthermore, beech leaves were not included in the calculations of nutrient export because beech in general is harvested in the winter half year. However, for all nutrients, except for Ca, the relative proportion of nutrients stored in wood and bark for beech is still higher, even though spruce incorporates a high amount of nutrients to the bark. Comparing beech and spruce at Kranzberger Forst nutrient export by harvest may be more efficiently reduced in spruce stands by leaving bark and crown material in the stand. For beech the potential for a reduction of nutrient export by optimized harvesting strategies is much lower.

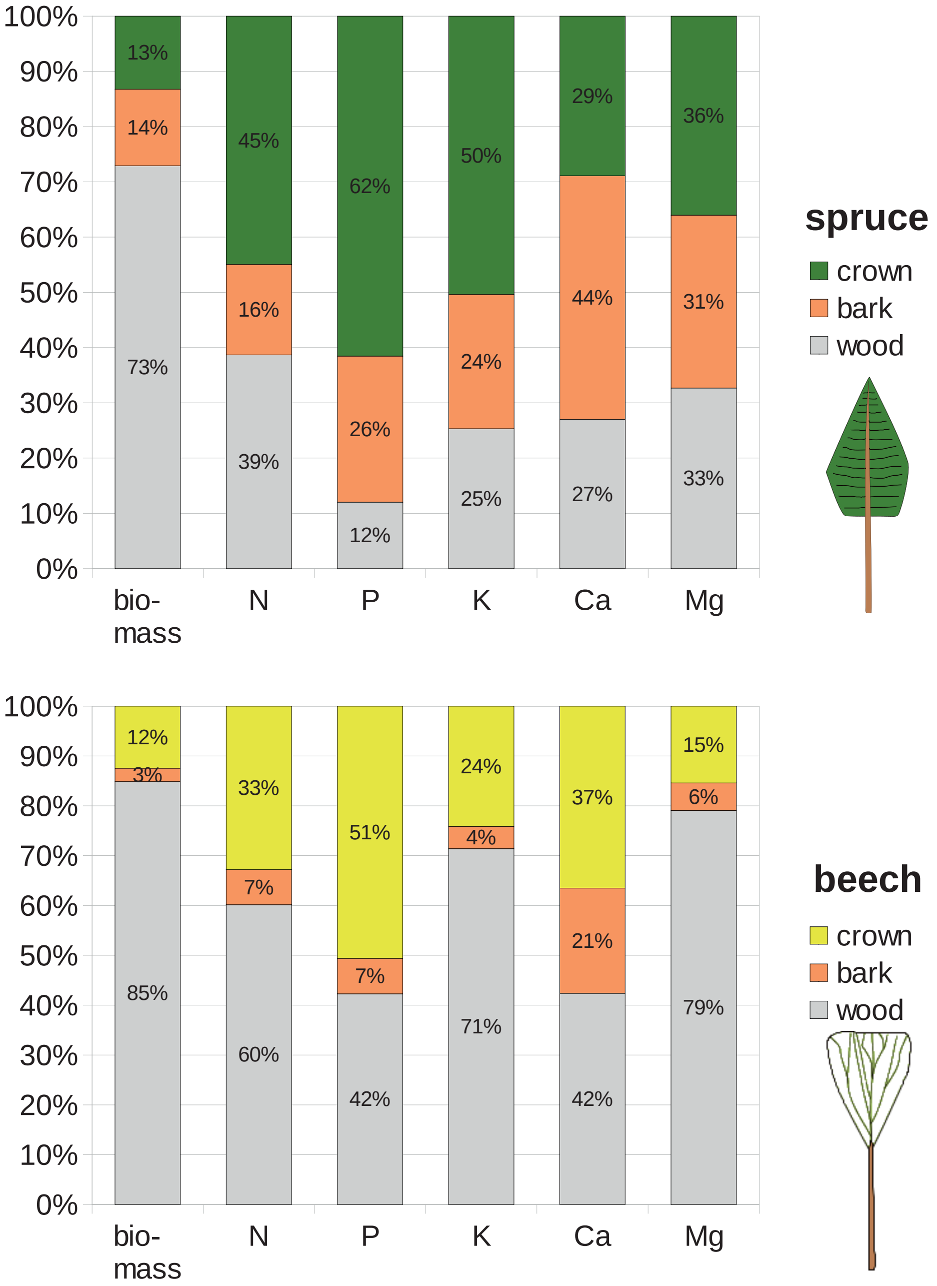

Fig. 2 Implication of different harvest intensities on biomass and nutrient removal; average value per tree; for beech the leaves were not included because beech is normally harvested in winter time.

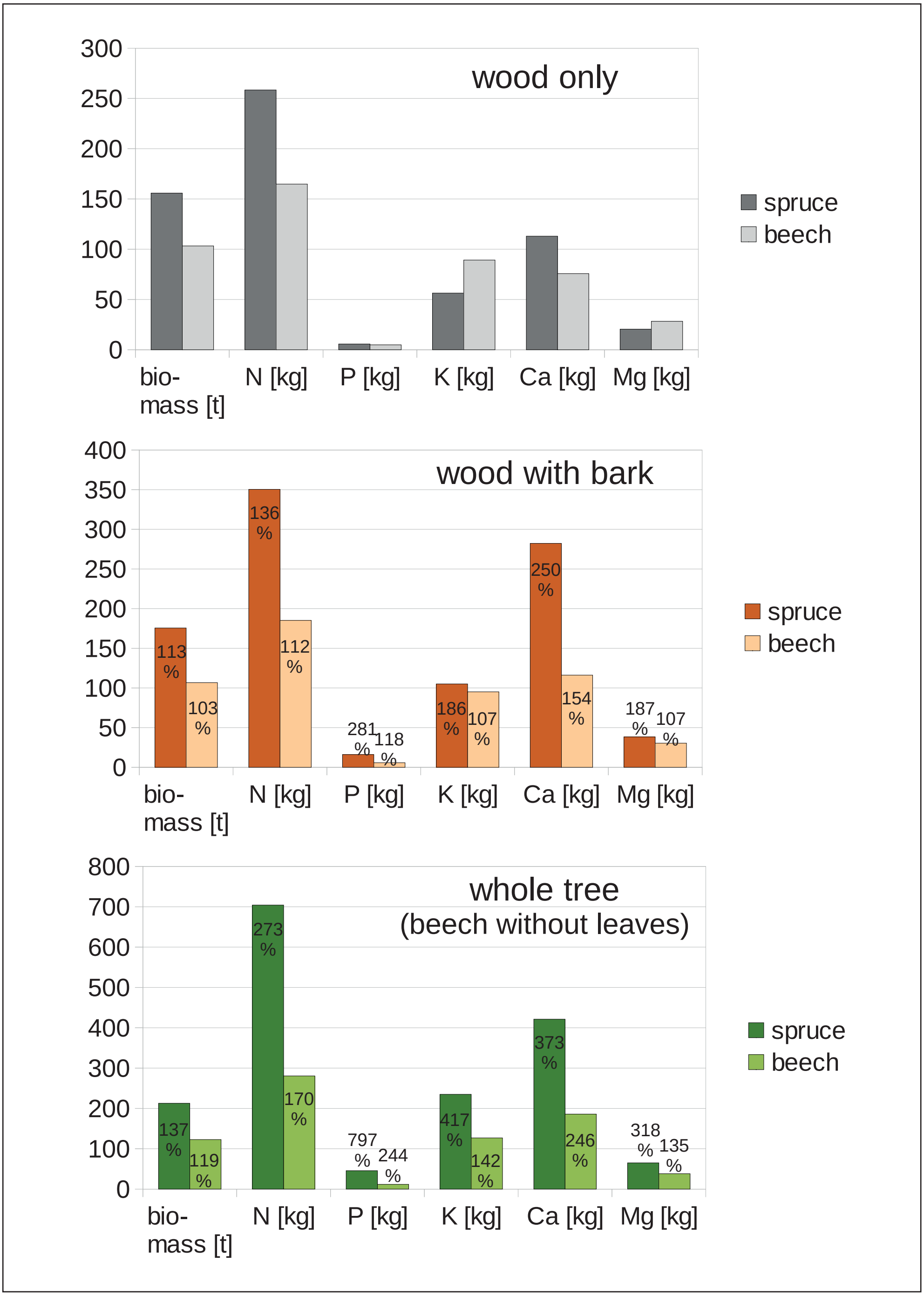

Fig. 3 Biomass and nutrient removal with different harvest scenarios for the existing stand, on a per hectare basis; per cent values refer to the wood only scenario.

Because there are more spruce trees per hectare, looking on an area basis things are changing (Fig. 3). Spruce produces more biomass, and thus with some exceptions also incorporates more nutrients. These exceptions are K and Mg for a wood-only harvest scenario, with beech incorporating clearly higher amounts of these two nutrients. For P the differences between spruce and beech are small for the wood-only scenario. With respect to hectare related values for the conventional harvest (wood including bark) a marginal increase of biomass (13 % for spruce, 3 % for beech) leads to a disproportionately increase of nutrient export with a factor around and greater than two for the elements P, K, Ca and Mg for spruce. For whole tree harvest, according to common practices calculated for spruce with and for beech without green biomass, the increase of biomass removal compared to the wood only scenario is 37 % for spruce and 19 % for beech. For spruce the nutrient exports were increased by a factor of 2.7 to 4.2 for the elements N, K, Ca, Mg and extraordinary for the element P by a factor of nearly 8. Because whole tree harvest of beech does not include the nutrient rich leaves the increase of nutrient export is much lower than for spruce with factors ranging from 1.4 to 2.5. Looking at the conventional harvest (wood including bark) nutrient export for a spruce stand is clearly higher than for a beech stand for the elements P and Ca which on the long run may also have consequences for soil fertility. At present, nitrogen input is high (e.g. HUBER et al. 2010, MELLERT et al. 2004), so that exports of N are of minor relevance for the ecosystem. Whole tree harvest of spruce with its very high nutrient exports has a strong impact on the nutrient budget of the stand which especially on nutrient poor sites my rapidly lead to a decrease of site productivity (HELMISAARI et al. 2011).

4. Conclusions

Tree-internal nutrient distribution and thus nutrient related efficiency parameters are very different for beech and spruce. Because beech only holds a low percentage of biomass and nutrients in its leaves, its efficiency of wood production and biomass maintenance by the green biomass is very high. On the other hand, due to the high nutrient content of wood its efficiency of wood production in relation to nutrients incorporated in wood is rather low. Because the crown radius of a beech was higher than that of spruce the advantages of beech in space related efficiencies are vanishing. Regarding the nutrient related efficiency for maintenance of total aboveground biomass per m² crown area the two species range in the same order of magnitude, which shows, that both species are able to optimize the utilization of energy input by solar radiation in a comparable way. Reducing harvest to a wood-only removal would clearly reduce nutrient exports for spruce. Although nutrient contents in plant tissues of beech are mostly higher than for spruce, the higher biomass production of spruce leads to clearly higher nutrient exports for spruce, when harvesting wood including bark or the whole tree. All these conclusions, however, are only valid for the well growing site Kranzberger Forst with its nearly optimal nutrient supply. Under different site or climatic conditions the relations between the two species will change, the more, the farer the conditions are from the respective optimum.

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Feierliche Einweihung des neuen Hauptgebäudes der Leopoldina
Jägerberg 1, Halle (Saale)
am 25. Mai 2012

Nova Acta Leopoldina N. F., Supplementum Nr. 27
Herausgeber: Präsidium der Deutschen Akademie der Naturforscher Leopoldina
(2012, 51 Seiten, 13 Abbildungen, 8,00 Euro, ISBN: 978-3-8047-3114-1)

Am 25. Mai 2012 übernahm die Leopoldina offiziell mit einer Feierlichen Veranstaltung ihr neues Hauptgebäude auf dem Jägerberg in Halle (Saale). Der Band enthält u. a. die Ansprache der Bundesministerin für Bildung und Forschung Annette Schavan und des Ministerpräsidenten von Sachsen-Anhalt Reiner Haseloff auf dem Festakt sowie den Festvortrag von Wolfgang Frühwald „‚Natur hat weder Kern noch Schale‘ – Naturforschung im Diskurs und vor dem Anspruch der Gesellschaft“. Außerdem einen historischen Rückblick von Karl Vocelka „Zur Rolle von Kaiser Leopold und seine Bedeutung als Förderer von Wissenschaft und Kunst“ sowie einen Bericht über ein Rundtischgespräch zur Rolle der Akademien im 21. Jahrhundert.

Wissenschaftliche Verlagsgesellschaft Stuttgart

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Synthesis of Section III: The Scales – Spatio-Temporal Pattern Formation

Eckart Priesack (München-Neuherberg)

The consideration of the growth of vegetation includes at least three different spatial scales, i.e., that (*i*) of the stand, (*ii*) of the individual plant and (*iii*) of the plant organ.

The stand scale is important to characterize the different interactions between neighboring plants that can be either negative or positive, namely, negative by competition for space enabling usage of light, water and nutrients or positive by facilitation through mutual protection against adverse impacts (e.g. from climate extremes or herbivores) and through provision of additional resources (e.g. *via* canopy leaching, microbial enhancement, mycorrhizal networks or hydraulic lift) (Brooker et al. 2008).

The individual plant scale is the one where the distribution of the allocated resources for plant growth within the plant is organized and where the effects of environmental factors on growth partitioning among the plant organs are to be considered (Priesack and Gayler 2009).

At the plant organ level the acquisition of resources occurs, and it is this scale, where related energy and matter fluxes are usually determined (e.g. light absorption, release of water vapor, and CO_2 exchange at the leaf level or uptake of water and nutrients at the root level). Moreover, it is the organ level where at the end of the growing season we discern the nutrient provision for the seeds as being indicative for the fertility of the plant. Of course, by investigating growth processes we always almost implicitly address the cellular scale, since the inspected change in plant biomass occurs by cell growth, cell division and cell death, which altogether determine the different growth rates of the plant organs and their diverse tissues.

In a way, section III opens new views to these spatial scales by presenting and analyzing various evolving growth patterns at the different plant levels. A review on the recent status of systems biology approaches to plant science shows how one might prepare the grounds for advanced understanding of the spatio-temporal organization of biological systems as based on an improved knowledge about dynamical processes in networks at the cellular scale (Hütt et al. 2013, this volume). Through interpreting pattern dynamics in relation, e.g., to high-throughput gene expression data metabolic models may be established to predict the interplay between cell properties and environmental factors. Guided by large empirical data sets the theory of networks differs from past efforts such as generalized systems theory or catastrophe theory and may therefore lead to much better, less limited results in explaining spatio-temporal pattern formation in complex systems (West and Grigolini 2011).

Above the cellular level, at the spatial scale of root systems, we recognize another manifold of networks, i.e. the diversity of ectomycorhizal fungi “regarding amount, organization and range of the extramatrical mycelium that extends” from the tree root “into the soil”

(Agerer 2013, this volume). Generally, the role of mycorrhizal networks is still insufficiently understood (Beiler et al. 2009) and, therefore, it is so important to comprehend the network architecture in relation to its functioning in terms of soil exploitation by uptake of water and nutrients. Moreover, up to now modeling approaches to simulate mycorrhizal growth and its contribution to water and nutrient uptake of trees have not explicitly described the three-dimensional network architecture and its interaction with the soil structure (Schnepf et al. 2008, Meyer et al. 2010), which amongst other aspects is probably due to the elusiveness of its spatial structure.

Differences in patterns of allocated mineral nutrients in plant organs are investigated in the case of European beech and Norway spruce at the scales of plant organs and of individual plants. The detected mineral nutrient distributions within the trees reflect the different demands and growth strategies of these two species and thereby also depict the more general differences of growth behavior between coniferous and broad-leaved trees (Göttlein et al. 2013, this volume). For a description of the nutrient uptake dynamics which result in the measured distributions a plant model at the whole organism scale is needed. For this purpose we can consider the plant from the inside as built up by its organs and take the plant architecture into account. If we then apply a functional-structural model, we may end up by describing water and solute transport dynamics on network graphs representing the plant structure to describe the observed formation of nutrient distribution patterns (Lacointe and Minchin 2008, Mathieu et al. 2009, Janott et al. 2011).

At the stand level the focus is on the assessment of the dynamics of pattern evolution as it depends on the initial diversity distribution of plant species at the beginning of the observation. Regarding the investigation of grassland ecosystems a new instrument is presented able to almost continuously monitor, in an automated non-invasive way, the growth of multiple traits of vegetation by use of different sensors. They can record spatio-temporal growth patterns taking high resolution photographs and capturing hyper-spectral reflectance as well as sun-induced fluorescence of plant and soil surfaces (Plückers et al. 2013, this volume). Also at the stand level, a numerical simulation study examines the development of mixed forest stands and demonstrates the impact of different spatial distributions of European beech and Norway spruce on resource allocation and growth using random, clustered or grouped mixtures (Rötzer 2013, this volume).

Population scale crop models describe interactions between plant parts (i.e. roots, shoots, leaves, fruits etc.) and physiological processes (i.e. photosynthesis, respiration, water and nutrient uptake) by conceiving the population of plants as a single entity (Lucas et al. 2011). In contrast plant growth models as the applied model BALANCE (Rötzer 2013, this volume) or PLATHO (Gayler and Priesack 2013, this volume) simulate the growth of individual plants. Both models are indeed based on representations of plant parts and physiological processes similar to that of population growth models, yet differ with respect to the description of the interaction with the environment. This plant-environment interaction is now based on a spatial representation of individual plant architectures and can therefore more realistically describe energy and matter fluxes across plant surfaces and thereby directly account for plant-plant interactions such as competition and facilitation within the stand. The main limitation to addressing the stand scale of such individual based functional-structural plant scale models has to be seen in the difficulty to provide sufficient experimental data for model parameterization and model testing (Lucas et al. 2011). Therefore monitoring instruments that can provide such large empirical data sets as presented by Plückers et al. (2013, this volume) are necessary prerequisites to further develop and test these functional-structural plant models and to establish crop models that specify the growth of each individual plant.

In conclusion, at each of the considered plant scales we have identified spatio-temporal patterns of systems and their interpretation by networks and related dynamic properties: at the scale of sub-cellular and cellular level the metabolic network, at the scale of the organ level and whole plant level the representation of mycorrhiza and plants by the network of their architecture, and at the stand level we have seen different spatial networks of interaction between the various individual plants. In this way we might view the evolving spatio-temporal growth patterns as results of dynamical processes on networks and thus relate the different studies of this session to systems biology as recently proposed by Lucas et al. (2011).

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Section IV
The Systems: Holobionts and Hierarchy Theory

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Growth Controls Photosynthesis – Mostly

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With 4 Figures

Abstract

In this essay, I am trying to draw attention to a number of misconceptions related to a mechanistic understanding of environmental controls of plant growth. In contrast to wide-spread assumption, growth constraints imposed by water shortage and low temperature are not resulting from reduced provision of carbon assimilates by photosynthesis, but from direct impacts on tissue formation, with both these constraints in fact causing carbon overflow. Similarly, effects of elevated CO_2 on photosynthesis do not translate into growth stimulation as long as soil resources do not match enhanced demand, which is unlikely in a natural setting where plants had always been competing for these resources. Thus, in most cases (outside horticulture), photosynthesis is fulfiling demands set by meristematic activity (rate of growth) and not the other way round. The essay also recalls that productivity is not commonly related to ecosystem carbon storage.

Zusammenfassung

In diesem Essay stelle ich einige eingefahrene Vorstellungen zur Steuerung des Pflanzenwachstums durch Umweltfaktoren in Frage. So wird allgemein angenommen, dass Wassermangel oder niedrige Temperaturen die Photosynthese und damit die Versorgung der Pflanze mit Kohlenhydraten limitieren, was dann die Pflanzen am Wachsen hindert, während tatsächlich das Gegenteil der Fall ist: Diese Umweltfaktoren limitieren zuerst die Gewebebildung (den Bedarf), was zwangsweise zu einem Überschuss an nicht strukturgebundenen Kohlenhydraten führt. Aus ähnlichen Gründen führt vermehrtes CO_2-Angebot über eine Photosynthesestimulierung nicht zu mehr Pflanzenwachstum, solange die Bodenressourcen nicht den höheren Bedarf für das Wachstum decken können. Dies ist in freier Natur sehr unwahrscheinlich, da die Pflanzen dort immer schon im Wettbewerb um diese raren Ressourcen standen. Deshalb deckt die Photosynthese im Allgemeinen einen Bedarf, den das aktive Bildungsgewebe (das Wachstum) erzeugt und nicht umgekehrt. Der Essay ruft auch in Erinnerung, dass Produktivität wenig mit ökosystemarer Kohlenstoffspeicherung zu tun hat.

1. Science is Driven by Tools

We explore and measure what we are able to explore and to measure, and we attribute value to what we do. This is how knowledge is created and how scientific value is attributed to that knowledge. The weight given to such findings is not always reflecting the actual weight, because other facets of reality had not been considered simply for the lack of tools. Our current understanding of plant growth is an excellent example.

One of the most significant discoveries explaining how this world functions was the identification of photosynthetic CO_2 uptake from the atmosphere as the main pathway of fueling the biosphere, at the end of the 18th century (works by Jan Ingenhausz, Theodore de Saussure, and SENEBIER; FARMER 2010). Before, it was assumed that plants take the substance of their body (and thus also our food) from the soil. It needed someone like DE SAUSSURE to put a potted plant on a balance and prove that the biomass accumulated largely came from air (DE SAUSSURE 1804, Fig. 1). The fact that plants 'eat' air, and by doing so, also release oxygen, became a schoolbook-type landmark discovery. However, the fact that the world would not work as it does unless roughly the same amount of CO_2 would be released (and oxygen consumed) by abiotic and biotic oxidation (i.e. respiration) was appreciated much later, remained expert knowledge and lacked fascination and public appeal. Thus, this latter kind of knowledge remained a hidden agenda for most people so that the wide-spread misbelief originated that forests are net oxygen producers. While experts have learned to think in long terms, large areas, and equilibria of biogeochemical cycles, much of the concerned lay people's belief is still anchored in the concept that plants and ecosystems exchange carbon for oxygen only. And further, a broad scientific community still assumes that leaf photosynthesis controls the rate of growth. Hence, implicitly, plants and ecosystems are considered to be carbon limited, always.

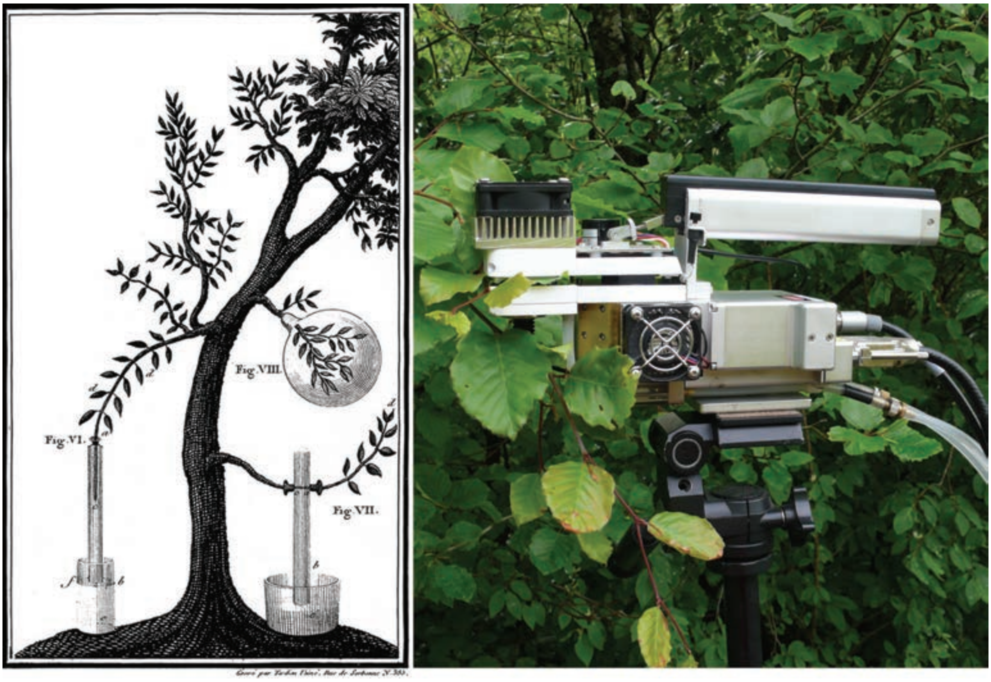

Fig. 1 Tools drive science. Theodor DE SAUSSURE's glass balloons flushed by air, with CO_2 trapped in potassium hydroxide, and then titrated for any rate of change in CO_2 concentration, have been replaced by computer controlled gas exchange chambers with open-path infra-red gas analyzers. Both measure leaf carbon metabolism. We have no such tools to measure carbon investment (e.g., growth), hence there is hardly any study of the direct controls of carbon investment.

Although, agricultural research back to the 1980s has disproven a direct linkage between carbon uptake per unit leaf area and growth rate or yield (e.g. GIFFORD and EVANS 1981, WARDLAW 1990), the availability of easy to use tools to measure photosynthesis is continuing to blind a broad community of the actual limitations of such data if one aims at explaining plant growth. The underlying assumption is that carbon is a growth rate-limiting resource, and the more CO_2 is taken up per unit leaf area (the more photosynthesis), the more a plant or an ecosystem will grow or produce. This view not only neglects the rules of stoichiometry of life (the proportional need of elements other than carbon), it also rests on the assumption that transport of photoassimilates, building of tissues (i.e. the body of plants), dry matter allocation to various plant parts, tissue duration and respiratory processes are negligible and/or constant when it comes to explain plant growth. Why? Because we have no ready-to-use tools to observe and measure most of these other processes. There is no other reason. In economy and industry, any process contributing to productivity has a process duration component. The cost and productivity of an engine is always seen as the net outcome of its rate of processing things and the duration of its functioning (amortization). Translated to plant sciences, the functional duration of leaves, roots or any other tissue has the same weight in determining the carbon and nutrient balance of these tissues than their specific metabolic activity. However, tissue duration is heavily under-studied in a carbon balance context (less so in a nutrient cycle context; Lüttge 2008, Givnish 2002, Turner 1994). The reason for that missing half of information is in large the boring nature of such census works, and the required long-term presence of the experimenter in the field.

Another example of the prevailing curiosity- or tools-driven concept of environmental controls of plant growth and ecosystem carbon cycle is the temperature response of mitochondrial respiration (CO_2 release). Since chemical reactions commonly run faster as temperature rises, respiratory metabolism rises instantaneously with temperature as well, provided the availability of substrate permits. However, in the case of living organisms, respiration not only needs substrate (e.g., sugar), it also needs a demand for the end products (in the case of mitochondrial respiration, ATP). In other words, organisms can be expected to respire ‘on purpose’ (demand), and any respiration stimulated by warming must meet a demand. Should warming stimulate growth, and should there be no developmental or other constraints to growth (e.g. flowering, dormancy, lack of meristems), then there will be more respiration. Without growth stimulation, a faster rate of respiration produces products for which there is no need, and, consequently, the rate will undergo down-regulation sooner or later. Hence, mitochondrial respiration is not a self-serving process driven by temperature, but can only be predicted *via* demand for its products.

Following a similar rationale, organic plant debris will be metabolized by micro-organisms at a faster rate with any rise in temperature, provided moisture permits. However, it is the amount of overall substrate (easily decomposable litter) that controls total respiration over longer periods. In humble language, there is only one ‘lunch’, but it can be eaten faster (if warm) or slower (if cold). So, annual ecosystem respiration will be driven by litter production and plant vigor, with temperature only triggering short-term responses. Soil respiration was found to be linearly related to annual litter input from Alaska to Amazonia, with no additional effect exerted by temperature across this vast latitudinal gradient (Raich and Nadelhoffer 1989, see also Caprez et al. 2012).

Hence, while representing a basic driver of metabolism (if moisture is not constraining), temperature is only one of the controls of respiration, and the three other controls, the availability of substrate (either life or dead), the demand for end products, and the developmental constraints are widely neglected, because they are not known or not measured. Had this biased weighting of the mechanisms that drive plant growth, respiration and ecosystem productivity been more widely acknowledged, we had seen less inappropriate reasoning and modeling in the context of atmospheric CO_2 enrichment and climatic warming research. In the following, I am presenting a few examples of traditionally applied, though misleading concepts related to environmental controls of plant growth.

2. Water Shortage and Low Temperature Control Tissue Formation

A carbon uptake focused view at drought stress and low temperature led to the assumption that both these constraints first act on leaf photosynthesis, which then restricts carbon supply to meristems and, as a consequence, will starve plants and reduce or even stop growth. Stomata are considered the starting point of such a chain reaction in the case of drought. The reality is quite different.

Of all processes related to plant growth, cell formation is the most sensitive to any decline in turgor, and tissue formation is constrained by drought long before carbon supply falls short because of drought induced limitations of gas exchange (Fig. 2). In fact, the immediate effect of water shortage is an accumulation of non-structural carbohydrates; not osmotically active ones, but insoluble storage reserves (Körner 2003, Würth et al. 2005). This knowledge was already around in the 1980s, but got completely overrun by technological developments that made it so easy to measure gas exchange that the opposing view, of a stomata-dominated cascade effect, prevailed. A recent review by Muller et al. (2011) re-established the actual interrelationships, referring to the classics in this field, while at the same time illustrating the newly emerging molecular basis for a dominance of sink limitation under drought, instead of source (stomata) limitation.

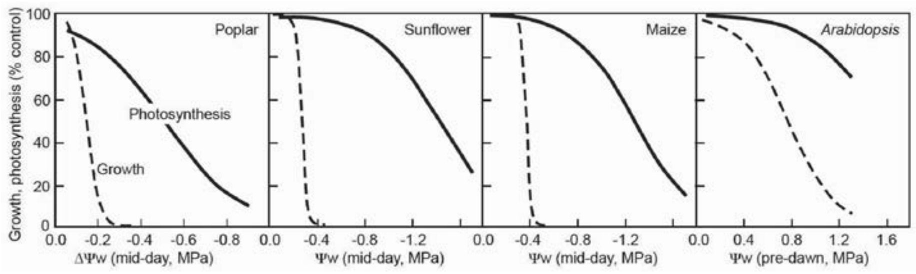

Fig. 2 The discrepancy of impacts of water shortage (tissue water potential) on plant CO_2 uptake (stomata) and growth, i.e. tissue formation. For poplar, the x-axis shows the difference in water potential between stressed and control plants (adapted from Muller et al. 2011).

Similarly, low temperature is affecting cell duplication or mitoses at levels that hardly constrain leaf photosynthesis. At 5 °C most cool-adapted plants perform >50 % of phoptosynthetic capacity, while growth is reaching an almost complete stand-still. No higher plant has ever been found to grow near freezing point, where net photosynthesis is still running at c. 30 % of maximum rate if water and light allow (Fig. 3; Körner 2006a). These constraints to tissue formation are specific to all cold-adapted higher plants, including winter crops (Körner 2008), and most likely are the cause also of alpine treeline formation (Körner 1998). The latter is evidenced by the accumulation of non-structural carbohydrates at treelines worldwide (no C shortage; Hoch and Körner 2011) and by detailed xylogenesis works that underpinned the 5 °C threshold for wood formation (Rossi et al. 2007). Trees are not more sensitive to low temperature than low stature plants, but due to their upright stature they are the first to be hit by declining ambient temperature (close aerodynamic coupling of shoots to air; Körner 2007). Roots also hardly grow below 5 °C (Alvarez-Uria and Körner 2007). So, similar to drought, low temperature is first constraining sink activity. As with the effects of water shortage, low-temperature exposed plants are driven into carbon overflow, a fact already observed in the 19th century using iodine starch assays (review by Körner 2003).

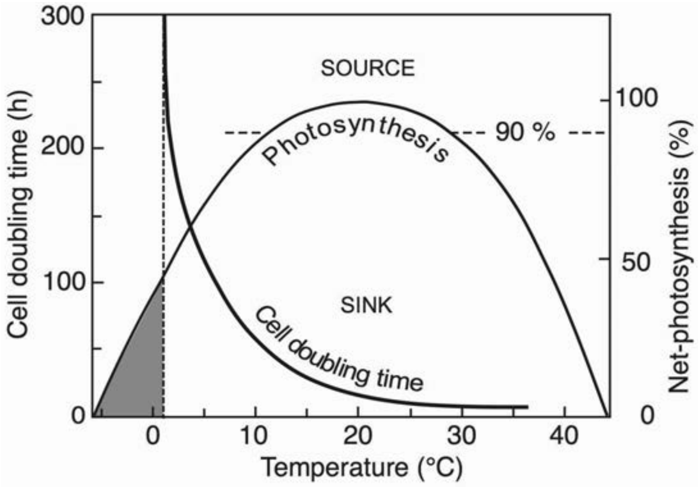

Fig. 3 The discrepancy between temperature dependency of source activity, i.e. net photosynthetic CO_2 uptake (relative scale) and sink activity, i.e. meristematic activity (tissue formation expressed as cell doubling time).

3. Effects of Elevated CO_2 are Nutrient-Constrained

Soon after it became established that plants need CO_2 for photosynthesis, atmospheric CO_2 enrichment was considered a means of enhancing horticultural production (de Saussure 1804, Lundegårdh 1924, Börnemann 1930). CO_2 fertilization of greenhouse crops (e.g., as a by-product of heating with propane) became a routine tool in indoor horticulture already early in the 20th century (Wittwer 1967). Yields of tomato or cucumber may increase by 30 % under optimal growth conditions. Since it became more widely acknowledged that humans are enriching the globes' atmosphere with CO_2, it was debated, whether this effect could enhance growth and productivity of the world's natural vegetation, forest plantations and field crops. Often, the CO_2-driven enhancement of productivity (should it occur) is confused with carbon sequestration (enhanced terrestrial bio-C-pools, see below). Results of early experiments, mostly in greenhouses, indeed seemed to suggest that there is a possibility of global CO_2 fertilization. Projections from such largely horticultural CO_2 enrichment trials led to expectations of a quadrupling of tree production (with proportional forest productivity inferred) in a CO_2-rich future (Idso and Kimball 1992) and a 40–50 % increase in crop yields (see below).

As soon as realism seeped into the debate through results of more realistic experimental trials, the 'euphoria' vanished. What had happened? Early tests had provided plants with unlimited water, nutrients and space to expand into. If an isolated tree is fed with a full nutrient solution in full sun, it may be responding to the relief of its remaining limitation by CO_2 by a 30 % greater biomass and canopy size within one season. And when that 30 % larger tree is allowed to grow for a second year with similar growth conditions and responses, we arrive at a 169 % biomass compared to the start, and in the third year, we arrive at 220 % and so forth with compound interest effects – provided resource supply and space are unlimited. By year 5 we have indeed quadrupled the biomass (experiment with sour orange trees by Idso and Kimball 1992).

In crops, with determinate growth and competition for space, no such 'compound interest' propagation with time is possible, but a growth stimulation up to 40–50 % is possible for a doubling of CO_2 concentration under optimal greenhouse conditions (e.g. Manderscheid and Weigel 1997, Ainsworth et al. 2002). Not surprisingly, a review of CO_2 responses of growth in plants exposed to Free Air CO_2 Enrichment (FACE) arrived at the conclusion that woody species are more responsive to elevated CO_2 than herbaceous species (Ainsworth et al. 2005). The above example shows how experimental conditions can lead to such results. Trees, if studied in an expansive phase, not only profit from exploring so far unexplored rooting space and, thus, nutrients, but they also will accumulate signals with time, whereas crops start from scratch every year.

Generally, results of all-inclusive meta-analysis of published works in this field are highly problematic, because they reflect the frequency distribution of experimental conditions under which CO_2 effects were examined. These are dominated by horticultural conditions, young plants (in the case of trees, seedlings or saplings), data from plants growing on disturbed soils with an enhanced nutrient cycle and without competition with neighbors. A minority of results comes from undisturbed test systems and/or for systems that have arrived at steady-state root and leaf turnover per unit land area. Such data for forests are available from two young, monospecific plantations in the USA only, and a dozen of mature forest trees in Switzerland, although forests are storing > 80 % of the biospheres' biomass-carbon (Körner 2006a). Two of those three experiments with forest trees arrived at no stimulation of growth (Körner et al. 2005, Norby et al. 2011, M. Bader et al. in prep.), and one, the pine plantation at the Duke FACE site, ended up with trees supposedly mining extra nutrients (Phillips et al. 2011), facilitating extra growth, but obviously, such nutrient mining cannot be sustained in the long term (see discussion below).

It is a truism that all organisms are composed of a broad spectrum of chemical elements that must be obtained from the environment in the right proportion for healthy growth and development. In the case of plants, soil nutrients of all sorts must meet the plant's demand for growth. The addition of only one resource, e.g. CO_2, can only enhance growth if the availability of all other resources is tracking the rising demand. In nature, this is a most unlikely situation. Soil minerals are representing a finite resource per unit land area, and plants have been competing for these resources since ever. Exept for nitrogen, none of these resources became more readily available in the last 150 years during which CO_2 concentrations rose by 40 %, nor is there any reason to expect that their availability will rise in proportion to the ongoing rise in atmospheric CO_2 concentration. Hence, there is little leeway for photosynthesis signals to translate into growth and productivity signals, except where such nutrient constraints do not come into action. One of such natural conditions is life in deep forest shade where absolute rates of growth are very low and carbon capture becomes decisive for survival. It had been shown that elevated CO_2 is shifting the light compensation point of photosynthesis to lower values, causing the relative stimulation of growth by elevated CO_2 to become substantial (HÄTTENSCHWILER and KÖRNER 2000, 2003, WÜRTH et al. 1998).

As discussed above, nutrient addition can facilitate a CO_2-driven growth stimulation. However, under realistic agricultural field conditions, effects on yield were still much smaller (less than a third) of what was expected from earlier trials in greenhouses. According to a review of results from FACE experiments with crops, mean responses for a doubling of pre-industrial CO_2 concentrations (c. 600 ppm) compared to con-current (c. 360–370 ppm during those years) came down to + 12 % (KIMBALL et al. 2002). In contrast, the green revolution with fertilizer application and pest management caused mean cereal yields in many parts of the world to rise from 1.5 to 6 t $ha^{-1}a^{-1}$, a quadrupling, completely marginalizing additional CO_2 effects of the above magnitude to be reached in perhaps 60 to 70 years. At the same time, the quality of cereals has been found to decline (less protein) under elevated CO_2 (FANGMEIER et al. 1999, HÖGY et al. 2009).

Given the minute effects in crops tested at field scale, it does not come at a surprise that natural, closed vegetation that has come to a steady state in terms of leaf area index and root turnover does commonly not take advantage from atmospheric CO_2 enrichment, once secondary effects such as those of water savings under artificial CO_2 enrichment are accounted for. Where CO_2 effects on biomass had been reported, these were either for young expanding systems where small initial effects could propagate as described above by compound interest effects, or where soils had recently been disturbed (and thus nutrients mobilized), or where stomatal water savings and thus, periodically higher soil moisture effects came into play. In addition, species were found to respond differently, causing some to gain and some to lose ground initially, but stoichiometry constraints will diminish net ecosystem effects in the long run (e.g., OREN et al. 2001, NORBY et al. 2010). Another exception are nutritionally open systems such as graminoid communities in an eutrophic estuarine in the eastern U.S., the longest CO_2 enrichment experiment to date (DRAKE et al. 1989), showing a continuation of enhanced annual productivity. However, in this system, nutrients are replenished by regular flooding of this extremely nutrient-rich semi-aquatic system.

The stomata-driven moisture saving effect that explains most grassland responses to elevated CO_2 (VOLK et al. 2000, MORGAN et al. 2004) relies on unchanged future climatic conditions and absence of atmospheric feedback (KÖRNER et al. 2007). Initial responses to a step increase in CO_2 concentration that have been observed in some systems, may take several years to fade. Even the orchard trees that initially quadrupled their biomass (see above) came down to an accumulated 70 % stimulation after 17 years, despite continuous provision of nutrient solution (KIMBALL et al. 2007). In a deciduous tree plantation in Oak Ridge, Tennessee, it took 11 years after a step increase in CO_2 provision for the system to return to zero stimulation (NORBY et al. 2010). In Mediterranean CO_2 springs it took CO_2 effects on the rate of forest re-growth (after coppicing) 25 years to disappear (HÄTTENSCHWILER et al. 1997). Mature, 100-year-old trees in a near to natural deciduous forest near Basel in Switzerland, did not show any indication of carbon limitation at current atmospheric conditions (KÖRNER et al. 2005, Bader et al. in preparation). Neither showed diverse tropical model ecosystems (Körner and Arnone 1992, Winter et al. 2000), nor conifers grown under high-montane conditions (which even showed a negative effect because of enhanced microbial competition for nutrients; Hättenschwiler et al. 1998), nor free-standing treeline conifers *in situ* a sustained advantage (Dawes et al. 2011). In the latter case, exceptionally warm years showed a CO_2 stimulation of growth in one of the two species tested (*Larix decidua*), but not in the normal, cool years, with *Pinus uncinata* never responding, irrespective of temperature (Dawes et al. 2011). Alpine vegetation (both, late and early successional; Körner et al. 1998, Inauen et al. 2012) neither showed a CO_2 effect on biomass production (some negative effects in the early-successional communities, again presumably related to competitive interactions with microbes). For the same reasons, it is also highly unrealistic to expect the single biggest biome and biomass-carbon-reservoir, the humid tropical forests, to incorporate more C in response to atmospheric CO_2 enrichment. In fact, due to a CO_2-driven growth enhancement in deep shade, lianas may become more aggressive and make the forest more dynamic and thus, induce a loss of carbon through a biodiversity effect (Körner 2004, 2009a). In this case, the biodiversity effect would revert projections made on the basis of physiological response curves (Körner 2004).

4. Conclusions

This was not meant to provide an exhaustive review on the nature of growth limitation under drought, low temperature and under elevated CO_2 concentrations. The message I wanted to get across is that processes directly involved in tissue formation are far more decisive for growth than primary leaf metabolism that can be readily measured, whereas direct tissue limitations cannot. For reasons, once more, related to tissue formation, the carbon cycle cannot be separated from the nutrient cycle, causing productivity in most ecosystems to remain unresponsive to elevated CO_2 in the long run, simply because soil conditions (nutrients) control productivity (Fig. 4). It is a most important and often confused aspect, that the ecosystem C balance is not related to plant growth in a straight forward manner. Fast growing forests tend to show lower C pools (e.g. tree plantations) and slower growing/older ecosystems tend to exhibit higher C pool. Hence, productivity must not be confused with carbon storage as is wide-spread custom (Körner 2009b). Biological model projections at the consequences of climatic warming and higher frequency of drought would take advantage from accounting for known constraints of plant tissue formation by these drivers than using extrapolations derived from responses of leaf metabolism that do not scale as assumed. The custom of using response functions of primary carbon metabolism originates in their availability rather than their actual predictive power.

With these few examples, I am advocating a more balanced view at plant growth, by accounting for allocation rules, nutrient stoichiometry, biodiversity and constraints that act upon carbon sinks, long before they act upon carbon sources. It is rather the norm than the exception that sink activity feeds back on source activity (e.g. through end-product inhibition in the chloroplast), causing growth (demand for carbon) to control photosynthesis, rather than the other way round. Further, at ecosystem scale, rates of growth in forest trees do not scale to carbon pool size, which is rather related to age distribution (demography) of stems or parcels (Körner 2000, 2006b, 2009a, b, Bugmann and Bigler 2011). It will take a while for such

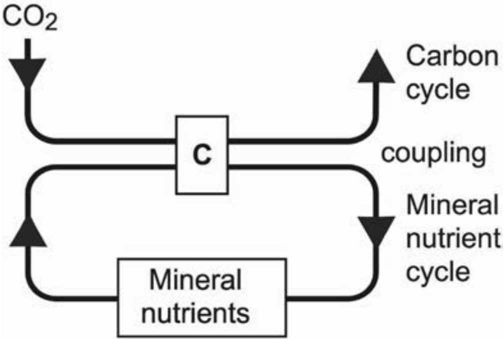

Fig. 4 The carbon cycle is tied to the nutrient cycle, both at plant as well as ecosystem level.

insight to become more widely acknowledged and become school book knowledge. I am afraid, the legacy of DE SAUSSURER's and SENEBIER's great discovery and the available tools in experimental plant sciences will continue to nourish a biased, source activity-based view at plant growth and ecosystem scale carbon relations. This little essay was meant to challenge some mainstream reasoning, and I hope it will do so.

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Vorträge und Abhandlungen zur Wissenschaftsgeschichte 2011/2012

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Der Band versammelt Vorträge aus den wissenschaftshistorischen Seminaren der Leopoldina einerseits und Abhandlungen zur Wissenschafts- und Akademiegeschichte andererseits. So werden Goethes Leiden und Krankheiten, Rousseaus Verhältnis zu den Wissenschaften und die Asklepiosmedizin in der römischen Kaiserzeit behandelt. Die Beiträge zu Institutionen untersuchen die Entwicklung der Max-Planck-Gesellschaft seit ihrer Gründung bis zur Gegenwart im Überblick, zeigen die Leopoldina in den DDR-Jahren im Netz der Staatssicherheit und folgen dieser Naturforscherakademie auf den Spuren Darwins. Außerdem werden die Anfänge der Leopoldina-Ausgabe von Goethes naturwissenschaftlichen Schriften dargestellt. Einen Schwerpunkt des Bandes bilden die Biographien hervorragender Forscher: des Botanikers Otto Renner (1883–1960), der als Genetiker und Leopoldiner gewürdigt wird, und des Biochemikers Otto Meyerhof (1884–1951), dessen Wirken als vielseitiger Gelehrter, Verfolgter des NS-Regimes und Emigrant analysiert wird.

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Complex Systems: Chances and Risks for Experimental Data Analysis

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With 3 Figures

Abstract

Complexity in biological systems challenges our traditional reductionist approach in science. There might be a deeper reason for such insufficiency which can be clarified considering insight provided by complexity theory. The lessons learned through the study of complex systems should then be reflected in the methodological approaches we are using for data analysis. We argue that methods of such kind are available. In order to demonstrate how these lessons are reflected in methods from data driven analysis, we consider kernel based support vector machines as an example.

Zusammenfassung

Die Komplexität biologischer Systeme fordert unsere traditionell reduktionistische Herangehensweise in den Naturwissenschaften heraus. Der Grund für diese Unzulänglichkeit scheint tiefer zu liegen. Er kann jedoch durch Einsichten aus der Komplexitätstheorie herausgearbeitet werden. Die Lehren aus dem Studium komplexer Systeme sollten sich dann wiederum in den methodischen Ansätzen widerspiegeln, die wir zur Datenanalyse heranziehen. Der vorliegende Text zeigt auf, dass solche Methoden zur Verfügung stehen. Ein Beispiel aus der datengetriebenen Analyse herausgreifend wird am Beispiel von *Support Vector Machines* gezeigt, wie Erkenntnisse der Komplexitätstheorie in der Methode Eingang gefunden haben.

1. From Data to Knowledge

Information on natural systems typically comes in terms of data and relations. The latter might be more or less implicit. The goal of scientific research is to infer knowledge on the hidden unknown of an observed system. In the western tradition, this goal is approached using the reductionist model of cognition. It is based on the assumption that there is something like an objective system which can be understood by considering its parts and their interrelations. In particular, the properties of the system result in a deterministic way from the properties of its parts. Knowledge thus is a mapping from the objective, real world into the conceptual world of our mind. Such models of the world are linear in the sense that the effect of a phenomenon is proportional to its cause (cf. Heylighen et al. 2007).

This general scheme has been applied in science for centuries and provides the basis of our understanding of many natural phenomena. Nevertheless, the more complex the systems are that we are trying to understand, the more data we can produce using modern experimental technologies, the more fundamental skepticism rises, questioning our approach as a matter of course. In particular in biology and social sciences many phenomena seem to defy themselves from any control resulting from simplified, idealized models. Such observations even led to the question of whether there are such things as laws in biology at all.[1] Even in cases where the reductionist approach has been applied successfully, there remains skepticism concerning the 'objective truth' of the findings, especially, since in modern science many aspects from 'out of science' further obscure the nature of the inference process.[2]

This does not imply that natural sciences are at an end following the reductionist approach. But there might be aspects of natural systems which force us to follow different approaches and to ask different questions. Such doubts concerning the traditions and scientific commons are not new at all. Neither are they restricted to certain disciplines. Quantum mechanics went beyond the scope of classical mechanics, providing a different view on the concept of a particle. Thereby, the classical basic assumption of determinism has been replaced by a fundamental assumption of randomness. In a similar way, all the classical foundations of reductionist thinking have in the meantime been questioned (HEYLIGHEN et al. 2007). Again, this does not imply the need for reductionism to be replaced, but to be broadened and aligned with alternative approaches.

Such an alternative view is provided through complexity. Many authors have argued that biology is intrinsically complexity science, searching for the principles and rules governing complex, evolutionary systems (e.g., KAUFFMAN 1995, SOLÉ and GOODWIN 2000, MAZZOCCHI 2008).

2. Lessons from Complexity

As a consequence of accepting biological systems as intrinsically complex, we should acknowledge the implications deduced from the theory of complex systems such as systems theory or cybernetics. In the following we will highlight some of them.

According to the theory of thermodynamics, complex systems tend to maximize their entropy. Since entropy is a measure for the randomness of a system, and life is a partially ordered state of a thermodynamic system, living systems appear to exist in a stable, low entropy state. Thus, from the second law of thermodynamics, living systems cannot exist as closed systems. They need to have an environment to act with and absorb entropy produced by the system. This apparently trivial observation has some important consequences.

In order to be able to properly define a system, we have to make precise what we consider as part of the system and what we consider as part of its environment, i.e., we have to define the boundary of the system. Such a choice is problem dependent and as such highly subjective.[3] This insight becomes crucial through by the huge rate of complexity that living systems exhibit. Being restricted to a limited view, either through experimental set-up or much more general through our limited way to capture living systems with our limited cognitive capabilities, we are forced to deal with a much simpler model of any complex system. This implies that there are plenty of other reduced models of the same system, which might at most partially coincide with the choice we made.[4]

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[1] Cf. SOBER 1997, DES AUTELS 2010 and the references cited therein.

[2] See IOANNIDIS 2005 to give an example.

[3] See also GERSHENSON and HEYLIGHEN 2005.

[4] Cf. ASBY 1956, HEYLIGHEN et al. 2007.

Therefore, being aware that any description of the system will include the subjective perspective of the observer is the first lesson we should take into account.

Let us now consider a lesson learned from the theory of non-linear dynamics. Even quite simple, non-linear dynamical systems show a behavior which is known as *deterministic chaos*. A defining property is that through non-linear interaction and feedback the trajectory of the system sensitively depends on the initial conditions. Since we will never be able to determine the initial conditions of the system with enough accuracy, it will practically be impossible to distinguish closed by trajectories, which themselves may lead to completely different phenomenological states in the future. Edward N. LORENZ coined the term *butterfly effect* in non-linear dynamics. It naturally occurs in dissipative systems, i.e., open systems which exchange energy and matter with their environment (cf. MAINZER 2005).

Thus, we should not expect to ever be able to completely determine the future state of the system from observing its present conditions.

This indeterminacy also works backwards: “It has become accepted that biological systems can only be understood in terms of their evolutionary history on Earth.”[5] But in the same way as we will not be able to fully capture the present state of the environment to uniquely determine the trajectory the system will follow, we will never be able to reproduce the state of the environment the system has gone through during the past. Thus, we are dealing with a unique state at a unique point in time.

Turning to the question how order of such an elaborate type as in living systems can occur at all, we have to consider *self-organization*. Stable patterns can result from the interaction of agents without a higher level steering principle. Evolution has generated a multitude of stable patterns on all levels of biological organization, all of which exist far from thermodynamic equilibrium (cf. COFFEY 1998). “Self-organizing systems spontaneously arrange their components and their interactions into adaptive structures with emergent properties.”[6] Randomness plays a crucial role within adaption. Indeed, adding a small amount of undirected energy to a system may significantly increase the chance of spontaneous order to emerge. This is what Heinz VON FOERSTER called *order from noise* (VON FOERSTER 1960). As a consequence, we have to face a certain amount of indeterminacy in every evolutionary system.

The case is further supported by the claim that randomness significantly drives adaption in evolutionary genesis. HEYLIGHEN et al. (2007) argue that adaption, i.e., the stable relation between the system and its environment, is realized through a trial-and-error strategy each component of the system is following. Being robust enough to maintain stability and at the same time flexible enough to quickly react to changes in the environment led Christopher LANGTON to the expression of *life at the edge of chaos* (LANGTON 1991).

Although close to DARWIN’s theory of evolution, the consequences reach beyond it. Fitness is understood to be an emergent property resulting from the individual struggle to maintain a certain stable state, i.e., viability. The latter can be seen as the temporal homeostasis of the living system within its environment. From the point of view of the individual, fitness is not a principle imposed on the system *a priori*, neither consciously nor unconsciously. Being emergent it cannot be explained through the individual. Fitness describes a phenomenon present on the higher level of the population.

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[5] VAN REGENMORTEL 2004a, p. 145.

[6] MAZZOCCHI 2008, p. 12.

One key insight is that within the framework of spontaneous order there is no need for the system itself to be aware of its farther environment. Therefore, from the internal point of view, there is no optimization of fitness in a direct way, although we tend to interpret the system's evolution following a fitness strategy on a higher level of interpretation.

Thus, rather than searching for laws in biology which provide some predictive power, we can search for the constraints, imposed by the environment onto the system ('downward causation') as well as in the other direction, constraints given through the system's components ('upward causation').[7]

3. Data Driven Analysis and Support Vector Machines

Let us now turn to the methodological side. Both views, the reductionist view and the holistic approach, have found their counterparts in statistical methodology. The underlying scheme is given in Figure 1. Basically, we consider the system to be analyzed as a 'black box'. An experiment then is a method which generates input x, e.g., through experimental design and variants, and observes an output y. Statistically, we try to capture the natural phenomenon mapping x onto y by using some conceptual model – in the wide sense – which mimics the observed phenomenon and at the same time can be analyzed using our cognitive system. There is always a risk of confusion. We tend to believe that the natural phenomenon is identical with our conceptual model, which is not true (cf. Gershenson and Heylighen 2005). Indeed, at the end of the day we study our conceptual model including its properties based on the assumption that there is an objectively 'true' relation between the conceptual model and the natural phenomenon we aim to understand.

We do not want to dwell onto the theoretical aspects of cognition here, but focus on the mathematical options to derive such a conceptual model. There are two different approaches.[8]

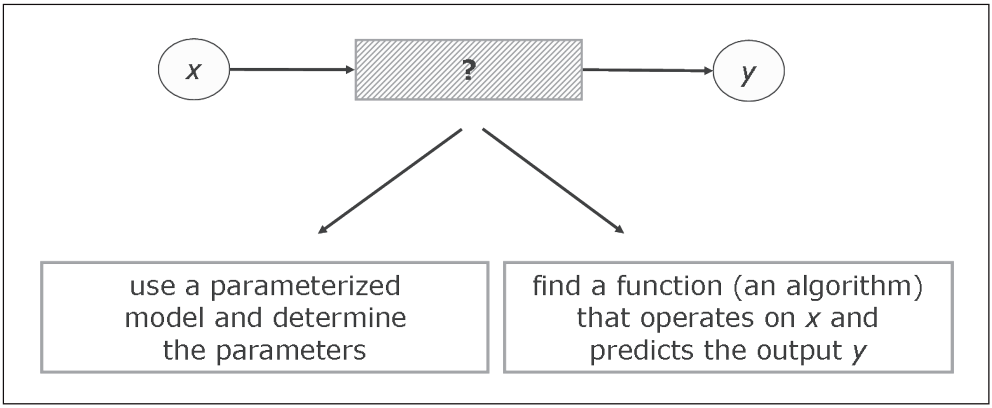

Fig. 1 The schematic concept of statistical inference: the model driven approach (*left*) and the data driven approach (*right*).

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[7] See e.g., Van Regenmortel 2004b.

[8] See also Breiman 2001.

The *model driven approach* is following the reductionist strategy. Setting up a mathematical model of the natural phenomenon to be described, abstracts from the complexity of the system and its environment, focusing on the essential components only. Such a model can either be deterministic, e.g., a set of ordinary or partial differential equations, or stochastic as, e.g., a stochastic process or a stochastic differential equation. In either case, the model is based on an assumption concerning the (mathematical) type of the relation between input and output. Commonly, this leads to a parametrized model of the system. Experimental data is then used to determine the model parameters. As mentioned above, within this framework inference has to be understood as the study of the model's characteristics and properties. Typically, such an approach is the basis in classical statistical testing.

On the other side, we have the *data driven approach*. It tries to impose as little assumptions on the nature of the data generating process as possible. The goal is to use the experimental data to infer an algorithm which allows predicting an output for unforeseen input values. This is done, being aware of the fact that the algorithm does not try to qualitatively model our understanding of the natural phenomenon. We rather learn the relation given implicitly through the data in either a supervised way, i.e., with a teacher, or an unsupervised way. Thus, inference means to mimic the system's behavior using our algorithmic tools. Our hope to gain understanding of the natural phenomenon then is worked into the inference task we are solving using our algorithm. This can, for example, be a classification task as well as model inference. Both approaches have their downsides and advantages. While the model driven approach is immediately directed towards the core of the natural phenomenon, trying to develop a thinkable model of it, data driven approaches can deal with much higher complexity (cf. ZU CASTELL et al. 2013). The latter commonly is inaccessible for the model driven approach due to our limited analytical capabilities.

For the purpose of the present article let us focus on a prominent example of the second class and put it into the framework of the lessons from complexity described in the previous paragraph. Doing so, we want to demonstrate how systemic thinking enters the world of statistical methodology in a natural way.

The example we consider are *support vector machines* which belong to the larger class of *kernel based algorithms* (see SCHÖLKOPF and SMOLA 2000). Let us briefly sketch the idea. Given data points of two different classes in the two-dimensional plane, we can build a classification algorithm by constructing a line such that the data belonging to one class lies on the one side of the line and the data belonging to the other class on the other side. The same can be done in arbitrary dimension using a *hyperplane*, i.e., an affine subspace of codimension one. Any hyperplane separating the data correctly is then called a *separating hyperplane*. Note that in most of the cases there are several separating hyperplanes. The question thus arises, which one of them is the best to choose.

In order to answer this question, let us consider the concept of generalization potential. Our goal is to find an algorithm – based on some set of training data – which can then be used to classify further, yet unforeseen data. The training set captures the 'true' nature of the two classes approximately, only. The approximation is assumed to improve with the amount of data available. Now consider the example depicted in Figure 2. Given four data points (two black squares and two white squares) both hyperplanes are separating the data correctly. But only the one on the right hand side does also provide the correct answer for the new data point (dashed white square). The reason why it does solve the problem correctly is that it does not lie as close to the set of white squares as in the example on the left hand side. Thus, assuming the data to be given with some noise and taking the fact into account that any set of training data can capture the 'true' nature of the classes in a fuzzy way only, it is a wise idea to choose a hyperplane which maximizes the distance to either class. Given linearly separable data, such a hyperplane exists and is called a *maximal margin separating hyperplane*. This is the key observation which led to the invention of *support vector machines* (cf. Boser et al. 1992). The idea is based on results from statistical learning theory. Support vector machines optimize the so-called bias-variance dilemma for the case of binary classification tasks. Let us briefly note that there are variants of the algorithm which can cope with non-linearly separable data, as well as versions to deal with other inference tasks, too (for further details and practical hints see Ben-Hur et al. 2008).

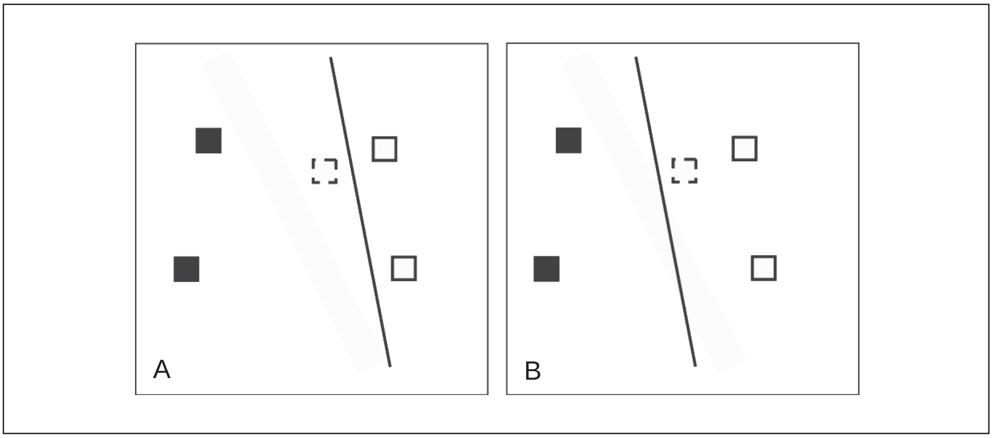

Fig. 2 (*A*) Classification using a separating hyperplane: an example with poor generalization potential and (*B*) good generalization potential.

Central concepts used for the support vector machine algorithm are the concept of distance and of a hyperplane. Both of them are generalized through the incorporation of a *kernel*. To be precise, we consider a positive definite kernel. Without going into details, kernels are similarity measures which can be used to map data into a commonly high-dimensional *feature space* where exactly the same idea can be used, as just described in the two-dimensional example. The kernel defines both, our measure of distance as well as the geometry of the feature space which is needed to construct a separating hyperplane. Thus, our simple geometric idea works in much greater generality. Note that the key ingredient we use is the kernel which has to be fixed in advance.

4. Kernel Based Methods from the Point of View of Complexity

Choosing a data driven approach from machine learning follows the adaptive feedback control strategy of a complex system within a dynamic environment. With incoming knowledge, the algorithm refines its classification strategy, using the experience gained in earlier trials. Such experience can be introduced through the supervisor in the supervised approach or gained within the learning phase for an unsupervised learning algorithm. In this way, the method captures the natural system as it exhibits itself through the data. Clearly, this will never be a complete view of the system, a problem which statisticians are well aware of. We will never be able to refine our algorithm beyond the resolution given in the data.

At the same time a learning approach accepts the lack of laws of predictive power. Being 'data driven' explicitly means, that the phenomenon is captured through the data only. In contrast to the model driven approach there is no need for the existence of an objective concept of the phenomenon which needs to be modeled *a priori*.

The maximal margin separating hyperplane is determined by a small set of data points lying closest to the hyperplane. These so-called *support vectors* show the locality of the method. It is the nearby environment determining the exact position of the hyperplane. Far away points just add non-active conditions in the resulting optimization problem.

At the same time, the sparsity expressed through a couple of support vectors implements a reduction of variety. William R. Ashby defined *variety* as the number of different states a system can adopt. In order to control a system, the controller requires at most the same amount of variety as the system to be controlled (*Ashby's law of requisite variety*; cf. Ashby 1956). Thus, it is indispensable for a system to attenuate the variety provided by its environment in order to be effectively able to react to dynamic changes. The reduction of the full data set to a much smaller set of support vectors demonstrates how the algorithm reduces the variety in the data to an amount which is sufficient to solve the classification task.

The hyperplane determined by the algorithm manifests a constraint, i.e., the discrimination boundary, determining what belongs to which class. As such, the learned concept will not be able to generate new data in the closer sense of prediction, but provides a corridor within which an educated guess is possible. The *margin*, i.e., the distance of the data classes to the separating hyperplane provides a measure of uncertainty. The closer a point lies to the hyperplane, the more uncertain is the prediction provided through the support vector machine algorithm. As such, indeterminacy of the system is directly reflected in the algorithm's outcome.

The discrimination of the data into two classes can be interpreted as an emergent property unravelled through the method. It is not the property of a single data point, since the point alone cannot define the class. It rather is the collection of several points together, which implicitly provide the concept of the two classes. The support vector machine algorithm searches for such an emergent concept exactly at the scale between the data points and the classes.

The above mentioned trial-and-error strategy can be followed directly using support vector machines in practice (see zu Castell et al. 2013). Given a high-dimensional data set, let us consider solving a variable selection task. To be precise, we want to determine the subset of variables which best discriminates the data into two classes. This can be done following a *greedy strategy*. The underlying scheme is shown in Figure 3. It is based on quality assurance *via* cross-validation type ideas. Thus, we randomly split the data into a training set and a testing set. We then train a support vector machine using the training set only and predict the class labels on the testing set. Doing so, we can then compare the predicted labels with the 'true' ones and obtain an estimate of the classification accuracy. Repeating the process several times stabilizes the estimated accuracy.

For variable selection, we can now analyze the discriminative power of every univariate set. Choosing the best variable, we can add a second one, again going through all possible choices. The best choice will be fixed and the search moves on to triples of variables. At the end, we have an ordered list of variables. Each variable is associated with a mean prediction accuracy obtained through fixing the variable together with all its predecessors. This way we can determine a collection of variables, which – put together – provide the highest discrimination power. Note that as for the natural system, the greedy step lies in adding the best variable while fixing the set of already chosen ones. This strategy might not lead to the overall optimal choice, but significantly reduces the complexity since a much smaller search space has to be searched through. In the same way, a natural complex system will react based on its present state. It will search for an optimal choice among its current range of options in order to deal with an incoming challenge through the environment.

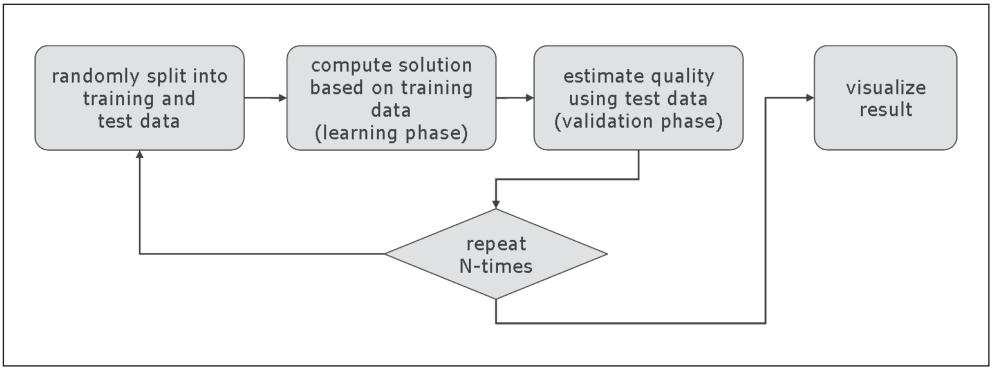

Fig. 3 Schematic concept of cross-validation type quality assurance

The most interesting aspect of the whole method is the choice of the kernel. It can be seen as model selection within a Bayesian framework (cf. Sollich 2002). But it is not a model in the sense of the model driven approach since it does not mimic the phenomenon to be analyzed. Being a similarity measure, the kernel constitutes our subjective choice of what we expect to be similar in the data. It is needed for the algorithm in order to make learning more efficient. Furthermore, it provides another way of variety attenuation since it reduces the data set onto a set of pairwise dissimilarities. At the same time it acts as variety amplifier through the feature space embedding. For most of the kernels, the finite dimensional data is mapped into an infinite dimensional space allowing for a richer geometry to be explored. For example, using a non-linear kernel allows extracting non-linear features with the aid of an affine linear hyperplane. In this direction, the kernel amplifies the limited discrimination capabilities of the separating hyperplane method.

Clearly, much care should be taken in choosing the kernel. As mentioned above, it is the instantiation of the subjective perspective of the observer within the support vector machine approach. It may combine multivariate information as, e.g., kernels for vector-valued data (cf. Beatson et al. 2011). Another option is to incorporate parametric components applying a semi-parametric learning scheme (cf. Berschneider et al. 2011). Several kernels, specifically tailored to inference problems in computational biology, have been developed in the meantime (cf. Schölkopf et al. 2004). Kernels further provide a nice way to synthesize data from different sources.[9]

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[9] For example cf. Yamanishi and Vert 2006.

A standard choice for data living in the Euclidean space is the Gaussian kernel which is the density function of the normal distribution. Commenting on this choice we point out towards another lesson: "Complex systems form structures, and these structures vary widely in size and ration. Their probability distributions are rarely normal, so that exceptional events are not rare."[10] Therefore, we should indeed be careful choosing the Gaussian kernel. It might turn out to underestimate the importance of rare data points.

Summarizing, we have argued that methods to tackle complexity are indeed available. The overwhelming success of many data driven methods in practical applications, in particular of support vector machine algorithms might be understood from them being intuitively adapted to deal with complexity.

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May Forest Functioning Help Agriculture?

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With 4 Figures

Abstract

The understanding of ecosystem functioning is central in both ecology and agronomy. This functioning is based on common processes such as photosynthesis, respiration, assimilate partitioning, water and nutrient uptake etc. Natural ecosystems began with life on Earth and have evolved with it. They have proven their sustainability over geological times and may thus be considered as useful examples for agriculture. There are major differences between them and agrosystems: the latter usually are composed of a single plant species, their soil is often ploughed and receives various inputs such as fertilizers and pesticides, and a large part of their production is exported for human needs. An inadvertent but important consequence of intensive agriculture is the release of pollutants in both the atmosphere and the soil. Comparing agrosystems to natural ecosystems may give guidelines for a better use of natural resources, thus decreasing pollution caused by agriculture and moving towards what has been called "ecologically-intensive" agriculture.

In what follows the comparison will be restricted to forests and crops functioning, considering the use of carbon, nitrogen and water.

Zusammenfassung

Die Funktion von Ökosystemen zu verstehen ist sowohl für die Ökologie als auch für die Landwirtschaft von zentraler Bedeutung. Diese Funktion basiert auf allgemein verbreiteten Prozessen, wie z. B. der Photosynthese, der Atmung, der Verteilung der Assimilate, der Aufnahme von Wasser und Nährstoffen etc. Natürliche Ökosysteme sind mit dem Leben auf der Erde entstanden und haben gemeinsam mit ihm die Evolution durchlaufen. Über die geologischen Zeitalter hinweg haben sie ihren nachhaltigen Bestand erwiesen und können deshalb als nützliche Beispiele für die Landwirtschaft angesehen werden. Es gibt aber größere Unterschiede zwischen ihnen und Agrosystemen: Die letzteren werden in der Regel durch eine einzige Pflanzenart aufgebaut, ihr Boden wird oft gepflügt und erhält verschiedene Zusätze, wie etwa Dünger und Pestizide, und ein großer Teil ihrer Produktion wir für menschliche Bedürfnisse exportiert. Eine unvermeidliche, aber wichtige Konsequenz intensiver Landwirtschaft ist die Freisetzung umweltverschmutzender Agenzien in die Atmosphäre und in den Boden. Der Vergleich von Agrosystemen und natürlichen Ökosystemen kann Hinweise für eine bessere Nutzung der natürlichen Ressourcen geben und dadurch die Umweltverschmutzung durch die Landwirtschaft verringern und das Fortschreiten zu einer „ökologisch intensiven" Landwirtschaft fördern.

Im Folgenden wird sich dieser Vergleich auf Wälder und landwirtschaftlichen Anbau beschränken und die Nutzung von Kohlenstoff, Stickstoff und Wasser betrachten.

1. Net Primary Production of the Biosphere: Past and Current Estimates

Measurements of net primary production of various ecosystem types have been a recurrent task among ecologists. From 1968 to 1974 the International Biological Program attempted to estimate net primary production (NPP) in various biomes and to explain the observed differences in terms of biological processes. At about the same time some scientists attempted to estimate global NPP of the continental biosphere. Lieth and Whittaker (1975) used NPP measurements from about 50 sites and assumed the main factors limiting NPP were temperature and water. They built regression lines between observed values of NPP and annual temperature (T) and precipitation (P). NPP for any given place was then computed as the minimum value of NPP(T) and NPP(P). Knowing the spatial variations of T and P throughout the globe, it was then possible to produce NPP maps and by summing to get a global value for NPP.

Up to the 1960s the only estimates of NPP were derived from measurements of harvested biomass. Several technological improvements allowed rapid progress in NPP measurements: development of CO_2 infrared analyzers for laboratory and field work, of small data loggers, launching of satellite sensors in the 1980s to monitor land reflectance. Ecophysiologists like Eckardt (1967) developed cuvettes to measure CO_2 exchange of small pieces of land while micrometeorologists such as Lemon (1960) and Monteith and Szeicz (1960) were able to measure CO_2 fluxes from vertical profiles of CO_2 and microclimate over crops or grasslands. Forests are rough surfaces with small vertical gradients that are difficult to measure, so another method was tried, the eddy correlation method that computes CO_2 flux as the product of the vertical component of wind velocity by the fluctuation in CO_2 concentration around its average value. The method required an open-path CO_2 gas analyzer, which was not commercially available until the 1990s. Flux measurements by the eddy-correlation method have become routine and are now performed over several hundred sites (http://daac.ornl.gov/FLUXNET/fluxnet.shtml) including all main vegetation types.

CO_2 flux is a net flux, i.e. a difference between CO_2 absorbed by photosynthesis and CO_2 released by vegetation and soil respiration. Measurements are more accurate during the day than during the night when wind and turbulence are low and often intermittent. Therefore, GPP (gross primary productivity or carbon fixation by photosynthesis) is easier to derive from CO_2 flux than NPP that contains a large fraction of respiration that can only be deduced from night-time measurements of CO_2 flux. Beer et al. (2010) summarized data taken over several hundred sites and combined these data with satellite information to derive GPP maps over the entire continents. They arrived at a global value of 122±8 Gt yr^{-1} in good agreement with previously published values (i.e. Saugier et al. 2001). The interest of monitoring CO_2 flux is to be able to study the effects of perturbations that occur in some years (drought, heat waves) and to document long-term trends related to global change such as increased atmospheric CO_2, climate change or nitrogen deposition.

It is estimated that roughly half of GPP is used for respiration, leaving about 60 Gt yr^{-1} for biomass production (NPP). Combining satellite data on vegetation index and on solar radiation, Nemani et al. (2003) reported a 6 % increase in global NPP from 1982 to 1999, explained by two main factors: globally the Earth becomes greener and there has been a decrease in cloud cover over tropical forests such as the Amazonia, leading to increased solar radiation and production.

2. Net Primary Production Used for Human Needs

In recent years some scientists tried to evaluate the fraction of NPP that was used for human needs, called human appropriation of NPP (HANPP). Haberl et al. (2007) computed that HANPP already represents 23.8 % of NPP. Of this estimate, about 53 % comes from harvested biomass, 40 % from productivity decrease related to land-use changes, and 7 % from human-induced fires. To estimate HANPP due to land-use changes, they ran a model of NPP with potential vegetation (that would exist in the absence of humans: NPP_0), and with actual vegetation NPP_a. NPP_0 was 65.5 Gt/yr and NPP_a was 59.2 Gt/yr so the difference 6.3 Gt/yr was attributed to land-use changes. Other estimates have been given and although the details may be criticized, humanity is using an important part of biomass production over land and this part is increasing with population growth and diet improvement. The FAO estimates that between 2000 and 2050 food production should increase by 70 % to satisfy our needs. It also considers that the increase in agricultural land will be quite modest (about 8 %). This means that most of this increase should come from an increase in yields. Cereal yields are still very low in some parts of the globe such as in most of Africa. But in the developed countries and in Asia, yields that have much increased in the second half of the 20th century are now rather constant at relatively high values. Moreover, the ecological movement in developed countries requires with reason that water and air pollution by fertilizers and pesticides should be minimized. How can agriculture maintain high yields with minimum damage to the environment?

It is interesting to compare production processes in crops and in more natural vegetation such as forests. Almost no inputs are used on forests, while fertilizers and pesticides are commonly used on crops, at least by farmers who can afford it. Yet forest NPP is usually higher than crop NPP: Haberl et al. (2007) gave values of 720 gC m^{-2} yr^{-1} for forests and 397 for crops (global averages). The ratio is 720/397 or 1.81. These values were respectively 776 for forests and 393 for crops in Saugier et al. (2001), which is quite similar. Crop values may have been underestimated so we may use GPP values quoted more recently in the supplementary material by Beer et al. (2010): 1419 gC m^{-2} yr^{-1} for world forests and 1095 for crops, which gives a ratio of 1.30. One may argue that the high value for forests comes from their high productivity near the equator, whereas crops are mainly located in temperate zones. Also these values include below-ground production, which is larger in forests than in crops. If we consider only the above-ground productivity of temperate forests, the result from Haberl et al. (2007) is 475 gC m^{-2} yr^{-1} against 340 gC m^{-2} yr^{-1} for crops. From Beer et al. (2010), GPP from temperate forests is 954 gC m^{-2} yr^{-1} or slightly smaller than that of crops with 1095 gC m^{-2} yr^{-1}. Since there are also crops in the tropical zone, we may conclude that in average world forests are at least as productive as world crops. How is this possible?

3. An Analysis of the Differences in Biomass Production in Forests and Crops

Monteith (1977) introduced a powerful method for interpreting differences in production of various crops. It has recently been used by Zhu et al. (2010) and may be applied to any vegetation type and. NPP is expressed as a product of various factors:

$$NPP = 0.487 \cdot S_t \cdot \varepsilon_i \cdot \varepsilon_c \qquad [1]$$

where S_t (GJ m^{-2}) is the total incident solar radiation during the growing season, ε_i the interception efficiency or fraction of that radiation that is intercepted by the crop, ε_c the carboxylation efficiency (fraction of energy in the intercepted radiation that is stored as chemical energy in the crop, resulting of canopy photosynthesis minus respiration).

ε_i increases with leaf area index and reaches a plateau for leaf area index (LAI) varying between 3 for a canopy of horizontal leaves and about 6 for a canopy of erect leaves. It is often modeled as

$$\varepsilon_i = 1 - exp(-k*LAI) \qquad [2]$$

with values for k ranging from 0.3 (erect leaves) to 1 (horizontal leaves). LAI variations for a decidous forest (Norby et al. 2003) and for a maize crop (Maddonni and Ortegui 1996) growing at similar latitudes are represented in Figure 1. Clearly the forest intercepts more light than the crop, keeping a green foliage (LAI > 3) from day 140 to day 275, i.e. for 135 days, while the corresponding period for the maize crop ranges from day 190 to day 260 or 70 days. So the forest clearly wins in terms of growing season and intercepted radiation. When calculated with the same incident solar radiation, intercepted PAR is 1147 W m^{-2} for the forest which is 1.6-times the value for the maize crop. This ratio would be much greater when considering the low crop densities that are still used in many countries which use very little chemical fertilizers, such as in most of Africa where LAI does not increase over 1, with corresponding low values for ε_i and yield.

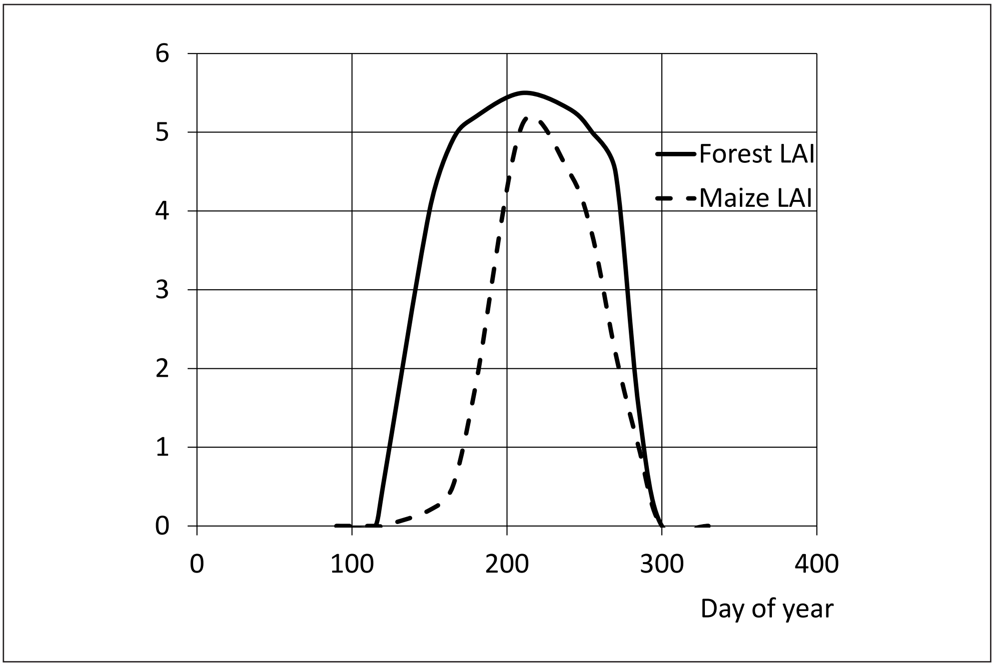

Fig. 1 Variations in leaf area index for a temperate deciduous forest and a maize crop (redrawn from Norby et al. 2003 and Maddonni und Otegui 1996)

What about ε_c the efficiency of photosynthesis and of respiration? Maximum photosynthetic rates (at high light and current CO_2 concentration) of tree leaves have usually been found lower than those of annual species (see for instance Ceulemans and Saugier 1991). Leaf photosynthetic rates range from 2 to 60 µmol m^{-2} s^{-1}. Most tree species have rates lower than 15 µmol m^{-2}

s^{-1} (and up to 25 for a few fast-growing species such as poplar or oil-palm) while cultivated herbaceous species have often rates above this value. However differences between trees and crops are strongly reduced when comparing canopy photosynthesis, as shown for instance by RUIMY et al. (1995) who analyzed responses of canopy CO_2 flux to solar radiation in various vegetation types. A complete canopy with LAI over 3 presents a more linear photosynthetic response to solar radiation than a single leaf (Fig. 2). As a result, maximum net CO_2 fluxes above closed canopies range from 20 to 25 µmol m^{-2} s^{-1} in forests and from 25 to 40 µmol m^{-2} s^{-1} in crops (Fig. 3). To these values ecosystem respiration should be added, a value around 5 µmol m^{-2} s^{-1}, a little more in forests and a little less in crops whose soils have a lower biological activity. So an average value for maximum photosynthesis would be around 27 (22+5) µmol m^{-2} s^{-1} in forests and 37 (32+5) in crops, giving a ratio of 37/27 or 1.36 in favor of crops. This ratio should be reduced somewhat since canopies do not always receive maximum radiation; when radiation is limiting, it is the quantum yield of photosynthesis that becomes important, an entity that is quite constant along a whole range of plant species (ZHU et al. 2010).

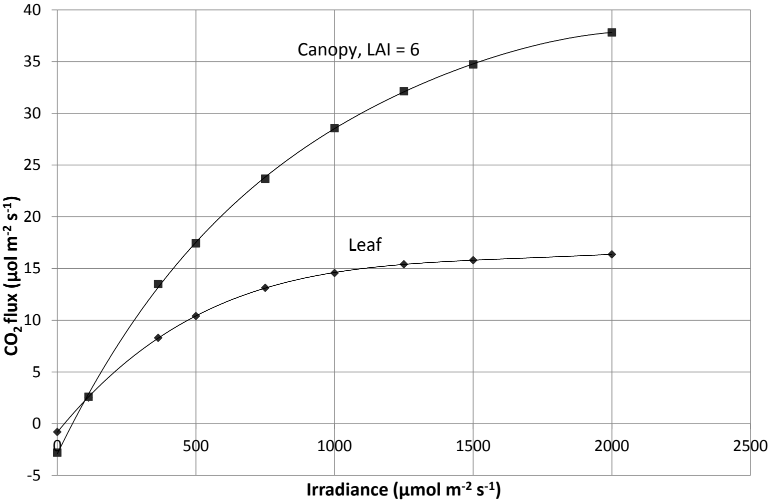

Fig. 2 Photosynthetic responses to light of a leaf (per unit leaf area) and of a plant canopy (per unit ground area) with a leaf area index of 6. From RUIMY et al. (1995), based on measurements on *Dactylis glomerata*.

In conclusion gross primary productivity should be similar in forests and in well-managed crops: the former have longer growing season but the latter have higher canopy photosynthesis. Published values of GPP are in agreement with this statement: for instance GRANIER et al. (2000) gave values ranging from 1000 and 1300 g C m^{-2} yr^{-1} for a beech forest in the North-East of France, while BÉZIAT et al. (2009) quoted values from 800 to 1300 g C m^{-2} yr^{-1} for various annual crops in the South-West of France.

If fertilized crops have GPP similar to forest GPP, non-fertilized crops have much lower values. We may thus understand why global values of GPP are smaller for crops than for

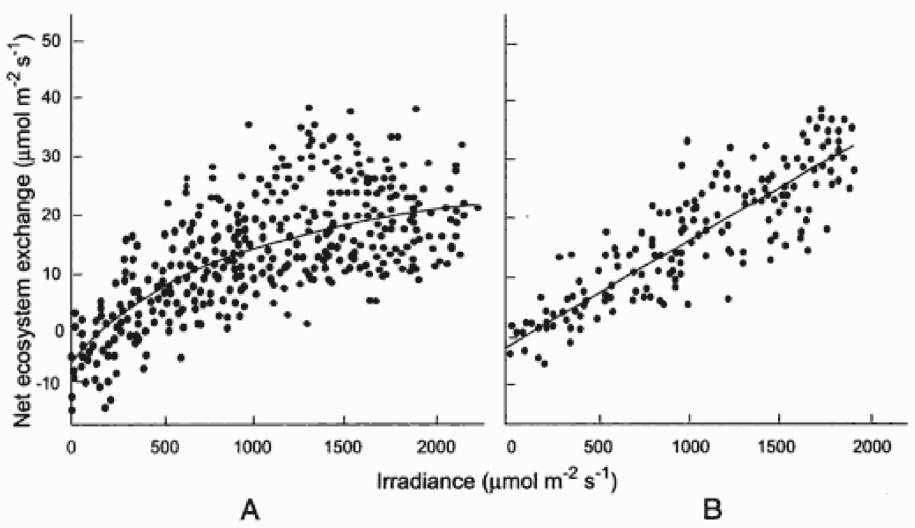

Fig. 3 CO_2 flux density (or net ecosystem exchange) as a function of incident light above forests (*A*) and crops (*B*). After Ruimy et al. (1995).

forests. Forests are not usually fertilized, and in temperate zones they are established on soils that have been found unsuitable for agriculture. Yet they maintain high LAI throughout the entire growing season, while crops require fertilizers to achieve high values of LAI for a limited time. Why is it so? Annual crops are harvested every year, the removed biomass is close to ½ of NPP, which exports from the agrosystem an important amount of nutrients (more than half, since the reproductive parts are richer in nutrients than the rest of the plant). In forests it takes several years between two clearings, and several decades between two harvests. Nitrogen concentration in wood is about 10-times smaller (0.1 to 0.2 %) than in grains or tubers (1 to 2 %). Since cumulated harvests of biomass are slightly smaller in forests than in crops, nitrogen exports are at least 10-times higher in crops, which explain the necessity of fertilization to ensure repeated high yields.

4. How to Minimize Damages to the Environment in High-Yielding Crops?

The increase in crop yield has been spectacular in many developed countries. The average wheat yield in France increased from 2 t/ha in 1950 to over 7 t/ha in 2000 and then leveled off. At world scale it increased from 1.1 t/ha in 1961 to 3 t/ha in 2009 (FAO data). The use of N fertilizers worldwide has grown from 10 MtN/yr in 1960 to over 80 MtN/yr in 2000 (85 in 2009, produced by ammonia synthesis, which requires 1.1 % of the world energy consumption from Dawson and Hilton 2011). In addition to that, it is estimated that biological nitrogen fixation by agrosystems amounts to about 40 MtN/yr (Galloway et al. 2008), which gives a global input of 125 Mt/yr of reactive nitrogen for agriculture. Industry and transport through fossil fuel combustion also release reactive nitrogen in the atmosphere, with a total production estimated by Galloway et al. (2008) at 187 Mt/yr. Since nitrogen in cereal grain harvests is about 33 Mt/yr (Raun and Johnson 1999), a large fraction of nitrogen fertilizer is lost to the atmosphere and to drainage water. As a consequence, there has been increasing amounts of N atmospheric deposits, from 34 Mt/yr estimated in 1860, to 105 Mt/yr presently. Local values, that were typically less than 3 kgN/ha before 1860, are now in excees of 5 kgN/ha in most parts of the continents, with large areas being over 10 kgN/ha (Galloway et al. 2008). Rivers in agricultural areas have also seen a strong increase in nitrate concentration of their water, due to fertilizer leaching. So we are faced here with a dilemma: without mineral nitrogen cereal yields are usually reduced by 30 to 50 % with respect to conventional agriculture,[1] which is insufficient to feed the growing human population. Yet we should clearly limit nitrogen pollution in the atmosphere and in water. We have pointed out nitrogen but intensive agriculture also makes a strong use of pesticides that also affect our environment, and of water, a limited resource in many countries.

Before the advent of mineral fertilizer, farmers were using organic fertilizers such as animal manure, they did not treat their crops, but yields were only a fraction of present ones, as shown by Goulding et al. (2008) on the famous Broadbalk wheat experiment in Rothamsted. With no fertilization at all, wheat yields with no rotation stayed between 1 and 2 t/ha depending on the variety used. With manure addition, they increased at 2 to 3 t/ha. Using herbicides and mineral fertilizer in addition to manure increased yields to 6 to 7 t/ha when wheat was planted every year. When wheat was planted in the first year in a crop rotation, then yields reached 9 t/ha. It should be noted that these improvements were not entirely the result of fertilization or herbicides, for new varieties with higher yields were progressively used. When wheat yield is plotted as a function of the amount of applied nitrogen (Fig. 4), the yield increase is high at low N and decreases at high N. This is well known, the crop makes the best use of applied N when it is low, and the N use efficiency (yield increase/ N fertilizer increase) decreases as the rate of fertilization increases, which means an increasing fraction of nitrogen is not used by the crop, but lost to the environment.

How to decrease these losses and to increase the fraction of fertilizer absorbed by crops? Firstly a slight decrease in the amount of N fertilizer has little impact on yield, and the economy made on the cost of fertilizer may compensate for the slight reduction in yield. Then it is now possible to locally adjust the amount of fertilizer to topography and soil properties in what is called precision farming. Genetics and breeding may also be used to produce new varieties with higher nitrogen use efficiency. Also nitrification inhibitors may be used to prevent nitrate leaching in periods of high rainfall. It should be also much desirable to make more use of biological nitrogen fixation. In the 1970s molecular geneticists claimed they would produce a nitrogen-fixing wheat within one decade or so, but four decades later this goal seems still remote, so in the meantime one may rely on rotations using nitrogen fixing species such as legume crops.

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[1] Badgley et al. (2007) compared cereal yield in organic agriculture (OA) and conventional agriculture (CA). He found a ratio (OA/CA) of 0.93 in developed countries, and 1.57 in developing countries, with the conclusion that OA could well feed the world. OA may be of great help in countries that use little or no mineral fertilizer, but the ratio of 0.93 seems in contradiction with many other studies and thus the conclusion seems doubtful (see for instance Goulding et al. 2009).

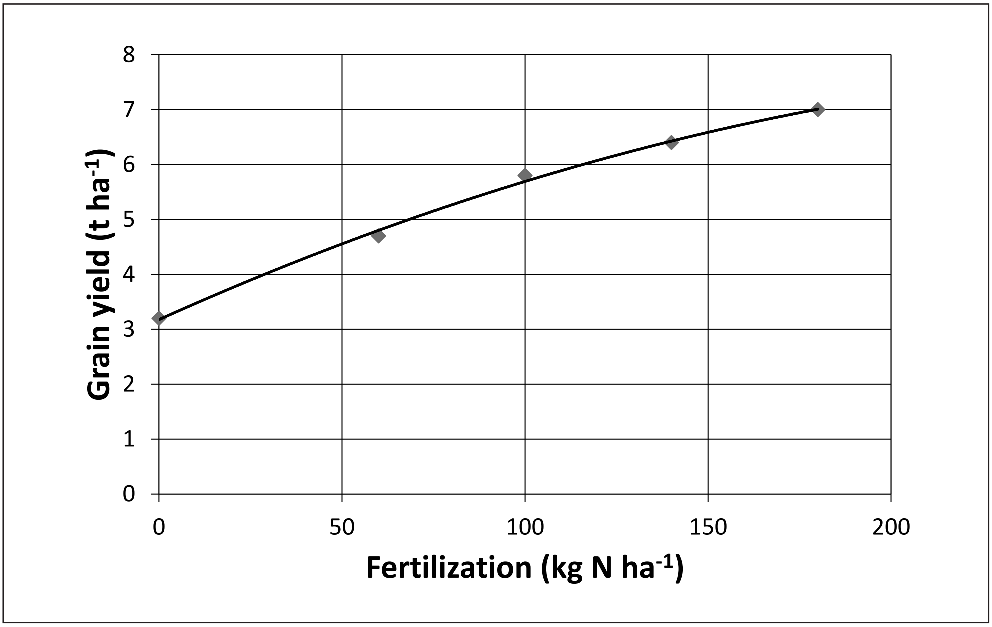

Fig. 4 Typical response of wheat yield to nitrogen fertilization. Redrawn from Charles et al. (2006).

5. Is it Possible to Reduce the Amount of Water Needed to Produce Biomass?

Forests are known to use much water by evapotranspiration: when a drainage basin is deforested, there is increased run-off, which enhances stream flow at the bottom of the basin. So they do not seem to be a good model for crops. However, forest trees have deep roots to withstand dry periods, and thanks to hydraulic lift (Caldwell et al. 1998) they prevent surface layers of drying out completely, which allows mineral uptake to go on. Forest soils also have high water permeability caused by the cavities left by fauna. This decreases run-off and maximizes soil water storage, thus making good use of available precipitation. Nitrate leaching is relatively rare under forest soils because tree roots absorb mineral nitrogen as soon as it is released by decomposition, which prevents nitrate accumulation. This gives at least two ideas to improve plants behavior in dry environments: to increase rooting depth by breeding, and soil biological activity through soil conservation. But is it possible to produce more biomass with less water?

As pointed out by Tardieu and Tuberosa (2010), biomass accumulation and transpiration are intrinsically linked *via* their main determinants, that is, stomatal aperture and leaf area, so there is a ‘built-in’ contradiction between biomass accumulation and stress avoidance *via* transpiration. Breeding can only optimize the terms of this contradiction, for example, by increasing the ratio of accumulated biomass to transpiration (water-use efficiency, WUE) or increasing the degree of root or leaf growth maintenance under water deficit. WUE has been studied through the $^{13}C/^{12}C$ ratio in biomass, both on trees and on crop species, but there have been very few examples of crops successfully bred for higher WUE, such as wheat under dry climates in Australia (Rebetzke et al. 2002). Tardieu and Tuberosa (2010) advocate the use of production models to determine what plant trait should be improved, i.e. WUE, leaf or root growth. It might also be desirable to manage the crop so enough water stay in soil during the critical reproductive period. The answer is obviously dependent on the species but also on local climate and soil. If it has been shown by modeling that maintaining leaf growth under water stress is the best strategy, then one may try to find quantitative trait loci, i. e. sets of genes that affect this trait, which means testing a large number of phenotypes to later select those useful for selection. Thus a careful combination of modeling and of experimentation may lead to some improvements, but no miracle should be expected. Passioura (2006) has given a realistic analysis of what can be obtained in the coming years: the hope that plants can be transformed by one or at most a few genes to grow well with very scarce water, is in my view misplaced. Functional genomics may have much to offer in the coming decades in relation to improving water productivity, but only when embedded in well-understood biochemical, physiological, and agronomic contexts.

Passioura insists on using water more efficiently through agronomic practices: reducing losses from soil evaporation, deep drainage and runoff. On dry land such as in Sahel, we may add run-off farming, which concentrates water in areas supplied with animal manure to improve plant growth. Such techniques have been used for centuries and were refined in Negev (Evenari et al. 1982) and recently developed successfully in Niger and Burkina Faso (Reij et al. 2009). Irrigation is sometimes considered as the main tool to improve food production in the coming years but it will not be possible to greatly increase the irrigated areas (presently about 280 million hectares worldwide i.e. 18 % of the total cropped area) and thus the development of dryland agriculture will be necessary and can be achieved at a fraction of the cost needed to implement irrigation.

6. Towards Sustainable Agriculture

Using the functioning of natural ecosystems as a basis to improve resource use efficiency of crops may give interesting insights. For Pretty (2008) the key principles for sustainability are to:

(*i*) integrate biological and ecological processes such as nutrient cycling, nitrogen fixation, soil regeneration, allelopathy, competition, predation and parasitism into food production processes,
(*ii*) minimize the use of those non-renewable inputs that cause harm to the environment or to the health of farmers and consumers,
(*iii*) make productive use of the knowledge and skills of farmers, thus improving their self-reliance and substituting human capital for costly external inputs, and
(*iv*) make productive use of people's collective capacities to work together to solve common agricultural and natural resource problems, such as for pest, watershed, irrigation, forest and credit management.

Only a few of these points have been discussed here, regarding nitrogen and water use to produce biomass. Item (*iii*) is quite important; for instance Reij et al. (2009) have shown that for farmers in the Sahel, digging holes in the ground during the dry season (when agricultural work is minimum) for collecting water in the rainy season is a very valuable investment that does not require much equipment but has a strong pay off. However, even the best techniques need a strong social organization to be adopted at large scale, and this is why item (*iv*) is also essential. This has been well illustrated by Ostrom (1990) regarding the use of common resources such as aquifers for irrigation in the county of Los Angeles. She showed that people do not necessarily exhaust a common resource, but if they have enough information on the state of that resource and on the consumption rate by each one, they may reach an arrangement (a reduction in resource use) that will be respected by each participant. This is quite typical of the attitude of modern man towards nature: we considered natural resources as virtually infinite, until we face serious problems, as shown several decades ago by the club of Rome with Meadows et al. (1972). We still have to learn sustainability in agriculture as in industry and in energy and water supply. Some people have proposed to reconsider the history of mankind with this goal in mind (Costanza et al. 2007, Hibbard et al. 2009).

7. Conclusion

Forests use water and nutrients in an efficient way since they produce as much biomass as crops that receive large amounts of inputs in terms of fertilizers and pesticides. The relatively low photosynthetic rate of tree leaves is compensated by a longer growing season and thus a greater light interception. However, nutrient export is much smaller in forests. Thus crops require mineral fertilizers to achieve high yields, but their use may be optimized through various techniques.

Water consumption by forests is at least as high as that of crops but forests manage to overcome long dry periods by making the best use of soil water thanks to deep tree roots and to high soil infiltration rates allowed by biological activity. Such a wise use of local water resources may be mimicked by crops to extend soil water use throughout the growing season instead of quickly exhausting soil water reserve without keeping enough water for the critical reproductive period.

The ideas presented in this paper are just examples of the changes required to shift our attitude towards natural resources, from predation to management. Thus developing renewable forms of energy become necessary with the coming exhaustion of fossil fuels. In agriculture water and nutrients should be used with parsimony to get sufficient yields with minimal damage to the environment. This requires more research in plant sciences (molecular biology and genetics, breeding, physiology), in soil sciences (physics, chemistry and biology, especially microbiology) and in integrative sciences such as ecology and agronomy to define and implement suitable farming techniques. But science by itself is not enough: it allows changes to be made but changes will occur only if people find interest in these changes, which requires some form of consensus that can only be obtained after discussion between all the interested partners. Thus the management of natural resources leads us to democracy.

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Information Theoretic Dissection of the Holobiont – Host-Virus Interaction as an Example

Kay Hamacher (Darmstadt)

With 4 Figures

Abstract

Evolutionary pressure and evolutionary dynamics shape the genomic information transferred between generations and between organisms that exchange genetic information. Information theory has proven to be of particular importance and utility for the analysis and quantification of emerging genomic patterns. Now, collectives of organisms, from host-pathogen collections to a holobiont comprised of hundreds of genetically diverse organisms, pose an interesting and important challenge to the notion of information theory: How to find the unit of selection? Options vary from the single entity to networks of coupled entities. We discuss different aspects of information theoretic analysis that might lead to a better conceptual solution to this question. In particular, we will use the concept of Jensen-Shannon entropy to assess the currently established thinking on interactions in evolving holobiontic systems, such as plants and their viruses.

Zusammenfassung

Evolutionsdruck und Evolutionsdynamik prägen die genomische Information, welche von Generation zu Generation und zwischen Organismen ausgetauscht wird. Informationstheorie ist ein wichtiger Zugang, diese Zusammenhänge zu analysieren und zu quantifizieren. Gruppen von Organismen, von Pathogen-Wirt-Gruppen bis hin zum Holobiont aus Hunderten von genetisch variierenden Organismen, bilden eine interessante und wichtige Herausforderung für die Informationstheorie: Wie bestimmt man die Einheiten der Selektion? Die Möglichkeiten variieren von einzelnen Einheiten bis hin zu ganzen Netzwerken von interagierenden Einheiten. Wir diskutieren hier verschiedene Aspekte der informationstheoretischen Analyse, welche zu einem konzeptionell besseren Verständnis dieser Frage führen können. Besonders eignet sich die Jensen-Shannon-Entropie, um die vorherrschende Meinung zur Interaktion in evolvierenden Systemen, wie etwa Pflanzen und ihren Viren, zu bewerten.

1. Introduction

Evolution is the driving force of biological dynamics. The question of the unit of selection is unanswered to this day (Dawkins 1976, Wilson 1994), but eventually it needs to include the notion of biological scales and collective entities, such as the holobiont or complexes of hosts and their respective pathogens, parasites, and viruses.

Understanding the mutually supportive interactions in a holobiont on a genetic/genomic level is difficult to this day due to the non-availability of all the sequences of a system at a quasi-ecological scale. As a first step into this direction, we therefore set out to investigate the evolutionary coupling of plant viruses and their respective hosts. These biological entities are engaged in various, complex interactions. Due to the known sequences of some dozen of plant viral genomes, we have therefore an ideal test case for information theory applied to different scales of biological systems.

Information theory (Cover and Thomas 2006) is a powerful tool to analyze general correlations and pattern in empirical data. In previous work, information theory was successfully applied by us

- to obtain reduced amino acid encoding (Pape et al. 2010),
- to extract co-evolutionary patterns within the HIV1 protease during its molecular evolution (Hamacher 2008), eventually suggesting drug targets that are subject to reduced evolutionary pressure towards resistance, or
- to point to (co)evolutionary hot spots in the molecular evolution of acetylcholinesterase (Weissgraeber et al. 2011).

Furthermore and most important, information theory is applicable to all scales and all "entities" that contain signals, thus effectively enabling multi-level and multi-scale investigations on the occurrence and correlations of evolutionary signals or information. This suggests the applicability to ecosystem-sized collectives such as the holobiont.

Research at the interface of information theory and computational biology is facilitated by available high-performance codes and efficiently implemented workflows to be used on, e.g., modern multi-core systems such as Graphical-Processing-Units (Waechter et al. 2011, Hoffgaard et al. 2010).

2. Method

We use the information theoretic based phylogeny method put forward by Hamacher (2010). This method uses distributions of protein domains encoded in a genomic sequence as a descriptor for the respective genome and then applies the Jensen-Shannon divergence to compute distances between genomes as information theoretical distances between those distributions. In the following, we describe the details of this phylogenetic approach: We first transcripted the genomic sequences into the three different open reading frames. The obtained amino acid sequences were then searched for signatures of structural protein domains by the HMMer[1] suite of profile hidden Markov models (Durbin et al. 1998). This gives rise to a so-called description vector v representing the protein domain content of a genome. The j-th entry v_j of v is the number of HMM-hits for protein domain type j of which there are N described in HMMer in total. We can then define the ratio or frequency of such a protein domain type j by

$$p_j := \frac{v_j}{\sum_{j=1}^{N} v_j} \qquad [1]$$

In a 'frequentist' interpretation p_j can then be regarded as the probability to find the protein domain type j when randomly selecting a region of the full genome. Two organisms, or a host and its virus are then described by two such vectors, e.g. p and q.

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[1] The HMMer software package implements Hidden Markov models to identify protein domains from amino acid sequences (Durbin et al. 1998).

Now, the Kullback-Leibler divergence (Cover and Thomas 2006) allows us to quantify the differences between any two probability distributions. A variant, the Jensen-Shannon distances, was discussed in (Hamacher 2010) as an approach to phylogenies of protein domain compositions of two genomes described by *p* and *q*, respectively. The Jensen-Shannon divergence $D_{JS}(p,q)$ between the distributions p and q for two data sources reads

$$\begin{aligned} &D_{JS}(p,q) := \tfrac{1}{2} D_{KL}(p \,\|\, m) + \tfrac{1}{2} D_{KL}(q \,\|\, m) \\ &D_{KL}(p \,\|\, m) := \sum_{j=1}^{M} p_j \log_2 \frac{p_j}{m_j} \quad \text{and} \quad D_{KL}(q \,\|\, m) := \sum_{j=1}^{M} q_j \log_2 \frac{q_j}{m_j} \\ &m_j := \tfrac{1}{2}(p_j + q_j) \qquad\qquad \text{and} \quad d(p,q) := \sqrt{D_{JS}(p,q)} \end{aligned} \qquad [2]$$

In Equation [2] m_j is an “intermediate” distribution of a hypothetical, evolutionary intermediary. $D_{KL}(p \,\|\, m)$ and $D_{KL}(q \,\|\, m)$ are the Kullback-Leibler distances between *p* and *m* or between *q* and *m*, respectively. A suitable metric on these distributions is then *d(p,q)* (Li 2004) of Equation [2].

As a first step, we computed the distances $d(p,q)$ between all viral genome pairs *p* and *q* for the set of viral genomes described in Section 3 (Materials). Secondly, using distance-matrix based clustering (Murtagh 1985) as implemented in the statistical software package R (*R Development Core Team* 2009) allowed us to derive a phylogenetic tree for the genomic sequences in the final step of the phylogenetic-information-theoretic analysis. It was previously argued that the Jensen-Shannon divergence for protein domains is related to an evolutionary model of higher-order operators (Hamacher 2010). Accepting the assumptions of this model, we can therefore even assign a molecular clock to the branch lengths of the clustering tree.

3. Materials

We used the *Chlorella* NC64A genomic sequence (Blanc et al. 2010), and plant viral genomes for the viruses AR158, ATCV-1, FR483, MT325, NY2A, PBCV1[2]. In these sequences there are unknown nucleotides to be found, each of which cannot be used in the translation to a specific amino acid, thus leading to ambiguity. They correspond to a very small fraction of some 0.7 % of all nucleotides in the genomic sequences. Therefore, we replaced all of the unknown nucleotides before translation by a “G” to avoid stopping codons, eventually accepting some noise in the data. Therefore, we expect the identification of potential protein domains to deviate from perfectness. Thus, we most likely need to accept hits with some elevated threshold of, e.g., *E* values.

4. Results

First, we analyze the number of identified protein domains as a function of thresholds on the statistical significance in the HMMs as expressed by the *E* values. In Figure 1 we show the results. From previous studies it is known, that viral genomes contain some 600 sequences

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[2] The author is grateful to G. Thiel for making these sequences available.

that show protein sequence characteristics (Van Etten et al. 2010). This is the gauging target we need: the HMM-identified sequences need to fulfill this property and not necessarily correspond to the number of proteins expressed and experimentally verified.

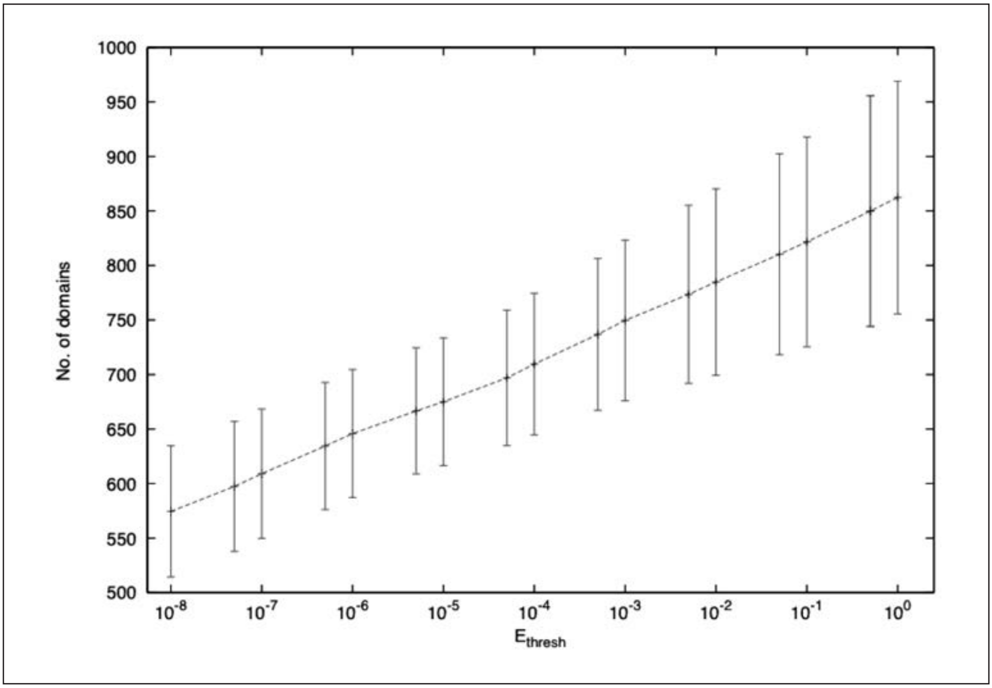

Fig. 1 The number of identified protein domains (y axis) as a function of the threshold on E-values (x axis) of the Hidden-Markov models from PFAM[3]. The points show the number of protein domains averaged over the viral genomic sequences; the error bars indicate the standard deviation of this number

From Figure 1 we conclude that a good choice for the E threshold is $E_t = 10^{-7}$. From hereon, we will choose $E_t = 10^{-7}$ as a selection criterion on HMMer results and for the derivation of p_i and q_i numbers.

Using Equation [2], we can now compute the evolutionary distances between the genomic sequences. We repeat this for the Jensen-Shannon divergence $D_{JS}(p,q)$ and the metric $d(p,q)$. While $d(p,q)$ is a metric and therefore usable as a distance measure between genomic sequences, the rationale to use also the Jensen-Shannon divergence $D_{JS}(p,q)$ was discussed in (Hamacher 2010): assuming Poisson processes in domain creation and annihilation within genomes, the Jensen-Shannon divergence is proportional to the evolutionary divergence times. Using the clustering algorithm implemented in R (*R Development Core Team* 2009) we obtain the clusterings in Figures 2 and 3 for the threshold $E_t = 10^{-7}$.

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[3] The PFAM database collects protein families, each represented by multiple sequence alignments and Hidden-Markov models (HMMs), available from http://pfam.sanger.ac.uk

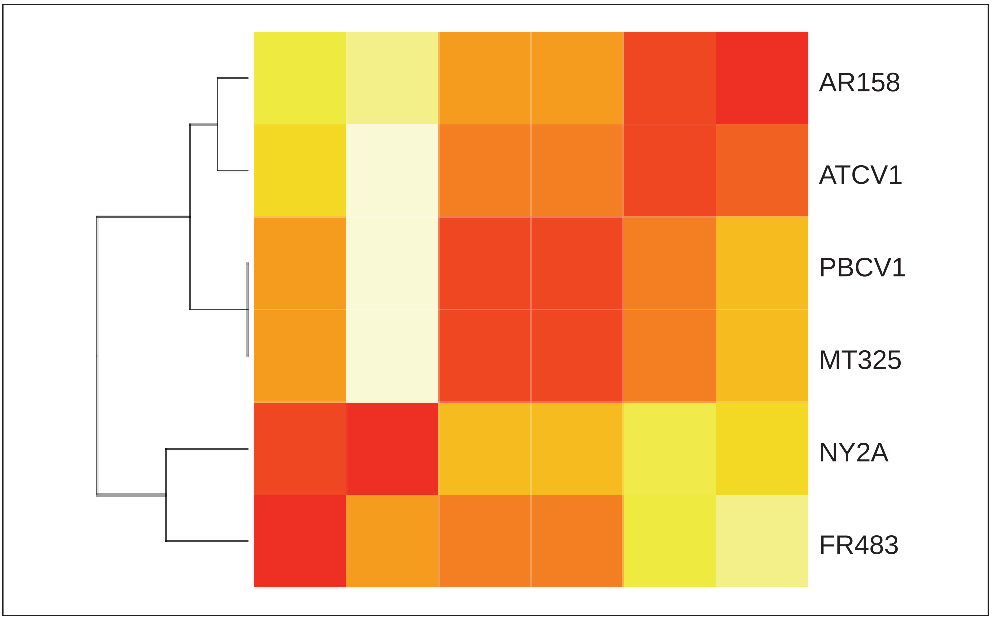

Fig. 2 Jensen-Shannon divergences $D_{JS}(p,q)$ between the identified protein domain distributions in the viral genomes as a matrix. The clustering on the left was obtained by an automated clustering approach (red=low values, yellow/bright = high values).

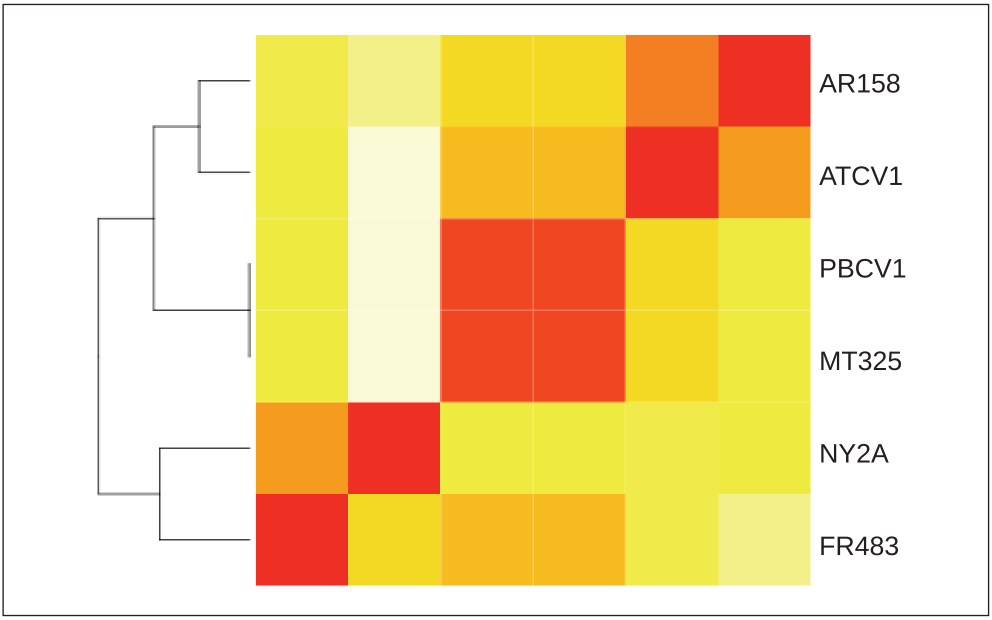

Fig. 3 The distances $d(p,q)$ between the identified protein domain distributions in the viral genomes. The clustering on the left was obtained by an automated clustering approach, color scale as in figure 2.

First, we note that both clusterings agree on the evolutionary relations of the viruses. While the subtle differences between *d(p,q)* and $D_{JS}(p,q)$ are noticeable in the colored matrices themselves, they result in the same phylogenetic relations. Nevertheless, clustering according to *d(p,q)* resulted in a more pronounced distance spectrum. This observation suggests, that *d(p,q)* will be a more effective phylogenetic distance measure in situations where more than just the six genomes from above are investigated. In such a set-up, subtle differences might lead to different phylogenetic clusterings and thus the usage of *d(p,q)* seems to be preferable; in particular, as *d(p,q)* is from an evolutionary perspective conceptually superior as it connects the distance values to a basal model of neutral evolution.

5. Discussion

In Figures 2 and 3 we obtained the phylogenetic relations of viral genomes for green algae viruses. Despite the subtle differences in the distance measures, the phylogenetic clustering agreed. The distance itself is based on an information theoretical analysis of the coded protein domains. The distance between two viruses is – due to Stein's Lemma (Cover and Thomas 2006) – related to the false-positive rate of the hypothesis that two proteasomes/protein domain description vectors coded in two viral genomes can be identified.

Furthermore, the distance is proportional to the evolutionary divergence time in a model of neutral evolution, in which – contrary to traditional molecular-phylogenetic models – the evolutionary operators are protein domain creation and annihilation. For long evolutionary time scales, such modeling is conceptually superior to "single-nucleotide" or "single-amino-acid" models with focus on rather frequent events and which can hardly model the sequence dynamics of various viral genomes, which might acquire functional units form their respective hosts or *via* cross-exchange during competitive infection (Hamacher 2010). Therefore, such a modeling might be more useful to model the interaction of virus – host, or any other parasite-host system in the holobiont.

The particular results in Figures 2 and 3 support this: the respective hosts are *Chlorella* NC64A for PBCV1, AR158, and NY2a; *Chlorella* SAG3.83 for ATCV1 and TN603; and *Chlorella* Pbi for MT325, CVM1, and FR483. Thus, the phylogenies we obtained do not correspond to host specificity. This implies that the viruses have acquired protein domain information *via* other routes.

To control for host specific environmental conditions, we analyzed the DNA polymerase coded in three viral genomes alone. The results are shown in Figure 4. Although, only a fraction of the viral genomes of Figures 3 and 4 could be related, we see that the phylogeny based on just this *single* coded entity (DNA polymerase) resembles the host specific environments in which the viruses evolved much more clearly. Just recently (Bose et al. 2011), an analysis of the disorder of the *full* genomes at the nucleotide level has shown host specific evolution of the viruses.

At first glance, we obtained two contradicting phylogenetic results:

- on the one hand, we found host specific evolution, compare Figure 4 and Bose et al. (2011),
- while on the other hand, we found non-specific clustering.

This superficial contradiction can be resolved. One 'just' needs to revisit the underlying assumptions and modeling approaches for the evolutionary dynamics – in particular, the units of

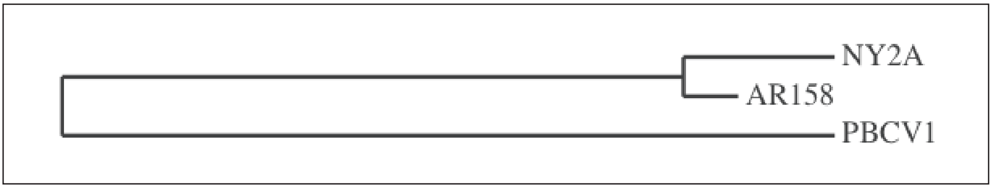

Fig. 4 Phylogenetic tree for the DNA polymerase coded in the viral genomes. The tree was obtained by the phylogeny.fr web service and its standard configuration (Dereeper et al. 2008, 2010, Edgar 2004, Castresana 2000, Guindon and Gascuel 2003, Anisimove and Gascuel 2006, Chevent et al. 2006). The search sequence was blasted *via* BLASTp Version 2.2.25 (Altschul et al. 1997) with the query sequence set as the DNA polymerase sequence from PBCV-1 (Grabherr et al. 1992). Note, that we could not obtain a positive identification in the genomes of FR483, MT325, and ATCV1; thus we left these genomic sequences out of the traditional phylogenetic analysis.

selection and the inclusion/exclusion of the environment formed by the 'holobiontic' regime, e.g., the viral host and its evolution.

For a single molecule as the DNA polymerase the typical change is modeled at the scale of a single nucleotide or a codon. Thus, typically time-scales modeled cover the neutral diffusion of single nucleotide changes. The selective advantage of the molecular evolution reveals itself in the function of a single protein, most likely even a single region within this protein such as a binding interface. Therefore, trees obtained from single molecule phylogenies resemble the *selection of the molecular function and stability.*

The same observation can be made for genome-wide studies based on properties of the nucleotides of the whole genome, when the analysis uses (implicitly) the unit of selection as any set of a few nucleotides. This is the case in, e.g., the study by Bose et al. (2013). Again, the *selective advantage* tends to focus more on local properties such as, e.g., *the ability to compress genomic DNA/RNA into viral capsids.* This, again, focuses on selective advantage of a single molecule.

Now, in this study we rather focused on the *combined selective advantage* of proteins and thus the selective advantage of the network of their concerted action and function. In contrast to the approaches on the nucleotide level (as above) the operator of change here is not a combination of nucleotides changes, insertions, or deletions. Rather, the operator of modification is the introduction or deletion of complete protein domains. This modeling is potentially much more suitable to cover molecular evolutionary events such as horizontal gene transfer or any acquisition of genetic material by a virus from its host(s).

Thus, the apparent contradiction might be resolved by an involved argument on the details of the respective modeling assumptions: the nucleotide-based analysis reveals evolutionary anchestory of *single, individual molecules* and the *evolutionary pressure on the full viral genome* (such as capsid packing). At the same time the *network* of potentially useful proteins (from the virus' perspective) is subject to a complete different evolutionary pressure – potentially a pure functional one. Eventually, these effects are overlaid and seem to set boundaries on evolvability simultaneously. The unit of selection seems, therefore, to be a rather opaque concept. Rather, the phylogenetic and evolutionary analysis needs to include several approaches, overlay them, and take on step back from a focus on just one individual aspect of the biological entity under investigation.

Our analysis can easily be transferred to ecosystems: one would first derive phylogenetic relations based on statistics of full genomic sequences on the one hand (as in e.g. Bose et al.

2013) and based on the 'full protein-universe' as identified by the procedure described above. In a second step, one needs then to investigate the differences between such results to extract the situations and cases in which the local selective pressure on full sequences differs from the selective pressure on coded domains. One can even imagine a protocol to analyze the sensitivity to perturbations: by omitting single or grouped organisms from the ecosystems and repetition of the two protocols and the difference assessment this is easily achievable, but computationally demanding. However, this remains an open question for future studies.

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2012 jährt sich zum 360. Mal die Gründung der heutigen Deutschen Akademie der Naturforscher Leopoldina – Nationale Akademie der Wissenschaften im Jahr 1652 in Schweinfurt, und am 7. August 1687, vor 325 Jahren, gewährte Kaiser Leopold I. der Gelehrtengesellschaft besondere Privilegien. Diesen Anlässen widmet sich eine Ausstellung, die eine große Anzahl von authentischen und einzigartigen Quellen zur Geschichte der Akademie aus den Archiven der Stadt Schweinfurt und der Leopoldina als Kooperationsprojekt zunächst in Halle (Saale) und später in Schweinfurt zeigt. Der Schwerpunkt liegt auf der frühen Internationalisierung der Akademie. Die Publikation beschreibt die vorgestellten Objekte und enthält darüber hinaus eine Edition aller kaiserlichen Urkunden von 1677 bis 1742.

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Stem Species: Plant Species that Function as Regenerating Cells of Gaia

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With 1 Figure

Abstract

The planet undergoes an environmental crisis with rising levels of greenhouse gases in the atmosphere and unprecedented rates of species extinction. Contemporary man begins to understand that such problems threaten his own survival. We examine this scenario under the light of the Gaia hypothesis that forwards the notion of dynamic stability of life in time, whether or not mankind persists on the face of the planet. For man to carry on being part of Gaia, biodiversity will need to "regenerate" in face of changes. Since higher plants amount to > 99 % of the biomass of the planet, they are collectively responsible for most such services. In organisms, stem cells act as a repair system for the body and can self renew to produce more stem cells. Focusing on higher plants, we define "stem species" as plant species that act as a repair system for Gaia, guarantee the production of more species and lead the protection of environmental services vital to man. This concept differs from the well-established concepts of engineer species or pioneer species or nurse plants for the fact that it operates also in geological time and not only in ecological time. We illustrate the concept with two examples from Brazilian vegetation: (*a*) *Clusia hilariana* Schltdl. (Clusiaceae), typically a hemiepiphytic strangler in rainforest that in geological time migrated to newly formed sandy plains to be a tree responsible for diversity in land, soil and water and for massive carbon storage; and (*b*) the case of spontaneous forestation of a portion of an Amazonian lake buried by bauxite tailings, with nitrogen-fixing plant species of flooded forests. We convey the following messages: (*1.*) for most species we do not know enough ecology, physiology and genetics to predict responses to current and future environmental changes; (*2.*) biological conservation of species still focus a lot on understanding rarity, but we know much less about regenerating power that can be an attribute of both rare and common plants; and finally (*3.*) habitat fragmentation and loss of connectivity can be at least as big a threat to such "stem species", and therefore to man itself, than climate change.

Zusammenfassung

Unser Planet erlebt eine Umweltkrise mit wachsenden Niveaus von Treibhausgasen in der Atmosphäre und einem nie da gewesenen Umfang von Artensterben. Der zeitgenössische Mensch beginnt zu verstehen, dass solche Probleme sein eigenes Überleben gefährden. Wir untersuchen dieses Szenario im Lichte der Gaia-Hypothese, die eine dynamische Stabilität des Lebens in der Zeit annimmt, unabhängig davon, ob die Menschheit im Antlitz des Planeten bestehen bleibt oder nicht. Wenn der Mensch weiterhin Teil von Gaia bleiben will, muss sich Biodiversität angesichts von Veränderungen „regenerieren". Da die höheren Pflanzen mehr als 99 % der Biomasse des Planeten ausmachen, sind sie kollektiv für die meisten solcher Dienste verantwortlich. In Organismen wirken Stammzellen in Reparatursystemen des Körpers und können sich selbst erneuern, um mehr Stammzellen zu produzieren. Indem wir uns auf höhere Pflanzen konzentrieren, definieren wir „Stammarten" als Pflanzenarten, die in Reparatursystemen für Gaia arbeiten, die Erzeugung von mehr Arten garantieren und zum Schutz der für den Menschen vitalen Umweltdienste führen. Dieses Konzept unterscheidet sich von den wohl etablierten Konzepten der Arten für Design, der Pionierarten und der Arten mit Hebammenfunktionen vor allem dadurch, dass es für geologische Zeiträume und nicht nur für ökologische Zeiträume gilt. Wir illustrieren das Konzept mit zwei Beispielen aus der brasilianischen Vegetation: (*a*) *Clusia hilariana* Schltdl. (Clusiaceae), typischerweise ein hemi-epiphytischer Strangulierer im Regenwald, der in geologischer Zeit in neu gebildete sandige Ebenen eingewandert ist, um als ein Baum für die Diversität auf dem Land, im Boden und im Wasser und für massives Kohlenstoffbinden verantwortlich zu sein. (*b*) Dem Fall einer spontanen Bewaldung des Teils eines Sees im Amazonasgebiet, der unter Bauxit-Abbauresten begraben wurde, durch Stickstoff fixierende Pflanzenarten von Überflutungswäldern. Wir wollen die folgenden Feststellungen machen: (*1*.) Für die meisten Arten kennen wir nicht genug Ökologie, Physiologie und Genetik, um Reaktionen auf gegenwärtige und zukünftige Umweltveränderungen vorherzusagen. (*2*.) Biologischer Artenschutz konzentriert sich sehr auf das Verstehen von Seltenheit, aber wir wissen viel weniger über die regenerative Kraft, die eine Eigenschaft sowohl seltener als auch häufiger Pflanzen ist. Und schließlich (*3*.) Habitatfragmentierung und der Verlust von Verknüpfung kann mindestens eine ebenso große Bedrohung für solche „Stammarten“ sein und damit auch für den Menschen selbst als der Klimawandel.

1. Introduction

The *Millennium Ecosystem Assessment* (2005) and the *IPCC 4th Assessment Report* (*IPCC* 2007) are unequivocal: changes caused in the planet due to anthropogenic activities have had, and continue to have, a seriously damaging effect on the ecological infrastructure. Rising levels of greenhouse gases in the atmosphere and a consequent warming of the planet take place in parallel to land use changes that result in rates of species extinction around one thousand to ten thousand times larger than one should expect if there were no changes caused by anthropogenic activities. This results from a development model that placed in opposite boxes nature and man. On the one hand, the stereotypical development advocate would argue that nature is an obstacle to progress, while the stereotypical environmentalist would argue, on the other hand, that man should be fenced away from nature. As extreme as this may seem, such antagonism is still present in much of the developing world, where most of the remnants of the planet's natural resources are located (Scarano and Martinelli 2010).

Clearly, this development pattern did not result in human well-being in every corner of the planet. Wealth is poorly distributed and many still live in absolute poverty. The world population has reached 7 billion and by 2050 it is estimated 9 billion people, 2 billion of which will enter the middle class and increase standards of consumption of the planet by several times. As it is, we currently lose the equivalent to the gross domestic product (GDP) of Japan (that ranks second in the planet) in habitat destruction (*TEEB* 2010), which is an obvious indication that prospects for the near future are bleak. Nature is paying back, and it increasingly looks like humans are the main threatened species.

We aimed to analyze this scenario under the light of Lovelock's Gaia hypothesis (Lovelock 1979) that postulates that Earth is a living organism with man as part of its large web of life (see also Matyssek and Lüttge 2013, in this volume). It forwards the notion of dynamic stability of life in time, whether or not mankind persists on the face of the planet. The present chapter starts from the premise that for man to carry on being part of Gaia, biodiversity – that underpins the environmental services vital to man's survival (climate stability, food security, water supply, health, etc.; McNeely et al. 2009) – must "regenerate" in face of changes. We thus develop a new concept – stem species – which is analogous to the concept of stem cells for organisms, only that it applies to the planet as an organism as proposed by the Gaia hypothesis. We then propose that such stem species are the "regenerating cells" of Gaia, we provide examples from Brazilian vegetation and we argue that we should urge to understand and preserve them.

2. Stem Species

It follows from the Gaia hypothesis that life should persist on the face of the planet, whether or not mankind does. Human survival and well-being are intrinsically dependent on services provided by the ecosystems, which are underpinned by biodiversity (McNeely et al. 2009). Climate stability, water provision in quality and quantity, food security, cultural diversity, human health are all services dependent on the existence of biodiversity. Since these are pre-requisites for human survival and well-being, it follows that mankind is directly dependent on biodiversity. As biodiversity declines at unprecedented rates, one should expect that for man to carry on being part of Gaia biodiversity will need to "regenerate" in face of such changes in the planet.

Regeneration within organisms is promoted by stem cells that act as a repair system for the body and can self renew to produce more stem cells. If we think of the planet as Gaia, as an organism, we could wonder if the planet has "regeneration cells". We propose that these should be more likely found among higher plants: since they amount to 99 % of the biomass of the planet (Trewavas 2003), they are collectively responsible for most such services. Thus, focusing on higher plants, we define "stem species" as plant species that act as a repair system for Gaia, guarantee the production of more species and lead the protection of environmental services vital to man. This concept differs from the well-established concepts of ecosystem engineer species (Jones et al. 1997) or pioneer species (Whitmore 1989) or nurse plants (Franco and Nobel 1989) for the fact that it operates also in geological time and not only in ecological time. Nevertheless, a stem species may share characteristics related to these three concepts (Fig. 1). The concept will be better understood as we examine some practical examples next.

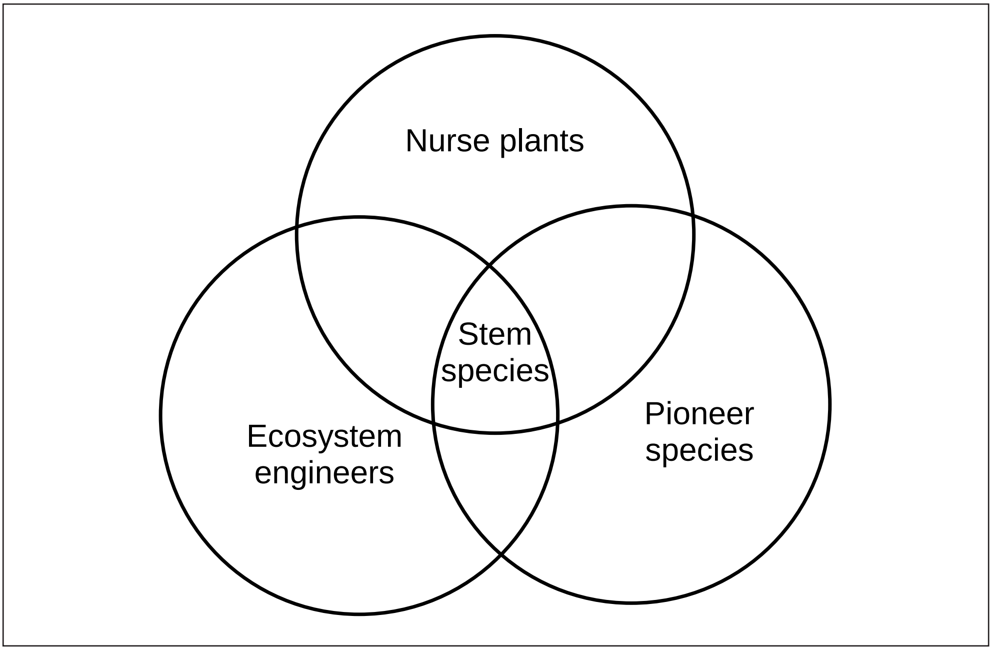

Fig. 1 The stem species concept shares many of the attributes of nurse plants, pioneer species and ecosystem engineers. However, stem species also operate at the geological time as a repair system of Gaia by modifying the abiotic environment and creating conditions for the establishment and development of other species.

3. Proof of Concept

We illustrate the concept with two examples from Brazilian vegetation: (*a*) *Clusia hilariana* Schltdl. (Clusiaceae), typically a hemi-epiphytic strangler in rainforest that in geological time migrated to newly formed sandy plains to be a tree responsible for diversity in land, soil and water and for massive carbon storage; and (*b*) the case of spontaneous forestation of a portion of an Amazonian lake buried by bauxite tailings, with nitrogen-fixing plant species of flooded forests.

3.1 Clusia hilariana in the Atlantic Forest Complex

The genus *Clusia* typically has many hemi-epiphytic stranglers and/or rupicolous species (LÜTTGE 2006) that live in rainforest habitats. In the case of the Brazilian Atlantic rainforest some such species migrated to sandy plains, known as restingas, which were formed at the coast during the Quaternary (SCARANO 2002, 2009). *Clusia hilariana* is one such species. However, the plasticity of the genus and of the species are such that in the restingas of northern Rio de Janeiro (SE-Brazil), this species occurs as a tree and can be as tall as 8 m (DIAS et al. 2006) and displays a number of peculiar features, such as (*1.*) dioecy (FARIA et al. 2006), (*2.*) seedling occurrence predominantly inside the tanks of terrestrial bromeliads (CORREIA et al. 2010), and (*3.*) CAM metabolism (LÜTTGE 2006). Moreover, it has been shown that through facilitation processes this species in the restinga is largely responsible for diversity in land (DIAS and SCARANO 2007), soil (KREUZER et al. 2007) and possibly even in adjacent water bodies (PIMENTEL et al. 2007). In addition to these positive effects on community diversity, *C. hilariana* plays a marked functional role: its aboveground biomass stock and understorey litter is comparable to the entire woody component of many neotropical savannas (DIAS et al. 2006). This species, therefore, fits our concept of stem species. It has enough ecological, physiological and morphological plasticity too, in time, colonize novel habitats and subsequently facilitate the onset of a diverse community. In other words, *Clusia hilariana* is one such regenerating cells of Gaia.

3.2 Nitrogen Fixers in the Flooded Forests of the Amazon

Another interesting example of such regeneration power of the planet comes from the Brazilian Amazon. From 1979 to 1989 bauxite washing tailings were continuously discharged into Lake Batata (State of Pará, Central Amazon, Brazil) and the surrounding *igapó* forest (i.e. forest seasonally flooded by low nutrient waters). When the discharge was halted, circa 30 % of the lake area with its marginal *igapó* forest was buried by a 4–5 m bauxite tailings layer (SCARANO et al. 1998). Frequent and prolonged exposure to full sunlight during the dry season has led to dehydration and consolidation of the bauxite tailings. The bauxite tailings substrate consists of 75 % clay, 21 % silt, 3 % fine sand, and 1 % coarse sand. It differs from non-impacted *igapó* soil in the proportions of clay (49 %), silt (37 %), and fine sand (13 %) (DIAS et al. 2012), and therefore may be considered as a new habitat. Perhaps surprisingly, many native *igapó* species began to spontaneously regenerate and grow on the top of this substrate, particularly in areas where water was more still during flooding. Vectors of seed dispersal in these forests are to a large extent water and fish (MANNHEIMER et al. 2003) and, therefore, sites with water currents and fast flow during flooding are less prone to establishment. Thus, a large scale reforestation program was set in place to provide forest cover to this new environment (Bozelli et al. 2000). After over twenty years since the impact happened and fifteen years since man-induced forestation started, it is now apparent that nitrogen fixing legumes (*Acosmium nitens* (Vogel) Yakovlev and *Dalbergia inundata* Spruce ex Benth.; Souza et al. 1994) are the most abundant species in the site, both due to spontaneous regeneration and to successful performance of planted seedlings (Scarano et al. 1998, Dias et al. 2012). Nitrogen fixation by these species in such a nutrient poor substrate can possibly be a factor contributing to the high diversity found in this new habitat, which would also fit them in our stem species concept.

4. Practical Implications

When technical experts first saw the damage caused by the bauxite tailings, the immediate first reaction was to imagine how to remove that, which was obviously not a viable operation. No one would expect that sandy substrate adapted plants such as the *igapó* plants would be able to acclimate, germinate and grow on the top of a very hard substrate with hardly any nutrients. However, many such species did so and, possibly, with much contribution from nitrogen-fixing species. Similarly, take an imaginary scientist (knowing all science we know nowadays) who observed over years the last marine regression, three thousand years ago, sitting on the top of the mountains in the Atlantic rain forest. He would possibly never have imagined that the new sandy plains, with their dry and nutrient poor substrate, would ever be colonized by rainforest species having a hemiepiphytic strangler as the main tree facilitating this process (see Section 3.1).

These two examples originate from tropical vegetation, in Brazil, but they have more in common than that. These plants demonstrate considerable ecological plasticity, have apparently low habitat requirements, they are facilitators and have long distance dispersal. They are also insufficiently known in regards to their ecology, physiology and genetics. Plants known to science under all those angles are often productive (agriculture or forestry), or model plants (e.g. *Arabidopsis*), or, to a lesser extent, rare or threatened species that are focus of numerous conservation biology studies. Common plants that are neither economically relevant nor rare, we claim, are largely understudied in the tropics. Some of the traits described above for stem species (high plasticity, long term dispersal, low habitat requirements) are often found in common species as well. Thus, we would argue, plants of the future and our stem species are likely to reside among common plants, with no known economic importance, which happen to be the species we know less about.

We believe that a scientific search for stem species should be in order. Interestingly, critics of the Gaia hypothesis have often argued that there are no real-world counterparts to the black and white daisies of the so-called Daisyworld model[1] (see debate in Schneider et al. 2004).

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[1] The Daisyworld model was developed by Lovelock to support Gaia's hypothesis claim that the biosphere can regulate the climate system by modifying the Earth's surface albedo. The essence of the model is that a trait (daisy albedo) affects the individual and the environment in the same way. A black daisy captures more solar energy, warming itself and its surroundings. A white daisy reflects more solar energy cooling itself and its surroundings. Under cool background conditions (i.e., below the optimum temperature for growth) black daisies have a selective advantage. They alter the environment (warm it) in a way that enhances growth but reduces their selective advantage. Under warm background conditions (i.e., above the optimum growth temperature) white daisies have

The stem species concept provides an operational basis over which ecologists may work by searching plant species (and their traits) capable to regenerate and create ecosystems in face of climate change and habitat loss. Thus, stem species concept might help bridge the gap between the Daisyworld model and the search for real-world daisies.

While some of the stem species traits defined here may account for regenerating power under a climate change scenario, increasing habitat fragmentation may be a more difficult hurdle to overcome, even for species with long term dispersal. For instance, the case of the Atlantic forest biome (rainforest plus associated ecosystems), where *Clusia hilariana* occurs, is particularly serious. This biodiversity hotspot (sensu MYERS et al. 2000) has been reduced to somewhere between 12 and 16 % of its original cover only and RIBEIRO et al. (2011) have recently shown that these remnants are scattered in 245,173 patchily distributed forest fragments of varying sizes. Most such fragments (83.4 %) consist of patches smaller than 50 ha, while only 0.03 % are larger than 10,000 ha. Practices to circumvent the fragmentation problem are not new: forest restoration and creation of protected areas. We hope in the near future, deeper knowledge on stem species could help define conservation priorities and species selection for restoration.

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We thank Prof. LÜTTGE and Prof. MATYSSEK for the kind invitation to participate in the Leopoldina Symposium, where we first forwarded and debated the ideas presented in this paper, under the title: "From plants to planet: integrating hierarchies to help solve planetary crisis". The paper benefited from these debates and from revision of Prof. LÜTTGE.

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a selective advantage. They also alter the environment (cooling it) in a way that enhances growth but reduces their selective advantage leading to a negative feedback selection. Nitrogen fixers are real-world examples of such negative feedback selection as the increase of nitrogen for their own supply also increases the amount of available nitrogen in the environment; this nitrogen availability for nonfixers reduces the selective advantage of nitrogen fixers (LENTON 2004).

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Gaia: The Planet Holobiont

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With 1 Figure

Dedicated to Prof. Dr. Otto L. Lange on the occasion of his 85th anniversary.

Abstract

The strict definition of holobiont is that it is a host organism (plant or animal) in interaction with all associated microorganisms as an entity for selection in evolution. This definition can be generalized when not only microorganisms internal of or endogenous to a host organism are concerned but any regular organisms which strongly interact between each other also externally. Such latter associations may altogether function as co-evolutionary entities at the community level, as can be concluded from their evolutionary history. A thorough inspection of habitats and ecosystems shows that such kinds of mutualistic associations prevail. Therefore, we can scale up examples of endogenous symbioses to ecosystems, biomes and the entire biosphere as holobionts, where the planet holobiont is Gaia. James Lovelock defines Gaia as the biosphere being a self-regulating entity that ensures the planet's capacity for harboring life by controlling the chemical and physical environment in conducive ways. Thus, the question is of whether Gaia manages a global equilibrium which sustains life on Earth. Possibly this holds for life as such but not for any specific forms of life as illustrated by several extinction waves of organisms during geological history. Such events were typically followed by the emergence of innovative new forms as arising from adaptive radiation into abandoned ecological niches. Will man be subject of the next extinction wave? Such a possibility raises ethical imperatives for man to sustain the biosphere *sensu* Gaia on which he not only depends, but also is part of. Holobiont research must aim at gaining advanced understanding, both in mechanistic and holistic terms, by extending the holobiont concept across the spatio-temporal scales of ecological organization. This implies to delineate the humble position of man in the biosphere and to explore conditions for man's sustained survival on Earth.

Zusammenfassung

In ihrer strengsten Form besagt die Definition von Holobiont, dass er ein Wirtsorganismus (Pflanze oder Tier) in Wechselwirkung mit allen assoziierten Mikroorganismen als eine Einheit für Selektion und Evolution ist. Es liegt nahe, die Definition so zu verallgemeinern, dass nicht nur interne oder endogene Mikroorganismen in einem Wirt gemeint sind, sondern generell typische Organismen, die auch extern intensiv miteinander interagieren. Assoziationen dieses Typs können zusammengenommen als in Co-Evolution geformte Einheiten auf der Ebene der Organismengesellschaften angesehen werden. Dies folgt auch aus der evolutionären Geschichte. Sorgfältige Beobachtungen in Standorten und Ökosystemen zeigen, dass solche mutualistischen Assoziationen sogar die Regel sind. Wir können deshalb von Beispielen endogener Symbiosen zu den Skalierungsebenen der Ökosysteme, Biome und der ganzen Biosphäre als Holobionten aufsteigen, wo die ganze Erde als Holobiont dann als Gaia identifiziert wird. James Lovelock definiert Gaia als die selbst-regulierende Einheit Biosphäre. Danach soll durch ihr Vermögen, unseren Planeten durch die Regulation der chemischen und physikalischen Umwelt gesund und lebensfähig zu erhalten, die Biosphäre *sensu* Gaia die Kapazität unseres Planeten sichern, Leben zu beherbergen. Die Frage, die also gestellt werden muss, ist, ob Gaia wirklich ein globales Gleichgewicht gewährleistet, das das Leben auf der Erde dauerhaft trägt. Dies trifft vermutlich auf das Leben als solches zu, aber nicht auf ganz konkrete Formen des Lebens. Der Gedanke wird durch die verschiedenen Aussterbewellen von Organismen in geologischer Zeit veranschaulicht. Auf solche Ereignisse folgten typischerweise immer Innovationen und das Auftreten neuer Formen von Organismen durch adaptive Radiation in verlassene ökologische Nischen. Wird der Mensch, die Art *Homo sapiens*, von der nächsten Aussterbewelle mit erfasst sein? Diese Möglichkeit stellt dem Menschen den ethischen Imperativ, die Biosphäre *sensu* Gaia zu erhalten, von der er nicht nur abhängig, sondern deren Bestandteil er auch ist. Die Holobiont-Forschung muss nach einem vertieften Verständnis der Mechanismen und holostischen Zusammenhänge trachten, indem das Holobiont-Konzept über die verschiedenen Skalierungsebenen ökologischer Organisation verfolgt wird. Die schließt ein, des Menschen bescheidene Position in der ganzen großen Biosphäre und als Teil von Gaia zu erkennen und zu lernen, welche Bedingungen unumstößlich sind, um das nachhaltige Überleben des Menschen auf der Erde zu gewährleisten.

1. The Concepts of Gaia and Holobiont

In ancient Greek mythology Gaia is the mystical goddess of the Earth. Broad-breasted Gaia is Mother Earth. The contemporary Gaia concept was advanced by the famous environmentalist James Lovelock in the 1960s to 1970s (Lovevlock 1979). He defines Gaia as “a complex entity involving the Earth’s biosphere, atmosphere, oceans and soil”, where Gaia is “a shorthand for the hypothesis […] that the biosphere is a self-regulating entity with the capacity to keep our planet healthy by controlling the chemical and physical environment”. Keeping the planet healthy means sustaining the planet’s capacity for harboring life. With “biosphere” we refer in this essay comprising all life harbored by lithosphere, hydrosphere and atmosphere at the surface of our planet. Thus, providing conditions supportive of life, the biosphere arrives at being a self-regulating living organism. The biosphere is a dynamic system, stabilized *via* feedback coupling mechanisms so that our entire planet is considered to be one single living being, a supra-organism. By using another term we may say that Gaia, i.e. the biosphere, is the ultimate case of a holobiont. This facilitates our approach because we can first ask what is a holobiont? Then we can look for examples of holobionts which allow extrapolation to Gaia.

The basic and strict definition of holobiont is that it is a host organism (plant or animal) in interaction with all associated microorganisms as an entity for selection in evolution.

One of the best examples for this is man himself. Man is the host of innumerable bacteria on his skin, in his mouth, in his intestine and elsewhere in the body. In our digestive system each of us carries a good kilogram of bacteria with him. This amounts to 10 to 100 bacterial cells per human body cell. It comprises several hundred different species of bacteria. The number of genes of the bacteria in our body is about 100 to 150 fold the number of genes in our own genome. Taken together we can name the integral of the genetic information provided by the host and its associated microorganisms the hologenome of the holobiont. Without these bacteria, not only our digestion would not function. They have many other beneficial effects. For example without these bacteria our immune system could not be established and attain functional maturation. Biologically man can only live and survive as a holobiont with all these bacteria (La Recherche 2010b).

If we underline the term *interaction* in the above definition and if we generalize from microorganisms to organisms in general – of the same or another species – we do not substantially modify the definition in reaching higher spatio-temporal scales of biological organization. However, with respect to man this already leads us to perform a step of enormous consequences. With the extension to man’s cultural means of interaction and globalization, arising from his biological evolution, the entire mankind as an abstraction may be considered as a holobiont.

Before we pursue this to eventually arrive at the entire biosphere and Gaia it is worth mentioning that there is a wealth of other examples when briefly viewing the animal kingdom. Among the mammals we can name the ruminants with their rich internal fauna of ciliates mediating digestion of cellulose, and other herbivores, such as horses or rabbits with bacteria in their intestinal appendices. These are examples of symbioses.

Let us take the term symbiosis very general as it was used originally by de Bary (1879) and as it is frequently done again now, i.e. meaning the association of different organisms closely living together. Thus, there are symbioses either with advantage to the partners (mutualistic relations) or with exploitation of one partner by another (parasitism) or with mutualistic and parasitic interactions of different partners at the same time. This evidently implies that symbiotic systems are holobionts. A holobiont already is a supra-organism with interactions and synergism of its parts (including parasitism and food chains in view of co-evolutions of host-parasite and predator-prey systems, respectively).

Resuming the above introduced generalized holobiont definition or concept, in addition to endogenous symbioses also biotic interactions between separate organisms in the same environment may be envisaged, e.g. systems which have been subject to very intimate co-evolution. The best examples are obligate mutualistic pollinator systems, such as bees and flowers. This generalization of the definition of holobiont is quite logical. Co-evolved systems produce competition and facilitation. Although for competition this may not be intuitively evident, in fact both may increase fitness of the organisms involved, which generalizes rather than widens the holobiont definition. Nevertheless, the reached scope of consideration paves the avenue for a gradual approach to the entire biosphere as the supra-organism Gaia. We shall explore this in Section 2.

When James Lovelock introduced the Gaia hypothesis he faced controversy from two angles, namely (*i*) from the scientific point of view that the history of life on Earth has shown that the biosphere is not that stable that one might be able to talk of stabilization or equilibrium, and (*ii*) the philosophical point of view that referencing Gaia as the mystical goddess of the Earth might imply moving from science to metaphysics. The first one is a matter of interpretation as we shall view it in Section 3. Regarding the second one Lovelock considers the concept purely scientific. When in his book (Lovelock 1979) there is conjecture, such speculations are cleanly scientific, i.e. open to investigation and experimental examination. Lovelock explicitly refrains from any metaphysical implications. However, as we shall see in Section 4, notably on the one hand man is biologically part of the biosphere and hence of the Gaia holobiont, and on the other hand, given his cultural evolution as a spin-off from biological evolution, man is performing as if he were the master of nature. Such a specificity of man is conducive to developing metaphysical projections, particularly in an ethical perspective.

2. Scaling Holobionts from Organisms to Biosphere

We can explore nature for examples of holobionts on different levels of scaling from microscopic organisms up to the biosphere and try in this way to advance from the holobiont concept in its original strict sense (see above) to the Gaia concept. The following examples will be chosen from plant life.

2.1 The Endosymbiosis Theory of the Eukaryotic Cell

The endosymbiosis theory of the evolution of eukaryotic cells was first conceived by A. F. W. Schimper (1883) and then elaborated in detail in 1905 by the Russian scholar K. Mereshkowski. A serial ingression is claimed to have occurred *via* phagocytosis by non-compartmented ancestral cells of prokaryotes capable of respiration and of cyanobacteria-like prokaryotes performing photosynthesis, respectively. As failure of digestion proved beneficial, the phagocytized cells were kept as endosymbionts, evolving to mitochondria and chloroplasts, respectively, during the further course of symbiogenesis. Originally, it was a daring hypothesis up to the 1960s when new cytological techniques, especially the advent of electron microscopy, opened new breakthroughs. The eucyte concept developed by Eberhard Schnepf (Schnepf 1966) with among others studying the extant endosymbiosis of *Geosiphon* (see Section 2.5), and subsequently modern developments of cytology, biochemistry, genetics and molecular biology made the endosymbiotic origin of eukaryotic cells a widely accepted theory (see Chapters 1.6.2 and 1.6.3 of Lüttge et al. 2010). Symbiogenesis and evolution make eukaryotic cells the best examples of holobionts in the strictest sense of the original definition, where a holobiont is a host organism constituting in interaction with all associated microorganisms an entity for selection in evolution. As a consequence actually all eukaryotic organisms from the very beginning of their evolution carry the holobiont syndrome along with them.

2.2 Virus-Infected Unicellular Organisms

Many photosynthesizing prokaryotic cyanobacteria and eukaryotic microalgae are infected by viruses. For example it is estimated that about 20 % of all such organisms in the marine phytoplankton have a virus (Suttle 1994). The scalar level of these associations is from fractions of micrometers (viruses) to several tens of micrometers (algae). The associations of cells and viruses are most interesting within a perspective of holosymbiont evolution. Viruses of the unicellular eukaryotic green algae of *Chlorella* contain a mosaic of prokaryotic and eukaryotic genes. They may be related to the last common ancestor. Algal viruses and their genes have a long evolutionary history (van Etten 2003).

2.3 Symbioses between Unicellular Organisms

Some *Chlorella* species live as hereditary endosymbionts with freshwater or marine organisms (Reisser 1992). They are also called *Zoochlorella*. An interesting example is the symbiosis of two unicellular eukaryotic organisms with the ciliate *Paramecium bursaria* being the host of endosymbiotic *Chlorella* (Karakashian 1975, Smith and Douglas 1987). Inside the cells of *Paramecium Chlorella* is protected from virus infection. The scalar level is 90 to 150 µm.

2.4 Lichens

Lichens are ecto-symbioses. They are holobionts of a fungus (mykobiont) and photosynthetically active symbiotic prokaryotic cyanobacteria (cyano-lichens) or eukaryotic green algae (chloro-lichens) as the phycobionts, living in very close extracellular association. Some lichens have both types of green phycobionts (and photobionts), i.e. green algae as well as cyanobacteria. The morphological appearance of lichens is determined by the mykobiont. It is noteworthy that the lichen holobionts attain a very characteristic gestalt. It is so specific that one can distinguish lichen "species" and perform lichen taxonomy, where the mykobionts are determining the names of the various lichens. The scalar level is in the range of centimeters to decimeters.

2.5 Geosiphon

Geosiphon pyriformis is an endosymbiosis of a fungus and cyanobacteria of the genus *Nostoc*. Again there is formation of specific gestalt. When the hyphae of the fungus get in contact with the appropriate strains of *Nostoc* they ingest the *Nostoc* cells phagocytotically and form characteristic bladders where they retain their *Nostoc* phyto- and photobionts. This is the characteristic gestalt of the *Geosiphon* holobiont which is only formed when the cyanobacteria are present. Beyond this, *Geosiphon* is also part of higher holobiotic integration. In addition to the cyanobacteria there are also bacteria of yet unknown phylogenetic affiliation in the bladders. In the field *Geosiphon* is closely related with bryophytes, such as *Anthoceros* and *Blasia*, which house the same *Nostoc* as exosymbionts and which may exchange *Nostoc* with *Geosiphon* and among each other. Moreover, all three of them are interconnected and may even be connected to grasses by mycorrhiza. The fungus of *Geosiphon* belongs to the phylum Glomeromycota and is closely related to the fungi which form vesicular-arbuscular mycorrhizas. With *Geosiphon pyriformis* itself we are at the scale of several hundred micrometers, but with the higher holobiont integration we arrive at the scale of mini-ecosystems of centimeters to decimeters (KLUGE et al. 2002, Chapter 29.2.1.4 in LÜTTGE et al. 2010).

2.6 Endosymbioses and the Evolution of the Various Lineages of the Algae

An establishment of holobionts related to evolution of species and associated with the formation of very characteristic gestalt is most impressively seen among the algae. The endosymbiosis theory of the evolution of compartmentalized eukaryotic cells states that mitochondria and chloroplasts originated from prokaryotic endosymbionts initially acquired by phagocytosis (see Section 2.1). Thus, eukaryotic cells can be considered as holobionts. Among the algae such holobionts were established frequently and polyphyletically as it is revealed by the structure of chloroplast envelopes. Chloroplasts with a double membrane envelope originated from primary endosymbiosis, where endosymbionts were prokaryotic green cells. Chloroplasts with a triple membrane envelope resulted from secondary endosymbiosis, where endosymbionts were eukaryotic green cells and the external membrane of the phagocytotically acquired symbionts was degraded. Chloroplast with a quadruple membrane envelope are due to tertiary endosymbiosis, where the endosymbionts obtained carried already chloroplasts with a triple membrane envelope. We can see that the staggered formation of endosymbioses or holobionts led to different divisions of the alga with the specific gestalt of their species ranging from microscopic scales to meters and even many tens of meters (see LÜTTGE et al. 2010), e.g.:

- double membrane envelope: rhodophyta, heterokontophyta and chlorophyta;
- triple membrane envelope: dinophyta, euglenophyta;
- quadruple membrane envelope: cryptophyta.

2.7 Cuscuta Linking Different Host Plants

The holoparasites of the Convolvulaceae genus *Cuscuta* (see Chapter of Ralf KALDENHOFF, this volume) form long thread-like pale shoots which wind around their host plants. For parasitism they form haustoria penetrating into the shoots of the host and making contact to both phloem and xylem. Individual *Cuscuta* plants can grow simultaneously on different host plants of different species. In this way they can interconnect host species between each other.

It is observed that even viruses can move along these connections from plant to plant. The consortium as a whole can be regarded as a holobiont. The scalar level is that of whole plants in the order of magnitude of meters.

2.8 Quorum Sensing and the Formation of Bacterial Biofilms

Many surfaces are covered with biofilms, which are mucilaginous excretions of bacteria embedding their colonies as a joint medium that provides protection and enables for metabolic communication. Biofilms occur on surfaces of and within other organisms, in some cases becoming pathogenic, e.g. *Pseudomonas aeruginosa* infecting the lung. In addition, biofilms are found on surfaces of buildings and other objects around human settlements.

Bacteria can sense their local cell density. This is called quorum sensing (WATERS and BASSLER 2005). Originally the term quorum comes from the times of the Roman Empire where it referred to a fixed minimum number of members to make the proceedings of an assembly or society valid. Bacteria use a variety of different molecules, named autoinducers (AI) or pheromones, as signals of cell density in a bacterial population. In Gram-negative bacteria the most common AI is acyl-homoserine lactone (AHL) (review: MATHESIUS and WATT 2010). The AIs act as transcriptional regulators once they reach a threshold concentration. Thus, quorum sensing is used by the bacteria to coordinate their gene expression and their behavior including the formation of aggregates and biofilms as their density increases. Quorum sensing can occur within a single bacterial species as well as between different species. Biofilms are often made up of a variety of interacting microorganisms.

Plants have evolved strategies to modulate bacterial biofilms and to defend themselves. They can interfere with the quorum sensing signals of bacteria by producing compounds which mimic quorum sensing signals. Such compounds either block or stimulate AHL-regulated quorum sensing in bacteria (review: MATHESIUS and WATT 2010).

Quorum sensing and the formation of bacterial biofilms are very illustrative with respect to the definition of holobiont. Individual bacterial cells are parts of a decentralized system which with its self-organizing communication networks can create a new supra-organism, i.e. the biofilm, with completely new properties and behavior. For example *P. aeruginosa* does not become aggressive as long as its cell density is too low to avoid the risk of being destroyed by the host's immune system. However, the population becomes pathogenic if the bacterial density is high enough to form a biofilm. Similarly, pathogenic bacteria on the surface of plants, e.g. in the rhizosphere, may escape the plant's defense before they can overwhelm it. Through signaling within biofilms, bacterial communities perform like multicellular organisms (MATHESIUS and WATT 2010) – i.e. in this way become holobionts.

2.9 Soil Crusts

Similar to biofilms are the globally important but more complex soil crusts (BELNAP and LANGE 2001), which represent layers of soil particles that adhere to each other *via* contacts with (micro-)organisms and/or their excretions. Such layers have a thickness of a few millimeters up to centimeters but can extend over quite large surfaces. They are found especially on apparently bare surfaces of the sand of dunes, in savannas and deserts and other dry sites where larger vegetation cannot get established or forms gaps. They constitute a complex community of organisms with cyanobacteria, eukaryotic algae, fungi, lichens and bryophytes and small animals (non-vertebrates). Also associations of such kinds may be considered as holobionts.

2.10 Rhizosphere and Forests

In the tropics plants of the genus *Ficus* may begin their life as epiphytes on trees. Subsequently they form aerial roots which gain ground-contact. With secondary growth the aerial roots supporting and carrying their crown can look like stems. Often many such pseudostems of one individual may appear like a forest. The most famous example is a tree of *Ficus bengalensis* described by Walter and Breckle (1984), which was only 26 m tall but had an average crown diameter of 170 m, a crown circumference of 530 m and a crown area of 22,000 m^2. However, such a pseudostem forest really is still one individual and therefore not a supra-organism or holobiont.

It may be a different matter with clones. In herbaceous plants they often originate in nature from ramet networks of rhizomes or stolons. The largest clones of vascular plants are found among trees of the genus *Populu*s. In the Qira Oasis of the Taklimakan desert (Hotan Province, Chinese Xinjiang Uygur Autonomous Region, China) a clone of *Populus euphratica* Oliv. of a size of at least 4 ha has been identified by molecular fingerprinting using amplified fragment length polymorphism (AFLP) (Bruelheide et al. 2004). The largest clones known in the world are of *Populus tremuloides* Michx., i.e. in the Wasatch Mountains in Utah, North America, with 42.9 ha (Grant 1993) or even larger elsewhere (81 ha: Kemperman and Barnes 1976). When we consider genetically identical members of such clones as individuals these clones are evidently supra-organisms or holobionts.

Still more closely covered by the holobiont-concept appear to be root symphyses between different tree individuals of same species. Such structures are direct anastomoses which result from intergrowth between roots and may form stand-level networks. They may compare in function with clonal rhizome systems, however, with the exception of being hetero-vegetative structures. Such networks evidently also represent holobionts, although their ecological meaning is poorly understood.

Viewing entire forests as holobionts certainly climaxes when we realize the intensive tree-microbe interactions and mycorrhiza in the rhizosphere. The roots of plants in the soil are surrounded by a myriad of microorganisms, some of which have evolved to form mutualistic or pathogenic relations. Others are helper bacteria for the establishment of the fungi/tree symbiosis of mycorrhiza. This is a network with complex rhizosphere signaling systems (review: Mathesius and Watt 2010). There is quorum sensing and microbes build up biofilms (see Section 2.8). The complexities of quorum sensing in structured microbial communities growing in the rhizosphere are quite sophisticated so that the concept of "efficiency sensing" has been developed (Hense et al. 2007). It combines (*i*) the functions of quorum sensing by regulating gene expression and coordinating group behavior in response to population density (see Section 2.8) with (*ii*) sensing distance by incorporating the sensing of diffusion by regulating gene expression in response to mass flow in the surrounding medium. Efficiency sensing takes into account that the AI concentration sensed by bacteria is a combination of AI synthesis, chemical and biological turnover and diffusion characteristics in the environment (review: Mathesius and Watt 2010).

The hyphae of the mycorrhiza fungi create extended networks with the fungi, bacterial biofilms and tree roots connected among each other in the soil. In the ectotrophic mycorrhiza with trees fungal hyphae grow around the fine roots creating a coat of hyphae which also penetrate into the apoplast of the cortex of the roots forming the so called Hartig net which serves the exchange of metabolites and mineral nutrients. Hyphae of individual fungi can interconnect different trees. In this way entire forests can become holobionts, where the scalar level may be up to square kilometers (Chapter 29.2.2 in Lüttge et al. 2010). This led to the association of "www = wood-wide web" to stress the capacity of hyphal networks to reach largely extending spatial scales.

2.11 Ecosystems

Ecosystems in general may be considered as holobionts. The living organisms in ecosystems develop a plethora of interactions, such as competition and facilitation including competition for space (Grams and Lüttge 2010), predation, symbiosis with both mutualism and parasitism, and many others. Life itself is always affecting its environment. Organisms occupy niches and have niche specialization. Their activity may create new niches. Thus, ecosystems are highly dynamic systems the conditions of whose existence can be stabilized by the complex interactions of all organisms present. Ecosystems are self-regulating to the extent that external perturbations do not become too severe. The scale of ecosystems in special cases may be quite small, e.g. in the cases of biofilms or soil crusts (see above), or may reach many square kilometers. At this stage, we are approaching the scale and concept of Gaia.

2.12 Biomes

After Walter and Breckle (1991, 1999) we globally distinguish nine zonobiomes from the equator to the Arctic and Antarctic. In addition, azonal orobiomes and pedobiomes exist as well as extrazonal local biomes. Biomes are composed of ecosystems and on such higher integrated level we may also consider them as holobionts. The zonobiomes attain global dimensions scaling at thousands of square kilometers.

2.13 Biosphere

Moving up the various scalar levels with integrations of increasingly complex subsystems, we systematically approach the biosphere globally integrating all life on the planet. Its immediate subsystems are the biomes. Interdependence of the biomes is vividly recognized and realized by the current global climate debate. The biosphere as the boundary on Earth between the atmosphere and the lithosphere organically is Gaia. Some forms of life are found almost everywhere on the Earth's outer surface including land and oceans so that its $510 \cdot 10^6$ square kilometers are the scalar level attained by the holobiont Gaia.

3. Gaia: a Global Equilibrium

We have seen that the central argument of the Gaia hypothesis is that the biosphere is a dynamic system with self-organization through feedback stabilization. The critique concerns the question if therefore equilibrium and steady state are to be presumed. In a strict thermodynamic sense only closed systems can approach equilibrium with the establishment of steady state. Conversely, all living organisms are open systems through which there is a continuous flow of matter and energy. This includes ecosystems and holobionts. They can at best approach some kind of dynamic equilibrium which is also termed pseudo steady state. A simple cybernetic technical example of an open system is a water reservoir with an inflow and outflow and a control device that should maintain the water table of the reservoir at a constant level. The control device is operated *via* a sensor which can check the actual level of the water table against the set desired constant level. Regulation by the control device occurs *via* taking into account the feed-forward of inflow and the feed-back of outflow. All feed-back and/or feed-forward systems intrinsically bear non-linear dynamics. Our reservoir, therefore, is also a system with non-linear dynamics. During its regulation oscillations of the water table to be regulated are occurring. When regulation fails the system may get chaotic. As long as this is not the case, the permitted amplitudinal size of oscillations around the pseudo steady state to still talk of acceptable regulation is only an arbitrary issue. However, it is implicit in these considerations that all living systems as open systems are governed by non-linear dynamics.

Evidently the biosphere as a whole also is such an open system. The consideration of oscillations in cybernetic regulation systems is highly pertinent to the Gaia concept. LOVELOCK actually takes the occurrence of such a mechanism as a proof for the possible existence of Gaia: "The discovery of such a system, operating on a global scale and having as its goal the establishment and maintenance of optimum physical and chemical conditions for life, would surely provide us with convincing evidence of Gaia's existence."[1] The goal here can be taken as meaning the set point of the cybernetic system. The critical question to be asked is whether the observed fluctuations of the conditions for and of the forms of expression of life itself in the biosphere are so large that we cannot accept the hypothesis that life stabilizes itself on our planet? Should we question stabilization of special forms of life, and if so which kind of forms? Or should we rather talk of stabilization of life as such in whatever form it may be expressed?

Indeed there where very large fluctuations of the conditions for life during the more than $3.5 \cdot 10^9$ years of the history of life on our planet (Fig. 1). Most important for the conditions of life are the atmospheric gases oxygen and carbon dioxide (BERNER 1994). In LOVELOCK'S (1979) concept of Gaia's self-regulation of environmental conditions favorable for life, the atmosphere plays a predominant role. He views the atmosphere as "an extension of the biosphere", i.e. *sensu stricto*, the part supportive of life. The accumulation of oxygen in the atmosphere is thought to be mainly due to the activity of life itself, i.e. as a consequence of photosynthesis. Presumably after the onset of oxygenic photosynthesis on Earth the O_2 evolved was first bound by oxidation of iron in the Earth crust and gypsum formation (SCHLESINGER 1997) before accumulating in the atmosphere. About $2 \cdot 10^9$ years ago atmospheric O_2 concentration was about 0.2 %. Then it began to rise with considerable fluctuations over the times (Fig. 1). Atmospheric CO_2 is consumed by photosynthesis and – across geological time scales – by sedimentation, from which CO_2 is currently released intensively back into the atmosphere by industrial activity of man. Also fluctuations in CO_2 were quite high during Earth history. Atmospheric CO_2 concentrations in the Earth's atmosphere were more than 20-times higher in the Ordivician and still 10-times higher at the beginning of the Carboniferous period than today (Fig. 1). Still, the climate stayed supportive of life, as the solar constant was lower at that time than today by about 5 %. Hence, one needs to consider atmospheric and solar fluctuations in concert to understand impacts on the biosphere. Perhaps, changes in the

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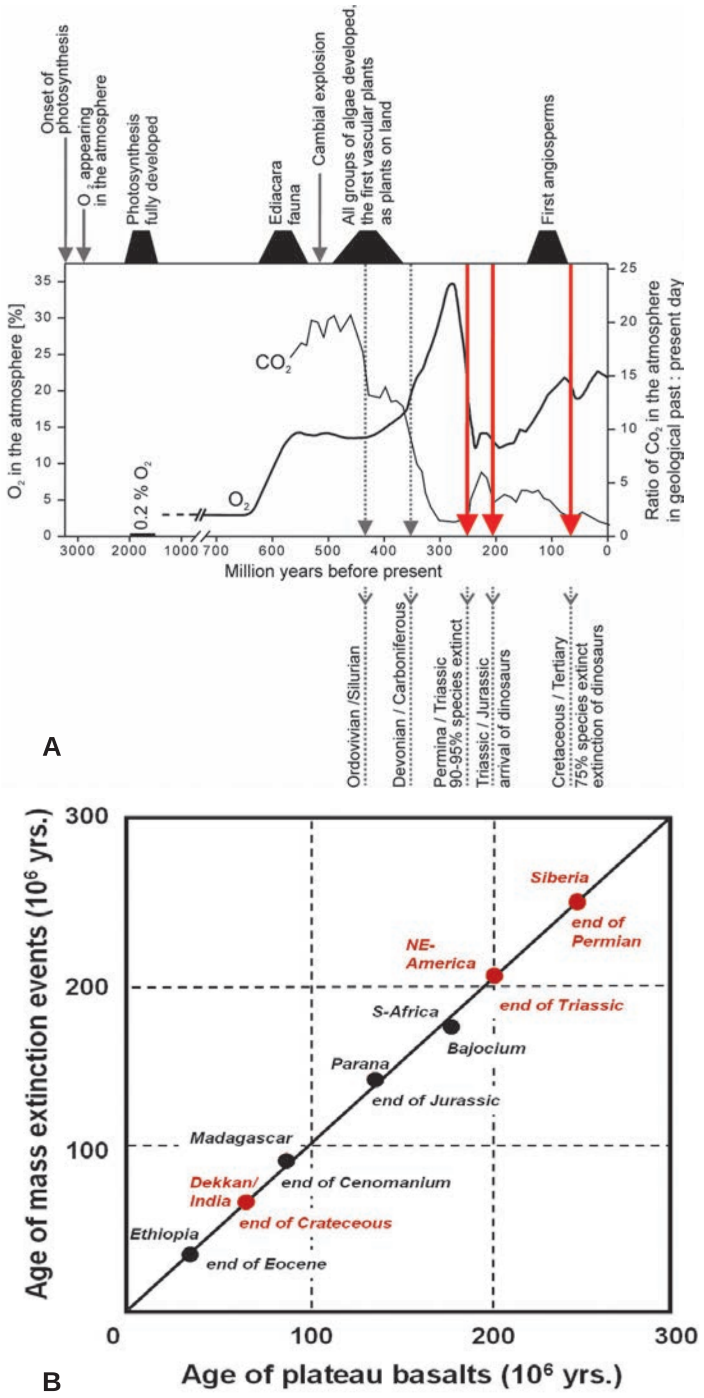
[1] LOVELOCK 1979, pp. 49–50.


Onset of photosynthesis
O 2 appearing in the atmosphere
Photosynthesis fully developed
Ediacara fauna
Cambial explosion
All groups of algae developed, the first vascular plants as plants on land
First angiosperms
O2 in the atmosphere [%]
Ratio of Co2 in the atmosphere in geological past : present day
CO2
O2
0.2 % O2
0
5
10
15
20
25
30
35
3000
2000
1000
700
600
500
400
300
200
100
0
Million years before present
Ordovivian /Silurian
Devonian / Carboniferous
Permina / Triassic 90-95% species extinct
Triassic / Jurassic arrival of dinosaurs
Cretaceous / Tertiary 75% species extinct extinction of dinosaurs
A
Age of mass extinction events (10^6 yrs.)
Age of plateau basalts (10^6 yrs.)
300
200
100
0
Siberia
end of Permian
NE-America
end of Triassic
S-Africa
Bajocium
Parana
end of Jurassic
Madagascar
end of Cenomanium
Dekkan/ India
end of Crateceous
Ethiopia
end of Eocene
B

atmospheric CO_2 level in response to the solar energy supply provide one strong argument in favor of the Gaia hypothesis. Conversely, major fluctuations in CO_2 are produced by geo-dynamical processes. On the one hand, volcanism liberates CO_2 from the Earth interior, while on the other hand, CO_2 is removed from the atmosphere upon crust formation (carbonates) and transport into the Earth interior *via* the process of subduction (as Earth tectonics force some continental plates to be submerged underneath others). Such geodynamic carbon cycling has time scales of around 10^5 years. It is unlikely, however, that changes in the solar constant and in geodynamics are intrinsically linked to each other.

Nevertheless, atmospheric temperature during early Earth history at times was that low so that the entire planet must have been covered by a thick layer of ice (snow ball Earth hypothesis: Kirschvink 1992, Etienne 2008). Such situations prevailed during the Proterozoic 750 to $575 \cdot 10^6$ years ago. Also, solar constant was lower at that time than today, promoting glaciation (Schröder et al. 2008). Probably, glaciation was reinforced by subduction in the absence of high volcanic activity, draining the atmospheric CO_2 concentration to a rather low level, and hence, cooling the global climate. Given that complete glaciation for millions of years would have disrupted all photosynthesis-driven life in the oceans due to darkness underneath the mighty ice shell, a snow slush rather than snow ball stage is to be assumed, with incomplete or missing glaciation in the tropics (cf. Nield 2007). Apparently, not even massive and prolonged planetary glaciation did eradicate life on Earth until eventually warming was re-introduced through huge global volcanic activity with an enormous release of CO_2 into the atmosphere. While the oldest known fossils of multi-cellular organisms discovered in Africa may date back $2.1 \cdot 10^9$ years (El Albani et al. 2010, Donoghue and Antcliffe 2010), this then led to an outburst of multi-cellular life, the Australian Ediacara fauna $630 – 542 \cdot 10^6$ years ago, followed by the Cambrian explosion of metazoic life $535–525 \cdot 10^6$ years ago (Fig. 1).

Due to the pronounced global fluctuations of climate there were also pronounced changes in the expression of forms of life (Fig. 1). We know of five large waves of massive species extinction during the last 450 million years occurring at intervals of 45 to 140 million years (100 million years on average), i.e.

- 444 million years ago at the change from the Ordovician to the Silurian,
- 364 million years ago at the change from the Devonian to the Carbonic,
- 251 million years ago at the change from the Permian to the Triassic, when 90–95 % of all existing species where extinct including the well-known trilobites,
- 206 million years ago at the change from the Triassic to the Jurassic, when extinction was associated with the appearance of the dinosaurs,
- 65 million years ago at the change from the Cretaceous to the Tertiary when 75 % of all existing species where extinct and with them the ammonites and also the dinosaurs, which led to the tremendous proliferation of the mammals on Earth.

Some of these waves of extinction appear to be correlated with changes of the atmosphere, i.e. the first one with increasing O_2 and decreasing CO_2, the second one with an acceleration of

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Fig. 1 (*A*) Changes of atmospheric levels of O_2 and CO_2 during the history of life on Earth, major forms and activities of life and important waves of extinction of species. (*B*) Correlation between events of mass extinction and outbursts of trapp volcanoes (along with their geographic locations), driven through “mantle plumes” in Earth magma (Courtillot 1995). Red color exemplifies major extinction events at the end of Permian, Triassic and Crateceous each.

these changes especially that of increasing O_2, the third one with decreasing O_2 and increasing CO_2, the fourth one with a dip of both gases (Fig. 1). Particularly the high O_2 concentrations are intriguing causes of the massive species extinctions of up to 95 %, in particular, hitting all those life forms during the early extinction waves adapted to an anaerobic world. Lovelock (1979) considers O_2 concentrations above 25 % as disastrous for life (leading to spontaneous self-ignition with open flame even of moist organic matter; Schlesinger 1997).

The atmosphere fluctuations most likely were predominantly due to volcanism. Thus, specific periodic activities in volcanism are regarded to be one probable cause of the extinction waves, i.e. the outburst of so-called trapp volcanoes. These originate from a number of "hot-spots" in the mantle layer of the Earth's interior (not related to the edges of tectonic plates as in the case of other volcanoes), inducing "mantle plumes" (in German "Manteldiapire"), which carry upward streams of huge amounts of magma (Courtillot 1995). Upon breaking through the Earth's surface in cracks of hundreds of kilometers in length, in the case of "super-plumes" areas of hundreds to thousands of kilometers in diameter can be covered through a continuous lava outflow over some hundred thousands of years. During such periods, the planetary environment is changed dramatically. Decisive events are (*i*) reduction of irradiance and initial cooling of the global climate through particulate accumulation in the atmosphere, (*ii*) as a consequence of exhausts destruction of stratospheric ozone with increase of UV radiation at the Earth's surface, (*iii*) alkalization of the upper oceanic water layer upon volcanic particulate deposition, (*iv*) long-term warming through atmospheric CO_2 accumulation, and (*v*) acidification of precipitation upon intense SO_2 release by the continuous and massive volcanic activity. Evidently, such changes represent tremendous impacts on terrestrial and aquatic ecosystems with fundamental effects on primary producers and food chains so that massive species extinction is the consequence. Ten of such "super-plume" eruptions are known since life has conquered land, occurring on average by 30 to 40-million-year intervals. Seven of these events synchronize conspicuously with mass extinctions (as compared to those three other events that occurred on the ocean floor), including the most prominent ones at the transitions from Permian to Triassic and Cretaceous to Tertiary. The initiation of such "super-plumes" appear to relate to marked re-polarizations in Earth magnetism, mediated through periodic processes in 2000 km depth at the boundary between the Earth's mantle and the magnetic core. It is believed, therefore, that the last mass extinction of Cretaceous/Tertiary (ending the era of dinosaurs) at 65 million years ago was not solely caused by an asteroid impact, rather this impact was coincidental and only accelerated the species break-down after preceding weakening by volcanism (Courtillot 1995). It also appears that in geological terms the next "super-plume" eruption is overdue.

Regarding the questions asked above with respect to special forms of the expression of life on Earth, the answer clearly is: No, Gaia does not stabilize the existence of particular forms of life. On the other hand life as such was maintained. Innovations always accompanied extinctions. The most notable innovation was the evolution of respiration in response to the formation of O_2 by the early green prokaryotes performing oxygenic photosynthesis. This O_2 production as the waste product of photosynthesis was the first dramatic global pollution because the O_2 was highly poisonous for the originally evolved anaerobic organisms. Evolution of respiration first served detoxification of the O_2 and then proved to be the most profitable choice for maintaining energy metabolism in the vast majority of extant organisms. Much more recent innovations were the appearance of dinosaurs 206 million years ago and with the extinction of the dinosaurs the proliferation of mammals 65 million years ago.

An interesting example of biochemical innovations are the metabolic CO_2-concentrating mechanisms for photosynthesis which began to evolve in the period of very low atmospheric CO_2 about 300 million years ago, towards the end of the Carboniferous period. (see Chapter 28.9 in LÜTTGE et al. 2010). Isoenzymes of the CO_2-fixing phospho*enol*pyruvate carboxylase (PEPC) emerged. This enzyme leads to the formation of the C-4 dicarboxylic malic acid, where the carbon atom 4 comes from fixed CO_2, hence the term C_4 fixation. This supported the innovation the CO_2-concentrating photosynthetic modes of crassulacean acid metabolism (CAM) and later C_4 photosynthesis. Between 300 and 150 million years ago the C_4-PEPC isoenzyme of monocotyledons was separated, between 200 and 100 million years ago the divergence of monocotyledons and dicotyledons occurred and the C_4-PEPC isoenzymes of dicotolydons and the CAM-PEPC were separated (Fig. 1; TOH et al. 1994, LAWS et al. 2002). CAM is rather old dating back to the Carboniferous. Extant relicts are succulent tropical ferns performing CAM. About 250 million years ago the genus *Isoëtes* with extant CAM-species branched off the other pteridophytes (LÜTTGE 2005). C_4 photosynthesis in single cells dates back 100 to 70 million years. C_4 grasses with a complex anatomical basis of C_4 photosynthesis evolved 25 to 12 million years ago and spread out about 8 to 6 million years ago while atmospheric CO_2 concentration declined continuously (Fig. 1). In the case of C_4 grasses, the high enzymatic CO_2 affinity was an ideal pre-adaptation to drought conditions, allowing for carbon gains similar to those of C_3 plants, but at reduced stomatal opening, and hence, transpiration.

From the point of view of biological evolution there is also no reason to assume a stable (pseudo-) steady state of the forms of life. Evolution of life is a vector in time. Species are not stable but subject to continuous change during Darwinian natural selection. This is independent of whether we accept DARWIN's gradualism, i.e. gradual evolution of new species by transformation of parent populations, or the punctualism of ELDREDGE and GOULD (GOULD 2002), i.e. rapid appearance of new species after longer periods of stasis. Terminating stasis new species arise from splitting of lineages and a small sub-population of the original parent population is the origin of the new species. Termination of stasis among other reasons may be induced by preceding mass extinctions (see above). In such latter terms, one may speak about the "survival of the luckiest", possessing the right features at the right time, rather than the "survival of the fittest", the specialized adaptations of which may become worthless through – in geological terms – "sudden" environmental discontinuities. It is exaptation *sensu* GOULD (2002) which then comes into play. Non-adaptive, i.e. effectively or nearly neutral features not directly built as adaptations for a current function, which persist the pressures of natural selection, can suddenly reveal evolutionary importance of a dormant cooption for utility in occupying new niches or *spandrels* in GOULD's metaphor.

The waves of extinctions and innovations and the time vector of evolution, both show that Gaia proved to be capable of stabilizing conditions such that they remained basically suitable for the existence of life on Earth. In this sense, the core of the Gaia hypothesis of LOVELOCK evidently is acceptable. So far! In a recent book LOVELOCK (2009) himself develops a more pessimistic view. Therefore, the next question is if life is imperative on Earth. This question leads to a consideration of the so called anthropic principle and touches the metaphysical domain as we shall see in the next section.

4. Imperative Life on Earth

The concept of the anthropic principle arose out of physics because precisely the very given values of physical constants of nature in our universe permitted the evolution and the persistence of life. The basic principal physical Planck constants of time, length, mass, energy and temperature which 10^{-43} s after the Big Bang began to determine causality describing the parameters for the velocity of light in the vacuum, the energy of photons and gravitation should not be even tiny bits different from what they are. The way they are allows the existence of life on our planet as we know it and possibly elsewhere in the universe. The weak anthropic principle says that our universe is built in a way making life possible. The strong anthropic principle says that its construction makes life unavoidable including the existence of thinking self-conscious beings such as man. Gierer (1998) calls it a meta-law of nature. The manifestation of the principle towards the initiation of life might be conceived then through the intrinsic capacities of carbon for building complex long-chained or cyclic molecular skeletons, readily reacting with O, N, P, and S as other elements of paramount importance for life, and most importantly, of interacting with liquid water in manifold ways. Of course, such characteristics are also subject to prevalent physico-chemical conditions allowing carbon to unfold its potential. The position taken by Eigen (1992) differs, in that life *per se* may not be an inherent property of matter (although being associated with it), but that specific conditions are required for life to express itself. Eigen does not claim a general physical theory to explain the origin of life – or, in other terms, if such a theory existed, then it should be able to mechanistically explain life to form reproducibly in its complexity (a pre-requisite currently not met). Being aware of the gradual transition between philosophy and natural science reflected by the above excursus, nevertheless, attempts to detect any traces of life in the universe might be associated with the search for carbon and liquid water in a physico-chemical environment similar to that on Earth (probably including appreciable amounts of molecular oxygen of photosynthetic origin in the atmosphere in view of the energy demand of life; Schlesinger 1997). In such a case, even encountering a self-regulated biosphere *sensu* Gaia would not appear to be *a priori* impossible.

One of the great virtues and the most sympathetic aspects of the Gaia concept is that it makes man part of the holobiont biosphere: "[…] if Gaia does exist, then we may find ourselves and all other living things to be parts and partners of a vast being who in her entirety has the power to maintain our planet as a fit and comfortable habitat for life"; " … from its origin the human species has been as much a part of Gaia as have all other species and … like them it has acted unconsciously in the process of planetary homeostasis."[2] Man arose from evolution within the biosphere, including his capacity of a cultural evolution. Apparently, it must have been an evolutionary advantage that his survival was not based exclusively on the development of specific anatomical structures or metabolic functions, but of intellectual capacities. Nevertheless, man depends on the biosphere. Most of its time, the biosphere was without man. Life on Earth began about $4 \cdot 10^9$ years ago. The oldest fossils of green organisms available are the $3.5 \cdot 10^9$ years old Australian stromatolites of cyanobacteria-like cells or biofilms. Man did not appear earlier than $2 \cdot 10^6$ years ago. There were several species of man some of which lived simultaneously, but now only one species, *Homo sapiens*, is remaining (*La Recherche* 2008, 2010a):

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[2] Quotations from Lovelock 1979, pp. 1 and 128.

- *Homo rudolfensis* 1.9 million years ago,
- *Homo habilis* 1.9 to 1.44 million years ago,
- *Homo erectus* 1.9 to 0.5 million years ago,
- *Homo heidelbergensis* 0.8 to 0.3 million years ago,
- *Homo neanderthalensis* 0.2 to 0.03 million years ago,
- *Homo sapiens* since 0.2 million years until present.

Perhaps, it is not surprising that eventually just one species of man has survived – given his evolutionary history as a tribal carnivore driven by territorial instincts in combination with his intellectual capacities (HESCHL 2009). It appears that the encounter of two similarly evolved species inevitably would have led (or would lead) to the aggressive elimination of one of them – not permitting the co-existence of an almost identical and similarly capable competitor. Such a consideration is speculative if previous companion species within the genus *Homo* became extinct due to other reasons (as the pre-history cannot be unveiled to the full extent). However, the evolutionary heredity and related tribal behavior of man may bear intrinsically his eventual failure and disappearance as a species, because of three reasons: (*i*) being highly evolved, i.e. specialized, but decoupled from natural selection so that further biological adaptation cannot occur, representing an evolutionary "dead end"; (*ii*) being caught in his inherited tribal behavior, which limits further cultural evolution; so that (*iii*) exploitation of the biosphere will continue to far beyond the ecological capacities, under which man's biological evolution had originally taken place.

Metaphysically JONAS (2003) has expressed the categorical imperative that man must exist on Earth – if evolution lasts long enough, given all the coincidences necessary, to eventually reach the required stage of highly complex biological organization. GIERER (1998) also thinks that the self-conscious man is an essential part of the nature of our universe. MORRIS (2003) much uses the phenomenon of convergence of the selective formation of structures and functions of organisms in phylogenetically separated lineages, e.g. the evolution of large brains in whales and dolphins and in man, to arrive at the conclusion that the evolution of man-like self-conscious thinking beings can occur repeatedly in a convergent way. It must not be overlooked, however, that the success of man does not only relate to his brain capacity, but also his ability to develop strategies of joint action with his conspecifics in highly abstracted ways (LOVELOCK 2009). MORRIS (2003) is convinced that if man would disappear from our planet new evolution would generate again thinking self-conscious beings. Moreover, he postulates that self-conscious beings like man must also have originated elsewhere in the universe. Notwithstanding such scientific reflections it appears that the quality of the argument that man must exist is metaphysical (JONAS 2003). From a purely scientific point of view we can easily imagine that life on our planet after a possible extinction of man might continue without man just as life on Earth was before man appeared. DIXON (1981) makes projections on a world after man into a future at about 50 million years from now, assuming mans' extinction upon degeneration due to the suspended natural selection. In parallel, DIXON accounts for principles in evolution which are derivable from the past in combination with the predictable continental drift and its global consequences for climate.[3] His approach, therefore, is not science fiction, but solidly anchored in our current knowledge on evolutionary processes. Winners in evolution after the loss of ecologically dominant species would be – as was the case

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[3] DIXON 1981, p. 124.

in the past – either species with exadaptive features *sensu* GOULD (2002) or species with low specialization in their adaptations, being modest and generalists in their ecological demands. Such species possess best pre-requisites for subsequent adaptive radiation into abandoned ecological niches. In the past, this was the case, as we know, e.g. for the early mammals after the extinction of the dinosaurs. Again exemplifying mammals, in the future winners might be e.g. rats or rabbits, which currently are lowly specialized generalists, so that they appear to be ideal candidates for a potentially upcoming adaptive radiation. They might be able to newly conquer manifold ecological niches by diversely differentiating into the broad range of herbivores, carnivores and omnivores across all climate zones. Such evolutionary trends in the future do not invalidate the Gaia-hypothesis as it promises stabilization of the conditions of life as such and not of specific life forms as we have seen above.

If a particularly catastrophic extinction wave were to comprise all forms of life with higher complexity including man evolution of new innovations may start again from the bacterial prokaryotes. In fact evolution of prokaryotes at the origin of life on Earth was subject to a severity of selective forces beyond comparison with what we have today. For example UV-radiation was extreme. Radioactivity was also high. As we can calculate from the radioactive half life of uranium-235 and a current stock of 0.72 % ^{235}U in the Earth's crust, the level of the radioactive element must have been 15 % ^{235}U $4 \cdot 10^9$ years ago (LOVELOCK 1979). By contrast to the suggestion by Paul CRUTZEN to call our era the "Anthropocene", Stephen Jay GOULD demonstrates that notwithstanding the evolution of high complexity, even to date the prokaryotic bacterial organization still is the most successful mode of life on Earth, considering "persistence in place and constant growth in height"[4]: "We do not live in […] 'the age of man' (1 species), or 'the age of mammals' (4000 species among more than a million for the animal kingdom alone), or even in 'the age of arthropods' (a proper designation if we restrict our focus to the Metazoa, but surely not appropriate if we include all life on Earth). We live, if we must designate an exemplar at all, in a persisting 'age of bacteria' – the organisms that were in the beginning, are now, and probably ever shall be […] the dominant creatures on Earth by any standard evolutionary criterion of biochemical diversity, range of habitats, resistance to extinction, and perhaps [...] even in biomass." Again bacterial biofilms may become the first holobionts leading to evolution of more highly complex supra-organisms.

Nevertheless, it must be clear that the existence of life on Earth is not infinite in time. When will life cease on this planet? With the Gaia concept discussed in this essay we restrict ourselves to life on Earth. So, the question of when life is ceasing on our planet Earth is identical with the question of 'when is the holobiont Gaia dying'? The age of the universe is $13.7 \cdot 10^9$ years. In $5 \cdot 10^9$ years the Sun will become a so-called red giant, heat the surface of the Earth to over 1000 K and absorb the Earth's orbit before eventually ceasing to give energy. However, the conditions favorable for life may be lost earlier than that – probably already in 10^9 years from now. At that time, during the long process of becoming the red giant and eventually swallowing Earth, the Sun will begin to significantly increase its energy load to Earth, so that the planet will gradually dry out, losing all its liquid water (SCHRÖDER and SMITH 2004). Another scenario are subduction processes of geodynamics removing CO_2 from the atmosphere towards the Earth interior within $0.5 \cdot 10^9$ years which may lead to insufficient CO_2 levels for photosynthesis as the basis of life (BOUNAMA et al. 2009). Thus, as life on Earth appeared $4 \cdot 10^9$ years ago it may already have consumed, in the worst case, about 90 % of its total avail-

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[4] GOULD 2002, Fig. 9–29, pp. 897 – 898.

able time of evolution. The much more recent *Homo sapiens* only used 0.05 % of his possible time. However, subduction as the "killer" of Gaia appears to be less likely than the warming up of the Sun towards becoming the red giant. Earth appears to have stayed "juvenile" as compared to the companion planets Mars and Venus, given by the still intense tectonics on Earth as manifested by the ongoing continental drift, of which subduction is one facet. However, subduction is hardly imaginable without volcanism, releasing CO_2 back into the atmosphere. In fact, subduction and volcanism are two sides of the same medal (NIELD 2007). In this context, the active "conventional" volcanoes along the "seams" of continental plates are crucial, and there is no indication that tectonics may cease in the geologically near future.

Man is not only biologically part of and dependent on the Gaia/biosphere but from his cultural position he also influences it. Man assumes he "is the possessor of the planet, if not the owner" rather than "the tenant". Conversely, "the Gaia hypothesis implies that the stable state of our planet includes man as a part of, or partner in, a very democratic entity".[5] Does man really perform like a partner? Human exploitation of nature is enormous and progressive, for reasons of man's evolutionary history (see above). There is pollution affecting the climate globally. The danger of wars with dreadful globally destructive nuclear weapons is not eliminated. Extinction through nuclear disaster, by purpose or accidentally, might even be an inherent part of man's evolution, which enabled him to discover nuclear fission (NIELD 2007). Perhaps, Gaia is naturally doomed even to terminate itself as a stringent consequence of having been able to evolve man. Such a risk is high, as Gaia over time, i.e. during its own process of ageing, has become highly differentiated, which increases vulnerability as compared to early developmental stages (LOVELOCK 2009). However, as pointed out above, bacterial life is tough, perhaps even in the worst case withstanding nuclear contamination.

One major immediate risk for man, however, arises from the continued unbroken increase of the global human population. Man behaves like an "r-strategist" in population dynamics due to his cultural evolution reflecting exponential growth progressively in exploiting the biosphere, although he did biologically evolve as a "K-strategist", adapted to make sustainable use of environmental resources by not exceeding a maximum population density. Catastrophic failure of population growth, however, is the ultimate fate of any "r-strategist" upon exhaustion of the available resources (some insect species are suitable examples). Until 1990 the arable land increased proportionally with the human population. Since then it stagnates at about $230 \cdot 10^6$ hectares while population continues to increase to a predicted $9 \cdot 10^9$ people by the year 2050. Arable land and resources, such as water and nutrients particularly phosphorus. For feeding them will become limiting. Will there be a sixth wave of extinction, a man made one in this case?

Evidently Gaia teaches us that we must further develop ethics. As biological part of the biosphere man shares many genes and evolution with the other organisms. Does that not create a strong ethical demand of protecting them? Due to man's activities extinction of species is rapidly progressing, each of which is an invaluable and irreplaceable treasure and living natural heritage. Gaia comprises world-wide mankind. We need global world ethics (KÜNG and KUSCHEL 1989) for maintaining the dynamic equilibrium of Gaia. This is essential for sustaining and not destroying the very basis of our own existence. Science and metaphysics merge with the holobiont biosphere being the nurturing Earth-mother identified as Gaia. In the quest of harmony, what appears to be more appropriate than ending this essay with the

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[5] Quotations from LOVELOCK 1979, p. 145.

words of James Lovelock, founder of the modern Gaia-concept: "It may be that the destiny of mankind is to become tamed, so that the fierce, destructive, and greedy forces of tribalism and nationalism are fused into a compulsive urge to belong to the commonwealth of all creatures which constitutes Gaia. It might seem to be a surrender, but I suspect that the rewards, in the form of an increased sense of well-being and fulfillment, in knowing ourselves to be a dynamic part of a far greater entity, would be worth the loss of tribal freedom."[6] In his most recent book, however, Lovelock (2009) begins to become doubtful, if man will succeed, given his strong ties in behavior as a species, as imposed by his evolutionary heredity.

5. Outlook

Evidently the generalized holobiont concept with supra-organisms at increasing hierarchical levels with the climax of biosphere or Gaia as developed in this essay is intriguing. It bears enormous challenges for research both theoretically on conceptions and empirically on observations. Concepts of such kind bear the fascinations of the web of life on Earth, even at the scope of Gaia (regardless of liking or disliking in terminology the allusion to a goddess). Future research must aim to unravel the background of these fascinations (*i*) to advance man's information and knowledge towards an understanding, (*ii*) to delineate man's humble position in the biosphere, and (*iii*) to explore the conditions of man's sustained survival on Earth, i.e. to give him a chance at least.

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[6] Lovelock 1979, p. 148.

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The Planet Earth: Can it Feed Nine Billion People?

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With 5 Tables

Abstract

The predicted human population size on the planet Earth for the middle of the 21st century is at least 9 billon people. It is an un-escapable fact that these need to be fed. This is a huge challenge where the present concern of the public about increasing atmospheric CO_2 concentrations is addressing just the tip of the iceberg. The sheer number of people presents a problem per se independent of global changes due to green house-gas emissions but aggravated by them. Current global agricultural production must be doubled by 2050. A failure results in a most horrifying scenario of apocalypse. A section of the essay surveys examples of vanishing resources. A further section lists socio-political ideologies and for each of them asks the question "can we afford" them in view of the challenge to feed 9 billion people? A final section discusses measures of biotechnology for crop enhancement. Such approaches of potential genetic modification as a promising avenue for increases of crop yields in the current literature mainly concern improvement of C_3-photosynthesis by genetic molecular engineering and expansion of C_4-photosynthesis. Photosynthesis is the only way of primary production of organic matter or biomass on Earth, and therefore, there is no other hope for mastering the challenge than by the aid of advanced photosynthesis together with partitioning and allocation optimized for enhancing yield. The essay is a plea for abandoning socio-political wishful ideologies – the most appalling ones concerning attitudes that prevent restriction of human population growth, selective views of energy supply and hostility to molecular engineering of crop plants – and urgent political reorientation and action.

Zusammenfassung

Die für die Mitte des 21. Jahrhunderts vorausgesagte Größe der menschlichen Population auf dem Planeten Erde ist wenigstens 9 Milliarden Menschen. Dies ist eine unausweichliche Tatsache, und diese Menschen müssen ernährt werden. Es ist eine riesige Herausforderung der gegenüber sich die Beunruhigung der Öffentlichkeit über den Anstieg der atmosphärischen Kohlendioxidkonzentration wie das Betrachten nur der Spitze des Eisbergs ausnimmt. Die schiere Zahl der Menschen stellt ein Problem ganz für sich dar. Die gegenwärtige landwirtschaftliche Produktion muss bis 2050 verdoppelt werden. Dieses Problem ist unabhängig von den globalen Veränderungen durch die Emission von Treibhausgasen, wird aber durch sie verschlimmert. Ein Versagen führt zu dem schrecklichsten Szenario einer Apokalypse. Ein Abschnitt dieses Essays bringt einen Überblick über schwindende Ressourcen. Ein weiterer Abschnitt zählt eine Reihe sozio-politischer Ideologien auf und stellt für jede von ihnen die Frage „Können wir uns das leisten?“ angesichts der Herausforderung, 9 Milliarden Menschen ernähren zu müssen? Ein letzter Abschnitt diskutiert schließlich biotechnologische Möglichkeiten der Erntesteigerung. Solche Wege möglicher genetischer Modifikation als vielversprechender Ansatz für die Steigerung der landwirtschaftlichen Ausbeute beziehen sich in der Literatur gegenwärtig vor allem auf die Verbesserung der C_3-Photosynthese durch molekulargenetische Manipulation und auf die Expansion des Anbaus von Kulturpflanzen mit C_4-Photosynthese. Die Photosynthese ist der einzige Weg der Primärproduktion von organischer Materie oder Biomasse auf der Erde, und deshalb gibt es keine andere Hoffnung, die Herausforderung zu meistern, als durch verbesserte Photosynthese zusammen mit einer optimierten Verteilung und Allokation der Produkte der photosynthetischen Primärproduktion zur Steigerung der Ausbeute nutzbarer Pflanzenteile. Der Essay ist ein Plädoyer, sozio-politische Ideologien und Wunschdenken aufzugeben. Die auffallendsten davon betreffen Einstellungen, die eine Einschränkung des Wachstums der menschlichen Bevölkerung der Erde behindern, einengende Bewertungen verschiedener Möglichkeiten der Energieversorgung und die Feindschaft gegenüber molekularer Manipulation von Nutzpflanzen. Politische Neuorientierung und politisches Handeln sind dringend.

1. The Principal Problem: Nine Billion People

The planet Earth currently is housing 7 billion people. Possibly it is also capable of feeding them. Perhaps not even that because according to reports of the Food and Agriculture Organization of the United Nations (FAO) right now 1 billion people are under the stress of starvation suffering hunger. In Sub-Saharan-Africa it is 30 % of the population. This may still be only a global political problem of management, trade and distribution. Nevertheless, it is a sign of what mankind is facing in the future where the prognosis is that by the middle of the 21st century the population on the planet will be nine billion people. Scenarios are presented by COHEN (2003). When fertility would remain at the current rates the population would grow to 12.8 billion by 2050. Thus, 9 billion may be even a conservative estimate. BENGTSON et al. (2006) have the number at 8.7 to 11.3 billion by 2050.

The estimate of 9 billion is based on the expectation that globally efforts of family planning will continue and will succeed (COHEN 2003). This is the optimistic part of the assessment. If it does not work, effects of recurrent catastrophes, where "natural evil" (MURPHY et al. 2007) multiplied by local overpopulation translate themselves into horrifying human tragedies, as well as deaths due to epidemics such as AIDS (COHEN 2003) will control size of global human population around 9 billion if not even initiating a decline (see below and Section 3.6). This sounds cynical. However, to the extent it is already observed to date it only foreshadows the apocalypse which we face when it turns out that the planet cannot even "only" support 9 billion people. This is the pessimistic part of the assessment. Has mankind already passed the point of no return? In view of the several waves of massive global extinctions during the evolution of life in geological history, including the rise and fall of highly developed species, in evolutionary terms an extinction of mankind would not be unequaled (MATYSSEK and LÜTTGE 2013).

Thus, we must consider the number of 9 billion people needed to be fed in less than 4 decades from now as given and as such being the most dramatic global problem. Can this be achieved? It means that agricultural production of food will have to be increased by 2 % every year. A working group of the French Academy of Agriculture has recently documented details of the problem and possible measures, where one of the major conclusions is that global political action is urgent and largely missing (*Académie d'Agriculture* 2009). Time is short and it may be running out.

The problem given by feeding per se of the number of people on Earth is exacerbated by other consequences of so many people living on the planet. Such consequences are unavoidable attributes of life. Any kind of life is modifying its environment. This was associated with the appearance of life on Earth right from its beginning in evolution. Life is shaping the geological surface of the Earth. The planet would not be what it is without its biosphere comprising at its surface life in the oceans, on land and in the air. With excessive use of space and resources mankind is now causing massive global changes. This comprises pollution including the release of the green house gases CO_2, CH_4, N_2O, O_3 and others threatening global warming. However, the current debate on anthropogenic increase of atmospheric CO_2 is only addressing the tip of the ice berg. Even more so the half hearted reaction of incapable global politics is deepening the nightmare.

James LOVELOCK in 1979 has conceived the Gaia concept (LOVELOCK 1979). Gaia, named after the goddess of Earth of antiquity, is the supra-organism of the entire biosphere. In a then (1979!) optimistic view regarding Gaia as a self organizing and self controlling entity

Lovelock has suggested that with its activities Gaia by itself would sustain conditions on the Earth hospitable for life including mankind. In another book thirty years later he is much less optimistic (Lovelock 2009). With the current threat of manmade global changes he thinks that mankind may survive but possibly with not more than a few hundred million people. We may recall that in 1700 the global population was 600 million people (Cohen 2003). In relation to his recent book, which he calls "a final warning", Lovelock is named "The prophet of environmentalism" (Jonathan Bate, *Sunday Telegraph*; quote from the book cover of Lovelock 2009). The process of selection of the survivors will embrace the most terrifying mass extinction one can possibly imagine.

Gaia then may be able to feed the few hundred million. When we return to the question if the planet can even feed the prognosticated 9 billion people we must note important threatening developments and we must ask a plethora of questions which will be the aim of the following sections of this essay. *First*, we must realize vanishing resources, which include space inhabitable for man, being progressively at risk through destruction by overpopulation pressure. *Second*, we must ask if we can afford the many socio-political ideologies currently governing influential attitudes to global change or even the ignorance of large societies about this threat. *Third*, we must fathom which possible measures may be given by biology, biotechnology and agriculture.

2. Vanishing Resources

2.1 Arable Land

The total area under agriculture world-wide currently is 1.4×10^9 ha. Arable land is continuously lost. One major problem is salinity caused by irrigation culture. The irrigated arable land used for agriculture under watering until 1990 increased proportionally with human population. Since then it stagnates at about 230×10^6 ha while population continues to increase exponentially. Globally 17 % of the area used for agriculture is watered while it provides 30–40 % of global food production. Due to evaporation leaving dissolved salt behind, even if the best fresh water is used for irrigation salinity is a paramount problem. Each year 200,000 ha are newly lost to salinization, total loss of land used by agriculture due to erosion and degradation is 10^6 ha per year (Lüttge et al. 2010, von Braun 2011).

Under a scenario of global warming a "migration of the North American Corn Belt into Canada" (Ainsworth and Ort 2010) might promise acquisition of new arable land. Destruction of forest associated with this will bring with it considerable liberation of CO_2, and hence, accelerate the vicious cycle of global increase of the green house gas. Moreover, it appears that the soils farther north are suboptimal and less productive (Ainsworth and Ort 2010). The idea that under the increasing demographic pressures large national parks will have to be sacrificed for agriculture (Tessier 2011), will only allow acquisition of proportionally smaller areas. However, it is symptomatically illustrating possible devastating ecological damage done wittingly and in desperation symbolizing loss of life quality and inhabitability of the planet. In any case, encroachment into natural landscape is in full progress. Let us consider here just one example, the Brazilian savanna-like cerrados. They belong to the 25 biodiversity hotspots of the world's vegetation (Myers et al. 2000, Oliveira and Marquis 2002, Gottsberger and Silberbauer-Gottsberger 2006). They originally covered 1,783,200 km^2 and are now reduced to 20 % of their original area (356,630 km^2, of which 22,000 km^2 or 6.2 % are protected). They have 10,000 plant species, 4400 of which are endemic (Myers et al. 2000). In the 20 years from 1975 to 1996 the cerrado area covered by the crops soybean, maize, rice and beans increased from 4.20×10^6 ha (15 %) to 9.17×10^6 ha (28 %). The numbers in brackets give the cerrado area in per cent of the total area which is covered in Brazil by these crops. This demonstrates that the whole increment between 1975 and 1996 of these crops in Brazil was due to cerrado cultivation (Resck et al. 2000, Gottsberger and Silberbauer-Gottsberger 2006). Massive destruction of tropical rainforests is more in medial lime lights than this cerrado example. Slash and burn agriculture in rain forests often is not sustainable for more than a few years due to exhaustion of mineral nutrients and invasion of weeds. Models of sustainability are those of shifting agriculture (Whitmore 1990) which only offer limited and modest use of the forest.

Arable land is a vanishing resource. Apparent new acquisition is both limited and destructive. Therefore, under the demographic pressure it appears that destruction of potentially arable land will be continued and even extended rather than halted. Independent of the current CO_2 debate, but potentially amplified by the green house effects, the consequences of new acquisition of arable land for agriculture now still occupied by other vegetation, such as mainly savannas and forests, are not foreseeable. Such new acquisition is not a way for sustained feeding of the 9 billion people to arrive.

2.2 Water

A major resource of agriculture is water. Of the fresh water available on the planet 70 % is used for agricultural irrigation, and 40 % of the total global production of agriculture depends on irrigation. In many regions fresh water is already under short supply. The problem will increase in the future globally. A prognosis is that by the year 2025 more than half of the world's human population will be facing severe problems due to shortage of water (Kulshreshtha 1998). The largest volume reserve of water is sea water, i.e. more than 10^9 km^3 (Tab. 1). Total fresh water amounts to 35×10^6 km^3, i.e. 2.5 % of the total water on Earth. Liquid fresh water available is just 0.76 % of the overall water reservoir. Procedures to produce fresh water technically by desalting sea water are highly energy consuming. Increased water use efficiency of agricultural production is a challenge of agronomic research to avoid a global water crisis in the future.

2.3 Nutrients

Natural resources of fertilizers are vanishing. Major nutrients are nitrogen and phosphorus. For crop fertilization N can be obtained technically *via* the Haber-Bosch procedure by reducing atmospheric N_2 to NH_3. This, however, is highly demanding of energy (Section 3.5). The situation is worse regarding phosphorus. Phosphates are non-renewable fossil resources. Currently 95 % of the phosphate rock mined is used in agriculture. The most economically exploitable phosphate reserves which can be mined for the production of phosphate fertilizer are estimated to be depleted within a period of 100–130 years (Cisse and Mrabet 2004, Cordell et al. 2009, Bünnemann et al. 2011, Vance and Chiou 2011). In principle global phosphate reserves for extraction of phosphorus appear to be sufficient but getting access to and exploration of new reserves will be costly and use much energy. Projects of recycling of

Tab. 1 Water reserves of the planet Earth (compiled after http//:www.theglobaleducationproject.org/earth/fresh-water.php)

	Volume (million km³)	% of total
Total global water	1400	100
Fresh water	35	2.5
Glaciers and snow	24	1.7
Fresh ground water	10.5	0.75
Ground ice and permafrost	0.3	0.02
Rain	0.1	0.007
Lakes	0.09	0.006
Soil	0.02	0.001
Atmosphere	0.01	0.0007
Wetlands	0.01	0.0007
Rivers	0.002	0.0001

phosphorus excreted by life stock and by humans back to agricultural land need to be developed (Oberson et al. 2011). Maintenance of soil fertility and mineral nutrition of crops is a major factor limiting future increases of agricultural production. Overall, provision of fertilizers will be highly energy demanding.

2.4 Vanishing Advance of the Development of Yield

We are so much used to taking continued increments of agricultural yields for granted. There is the outstanding historical experience, e.g. by the successes of breeding and cultivating wheat. In the times of antiquity for one grain sawn 3 grains could be harvested, in the middle ages these were 6 grains and now they are 50 grains. There is the experience of the "green revolution" in the middle of the 20th century where rigorous application of conventional breeding methods generated cereals with high yield potential and grain production (Hibberd et al. 2008). Between 1944 and 1956 breeding of wheat at CIMMIT (*Centro International de Mejoramiento de Maiz y Trigo*) under the direction of Norman Borlaug changed Mexico from a wheat-importing to a self-sufficient country and won Borlaug the Peace Nobel Prize. Yield increments of many different crops were a continuous trend of the second half of the 20th century. Now due to vanishing resources, increasing adverse environmental changes and pollution, where increasing levels of ozone are a major threatening (Ainsworth et al. 2008), increment of yields is decelerating or stalled and yields are even declining. It was documented for winter wheat and colza in France (*Comptes Rendus de l'Académie d'Agriculture de France* 2010) and also for wheat worldwide where between 1987 and 1997 per area production rose by 20 % and between 1997 and 2007 declined by 1 %. The figures for rice are increments of 17 % 1987–1997 and of 2 % 1997–2007, respectively (Long and Ort 2010). A particular concern are also potentially rising temperatures because many reactions in photosynthesis are heat sensitive and this will affect reduced productivity (Ainsworth and Ort 2010). The predicted declines of yields of crop plants including maize and soybeans in some models are dramatic being 30–46 % or even 63–82 % depending on the warming scenario applied (Schlenker and Roberts 2009). There are serious doubts that the rising

CO_2 concentration of the atmosphere, also called "CO_2 fertilization" will offset losses due to other perturbations, especially in the absence of simultaneous heavy use of mineral fertilizers (nitrogen) (Long et al. 2006).

3. Socio-Political Ideologies

The fastest growth of populations occurs in the less developed poor countries (Cohen 2003). By the year 2050 they will still grow by 0.40 % annually while the population of the developed countries will decline by 0.14 % each year (Tab. 2). Famine of large populations is currently restricted to the developing world. However, with the threat of changes ahead the so-called developed world may rapidly be included. We may have to take the pessimistic scenario of Lovelock (2009) with only a few hundred million people surviving a mass extinction as a serious possibility.

Tab. 2 Annual growth (%) of human population on Earth (compiled from Cohen 2003)

	Global	Developed countries	Poor, less developed countries
2003	1.22	0.25	1.46[1]
2050 (predicted)	0.33	–0.14	0.40

[1] Least developed 49 countries: 2.41 %.

Current global politics is largely overlooking this threat or just wittingly avoiding realizing it. By contrast, global politics is most discouraging as long as it strives for putative tribal or as it were national advantages in cases underpinned by conventional or nuclear armament, and where even a few fanatic individuals may develop the power to destroy the welfare and future of all mankind. Mankind cannot afford this.

We need all possible measures of scientific and technological research and applied technical tests to avoid realization of the pessimistic Lovelock scenario. We cannot live with the nostalgic attitudes of past centuries. Hence, we must ask if we can afford wishful thinking of socio-political ideologies currently hindering the necessary scientific and technological developments by focusing on some dangers which may appear minor in view of the apocalyptic horror of a possible mass extinction. With examples below I am asking repeatedly the question: "Can we afford?" There are enormous challenges. It is the challenge for politics to search for the answers to "Can we afford?" and to take action within a frame where ideological bias is completely excluded. Politics must create a solid and reliable fundament for science and technology to work on their own challenge to find solutions.

3.1 Can We Afford "Bio"-Agriculture?

In what can be subsumed by "bio"-agriculture, we must distinguish at least three different levels, which we may name (*i*) biodynamic agriculture, (*ii*) organic farming and (*iii*) agro-ecology. (*i*) is anthroposophic occultism (Treue 2002) and by great contrast (*iii*) is a serious interdisciplinary approach applying ecological principles to agriculture (*Wikipedia* 2011a). Thus, regarding the question "Can we afford?" we may exclude (*i*) because it is irrelevant. A critical inspection of can we afford (*iii*) is overrun by the promising expectation that agro-ecology at this very stage may well be prepared to generate important research and discussion in pursuing the aims in the necessity to find ways of feeding increasing populations and sustain functioning of agro-ecosystems. Organic farming (*ii*) needs closer inspection.

Notably the definition of yield potential is "the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting, and with pests, diseases, weeds, lodging and other stresses effectively controlled" (Evans and Fischer 1999, see Long et al. 2006). This contention of yield evidently is based on practice of conventional agriculture. Conversely, organic farming (*Wikipedia* 2011b, i.e. (*ii*) above) is agriculture excluding manufactured fertilizers, pesticides (herbicides, fungicides, insecticides), hormones, antibiotics and basically – but apparently not exclusively – genetically modified organisms (see Section 3.4). It is highly expanding worldwide and acquiring markets for its products. It occupies about 1 % of the world's total farmland and 8 % in Europe (Maeder et al. 2002). Increasingly many people select it as a sympathetic approach. It supports the development of higher biodiversity (Maeder et al. 2002). Nevertheless, it is highly controversial. It is frequently found to have significantly lowered yield as compared with conventional agriculture (Maeder et al. 2002). This will increase land use, where arable land is a vanishing resource (Section 2.1). The benefits of organic farming will be counteracted by lowered productivity. The quality of its products can be reduced, particularly where quality is related to mineral nutrition. Proteins of vegetables and cereals may be suppressed due to lack of N fertilization. Organic farming is not completely free from causing disturbance of the environment and from other risks although generally looking more healthy (Trewawas 2001). It can become destructive for soils of agro-ecosystems. Where no fertilizers are added the soil is exhausted of nutrients like a mine and organic farming is not sustainable (Tessier 2011).

Actually, the results of examinations holding organic farming *versus* conventional agriculture are diverse. Depending on crops and on (geographical) location organic farming may compete (Stanhill 1990). However, when we focus on the question "Can Earth feed 9 billion people?" the issue is not whether organic farming is a sympathetic approach to agriculture and right now adequately sufficient. The needed qualitative and quantitative assessment is, can we afford it with the challenge to increase agricultural production by 2 % every year? Considering a wide range of crops on average a 10 % lower yield as compared with conventional agriculture may be obtained by organic agriculture (Stanhill 1990). The Nobel Peace Prize laureate Norman Borlaug who with his cereal breeding programs became father of the "Green Revolution" assessed organic farming to be able to feed a maximum of 4 billion people (*Wikipedia* 2011b). We already have 7 billion inhabiting the planet.

3.2 Can We Afford Increasing Consumption of Meat in the Diet of World's Populations?

In ecological food-webs or chains of feeding at each trophic level the energy used in respiration and maintenance processes of the respective organisms is liberated in the form of heat. Thus, in fulfillment of the second law of thermodynamics the degree of entropy of the entire food-chain or food-web system is increased. Therefore, it is in fact a waste of resources and energy if we consume meat rather than eating vegetable protein. With the consumption of meat we are at best the third level in the food chain after the primary producers, i.e. plants, and the secondary producers, i.e. life stock. An example is given by Tessier (2011). For the production of 1 kg of chicken meat 4 kg of grains is required. In terms of the resource water this implies that 400 to 500 l are needed for one kg of flour or bread but 2000 l for 1 kg of meat. Evidently man's evolution is not vegetarian but omnivorous. This evolutionary heritage must be borne in mind when considering of how large global meat consumption by man can be afforded.

3.3 Can We Afford to Grow "Energy Plants"?

When crops are used to produce fuel this is a direct interference with feeding human populations as we can witness even right know in ongoing competition of food or fuel (Cassman and Liska 2007). In Central and South America the production of ethanol from maize is intensifying the problem of famine among the poor. In Europe the ethanol currently mixed into gasoline for cars comes from maize, wheat and sugar beets. However, even if we realize that such a problem will be under control with the advance towards second and third generation "energy plants" the question must be seriously studied if we can afford it and if its substantial subsidy by many governments is sound politics. Is it ethically acceptable to subsidize bio-fuel rather than recombinant DNA engineering for the production of more food (Cassman and Liska 2007)? The question is not mainly whether growth of "energy plants" can ever be quantitatively meaningful in view of increasing energy demands including those of agriculture itself. Any growth of "energy plants" will either compete with the use of arable land, a most scarce resource (Section 2.1), or it will lead to further increased destruction of cultured land and remaining natural ecosystems much faster than perhaps even needed for feeding the 9 billion people (see Section 2.1). Much tropical rain forest is currently destroyed to grow oil palms for satisfying the growing demand of the market of so-called "renewable" energy from plants. The overall cost-benefit balance is that much more CO_2 is liberated than by equivalent use of fossil energy sources. The destroyed rainforests are not renewable. It is an environmental disaster. The limits of bioenergy given, its chances must be seriously questioned (*Leopoldina* 2012).

3.4 Can We Afford Hostility Against Genetically Modified Organisms?

Here we need to distinguish two different types of socio-political worries generating hostility, namely (*i*) economic monopoly and (*ii*) environmental threats. As regards the first worry, a very small number of firms are developing a monopoly status backed up by patents on "living resources" that might well result in the power to take mankind as a hostage, inasmuch as the real increase in populations to be fed occurs in financially under privileged areas of the world (Tab. 2). It would, however, be the wrong reason for curtailing the research and development of genetically modified organisms (GMOs) needed now to secure mankind's food in the future. Economy-politics must take care of this. It is not a scientific problem but calls for the ethics of economy where politics can handle this out of itself without science. This will occur naturally as soon as the signals warning about the possible apocalypse become more manifest as they are even now. The second worry needs scientific evaluation. It appears that the vast majority of researchers involved in GMO work are convinced that environmental threats are under control. Environmental politics need to enforce strict rules for assessments.

When one is reading the current literature of plant physiology, biochemistry and molecular biology it is regular and indeed very frequent to encounter statements referring to the importance of the research presented with identifying genes for genetic modification of plants by recombinant DNA technology, and thus, for creating a momentum for favorable biotechnological developments. A plethora of problems is addressed in this way where GMOs will allow striking advancements such as resistance to various biotic and abiotic stresses, control of diseases, improvement of the use of resources including mineral nutrients, increased productivity, increased quality and nutritional value of crops etc. (for an overview also see CENTURY et al. 2008). We shall look at some specific examples from plant physiology in Section 4.2. For a general consideration it is essential to note the abundance of such statements in published research articles even in publications where molecular identifications address basic and not applied knowledge. It demonstrates that the scientific community at large and very broadly is convinced that every effort is necessary to use genetic engineering techniques to tackle the plethora of problems crop growth is faced with.

If we compare this with the socio-political scene, especially in Europe, we are faced with the most fundamental discrepancy we can imagine. There is extraordinary hostility of media, politics and large human populations against GMOs. This includes governments, decision makers and legislation. We are in the irrational situation of having extensive research on one side and at the same time precluding any practical realization on the other side. There are conspicuous geographical differences though. The situation is favorable for GMOs in the USA and Canada as well as in many Asian countries including India and China while hostility is pronounced in Japan and also in many countries of Europe including France and Germany (*Wikipedia* 2011c). The most promising approach for increasing agricultural productivity at the required rate for eventually feeding 9 billion people is the GMO technique. Therefore, the hindrance or even prohibition of actual applications including the necessary field trials deprive us of one of the most reassuring causes of hope for being able to master the challenge. It is irresponsible politics. If we develop ethical rules for attitudes towards the GMO technique we must bear in mind the demands in the future. The ethical question is pertinent not alone to considering the situation to date but much so also to developing measures which may not be needed right now but will become essential tools for humane survival in the future to come. In the view of Lothar ALTSCHMIED (Gatersleben)[1] this implies that as we will massively over-explore the ecological systems by human population growth we shall have to solve these problems first before we can discuss the ethical bases.

A change is necessary, and it is very urgent. Media, legislators and governments must stop hostility right now. No better way to illustrate this is to cite authors who publish a lot in the field, LONG and ORT (2010): “2050 may sound a long way away and a chance for several more years of discussion. However, given the time required to translate findings in model organisms to crops, complete the long regulatory process for releasing new transgenics, and then bulk seeds for commercial release, even starting now might only be just in time.”

It is promising that there appear to be even some organic farmers who seem to accept genetic modification as a biological technology consistent with the principles of organic farming (*Wikipedia* 2011b). For example GMOs appropriately engineered for resistance towards diseases and herbivory or more efficient acquisition and use of mineral nutrients can be crop plants better suited for the methods and objectives of organic farming. Examples for the potential which GMOs have for protection of the environment, i.e. reduced application of pesticides and production of the greenhouse gas CO_2 are given in Table 3. What a great organic choice is GMO as compared to synthetic food (Section 4.1.2)!

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[1] Round table discussion, p. 227, in WOBUS et al. 2010.

Tab. 3 Biotechnology-engineered crops and their benefits to the environment. (After Ainsworth et al. 2008 who used data reported in 2006 by Brookes and Barfoot)

Transgenic crops	Total area occupied
Maize (*Zea mays*)	
Sweet maize (*Zea mays* var. *rugosa*)	100×10^6 ha
Canola (*Brassica napus*)	
Cotton (*Gossypium hirsutum*)	
Soybeans (*Glyxcine max*)	
Papaya (*Carica papaya*)	
Sugar beets (*Beta vulgaris*)	
Squash (Cucurbita pepos)	
Environmental benefits	
Reduced pesticide application	224×10^6 kg
Reduced environmental pesticide impact	14 %
Reduced liberation of CO_2[1]	960×10^6 kg

[1] Ca. 0.01 % of the current annual anthropogenic CO_2-production of 8×10^{12} kg/year.

3.5 Can We Afford to Reject Nuclear Energy?

The energy demand of mankind shall increase enormously in relation to population growth and quite specifically with the extraordinary measures required for providing enough food. With respect to nuclear energy there are two questions. Without nuclear energy shall we have (*i*) enough energy and (*ii*) energy without excessive output of CO_2. It is intriguing to read Lovelock (2009). He is environmentalist. He is an outspoken and confessing green and as such esteemed as a prophet “who deserves every honour the human race can bestow” (*Guardian*, quote of book cover: Lovelock 2009). However, he is a green without the green ideology which he considers “damaging to the real environment” (Lovelock 2009). The major conclusion from his book is that we will not be able to solve the planet’s problems without nuclear energy. Regarding the output of the green house gas CO_2 it is worthwhile to inspect the Table page 69 of Lovelock (2009). According to the UK Government’s Energy Technology Support Unit Report the CO_2 output per kWh produced by nuclear is 0.5-times of that of wind, 0.24 that of energy crops, 0.03 that of solar, and 0.009 to 0.004-times that of fossil sources.

An important problem related to the question if we can increase global agricultural production by 2 % every year to be able to feed 9 billion people in 4 decades is if we shall have sufficient energy. We need technically highly developed “high-tech” agriculture which is energy demanding. For high yield agriculture it is important to have fertilizers particular for providing the nutritional elements nitrogen and phosphorus. Natural resources are vanishing (Section 2.3). Exploitation of new resources is energy demanding (Section 2.3). A virtually unlimited resource of nitrogen is the 80 % N_2 in the planet’s atmosphere. For biological utilization it needs to be reduced to ammonia, NH_3, which is technically achieved by the Haber-Bosch procedure. This requires high pressures between 250 and 350 bar and a temperature near 550 °C, and it is needless to say that this is energy consuming. Thus, producing N fertilizer is not a question of N resource but a question of energy. Even at present already 1.4 % of the world’s energy consumption is due to the Haber-Bosch procedure (*Wikipedia* 2011d).

Should it really come to the necessity of producing synthetic food (Section 4.1.2) energy demand may prove to be far beyond present assessability.

So the question remains if various forms of energy supply including so called renewable ones will ever be sufficient. If cold nuclear fusion turns out not to be feasible as it looks to date, can we afford to reject the use of nuclear fission energy in addition to other approaches to satisfy the energy demand? Notwithstanding the deep tragedies of horrifying accidents, will the use of nuclear energy turn out to be the lesser risk accelerating the decline of mankind as compared to that of the extinction of mankind due to other failures as it might be in the energy supply for the production of food?

The aggravating problems with nuclear energy are safety and waste. In view of the accidents due to technical failures and natural catastrophes some countries now decide for completely abandoning nuclear energy without any compromise while others opt for research improving security (Jacques Foos: "Il n'est pas question de renoncer au nucléaire … Mais il faut revoir notre culture de sûreté."; Delbecq 2011). Most likely safety already could be highly improved if current technological know-how and capabilities were used without compromise regarding the costs. Furthermore, intensive technological research should be able to arrive at innovations improving the situation. If we reject nuclear energy and with this renounce supporting research for better new generations of nuclear power plants to replace the present unacceptable ones we give up opportunities which we may recognize as badly needed when it is too late. It is a typical example of the dilemma weighing technology-anxieties against the apocalypse of not being able to feed 9 billion people. Wherever we look the approaches required to solve the problem of increasing agricultural production are energy demanding.

3.6 Can We Afford a Sexual Moral Supporting Unlimited Increase of World's Population?

Can we afford a sexual moral narrowly fixed on an idea of human life given by birth and death like the one of a large globally dominating Church? This implicitly bears out unlimited increase of the planet's human population. It has no intrinsic let alone outspoken means of controlling population growth. Population growth is the core of all problems endangering the survival of mankind on the planet. While we are threatened by the challenge to feed the unavoidable predicted 9 billion people, it appears vain to envisage feeding even more.

Population ecology has many examples, where the exponential growth of populations associated with maximum exploitation of resources led to the loss of the basis of existence and extinction. A sexual moral is needed which considers the idea of human life in ecological terms, in relation to the environment and in relation to what the planet can carry without running into the inhumane apocalypse of mass extinction (Mieth 1989).

4. Measures

4.1 Biotechnology

4.1.1 GMOs

Conventional breeding remains important. However, it is limited by the amount of genetic diversity in the germ-plasm of crop plants (Century et al. 2008). Therefore, the use of GMOs is one of the most promising measures for advancing fast enough with increasing agricultural production on the background of vanishing resources and declining yields (see Section 2). Some advancement is currently made (Tab. 3) underlining that specific genetic engineering can potentially

- increase yield in various ways;
- develop new crops resistant to stresses, such as of temperature, drought, nutrient limitation (particularly P: Vance and Chiou 2011), salinity, high irradiance, toxic metals, and therefore suitable for growth on less favorable land, thus helping to acquire new arable land, where e.g. genetic engineering for drought tolerance shows considerable promise (Century et al. 2008, Grene et al. 2011, Scheibe and Beck 2011);
- reduce losses to pathogens and herbivores, thus helping to reduce the use of pesticides, where an important already realized modification provides transgenic crop plants expressing the gene for the toxin of *Bacillus thuringiensis* (Bt) against the larvae of insects, which is a very promising example;
- increase the resistance to viruses (e.g. the realized development of virus resistant *Papaya*);
- minimize often very considerable post-harvest losses (e.g. the already achieved development of tomato with disturbed ethylene biosynthesis and better ripening control);
- increase food quality by increasing contents of essential minerals such as iron and selenium, vitamins (such as β-carotene for vitamin A by the introduction of "golden" rice), or decreasing contents of unhealthy saturated fatty acids (as in genetically engineered soybean, *Glycine max*, or colza, *Brassica napus*).

GMO-agriculture is expanding (Tab. 3). Worldwide 15 % of the arable land is already used for GMOs, 50 % of soybean and 30 % of maize cultivation is with GMOs. A conspicuous example of the irrational consequences of GMO-hostility (Section 3.4) is that Bt-transgenic crop plants would be prohibited while massive spraying with the *Bacillus thuringiensis* or its toxin would be allowed. GMO-agriculture will continue to expand. The challenge of to be or not to be able to feed 9 billion people in the future will not know geographical barriers.

4.1.2 Synthetic Food

With declining yields, with crop failures, with the scarcity and with even progressing loss of arable land a view is coming up which may look extreme, namely that we would have in the future to synthesize food. It is in fact discussed ardently and intensely serious by James Lovelock (2009). With water and CO_2 and nitrogen from the atmosphere and some minerals food could be produced using tissue culture. One can even think of purely chemical synthesis of amino acids and sugars. In desperation it could come up as an ultimate solution. Evidently it would have an enormous demand of energy creating completely new challenges for the debate concerning sufficient approaches of energy supply (Section 3.5). It would need biotechnological research and time to develop. Time may be short.

4.2 Plant-Biology's Physiology, Biochemistry and Molecular Biology for Primary Production by Photosynthesis

Photosynthesis of green plants is the primary production of any organic matter or "biomass" and hence of any food available on the planet Earth. Only improvement of photosynthesis can serve the required increase of food supply. This must include consideration of balanced acquisition of carbon and mineral nutrients, particularly N and P, as well as partitioning and allocation towards optimization of yield formation. It is known from cereals such as wheat that the photosynthetic capacity of elite cultivars is underutilized during grain filling, i.e. for securing yield (Reynolds et al. 2009). The yield potential (YP) is a function of the irradiance intercepted (IP), the radiation-use efficiency (RUE) of photosynthesis and the harvest index (HI), which is dependent on biomass and its partitioning to yield, e.g. grains in cereals:

$$\mathrm{YP} = \mathrm{IP} \cdot \mathrm{RUE} \cdot \mathrm{HI} \qquad [1]$$

One essential aim is to increase RUE and biomass and its conversion to yield under agronomic conditions (Reynolds et al. 2009). Given the genetic complexity of YP the major hurdle is that the overall process comprises various levels of complex reaction networks which are interlinked and highly regulated. Therefore, single gene manipulations can hardly find their targets. Conventional breeding remains essential. Conversely, many authors now begin to concentrate on increasing leaf photosynthesis as an "attractive avenue to drive increases in crop yields" (von Caemmerer and Evans 2010). While many traits of plants may get in the focus of genetic engineering promising increased food production (Section 4.1.1), genetic engineering of photosynthesis evolves as a prime target. This places plant physiology, biochemistry and molecular biology of photosynthesis in the center of expectations and responsibility of research and development. The possible opportunities of improvements in photosynthesis for increasing crop yields are surveyed and assessed in a seminal review by Long et al. (2006). However, the major hurdle is that the overall process of photosynthesis also involves complex interacting reaction networks. Moreover, mineral nutrient limitation will also have negative feedback effects on photosynthesis. It has therefore been suggested for genetic engineering to look for transcription factors which are master regulators of interlinked cascades of genes (Century et al. 2008). Naturally it is much easier if monogenic traits can be used for the improvement of photosynthesis. As suggested by von Caemmerer and Evans (2010) few single-gene changes may already be effective. Improvements based on single-gene engineering may take between 0 and 5 years, whereas manipulation of gene cascades will take decades (10 to 20 years; Long et al. 2006). I shall consider this with respect to C_3 photosynthesis and then inspect C_4 photosynthesis as a high-productivity natural modification of photosynthesis and the ambitious project of engineering for expression of C_4 photosythesis in the C_3 crops rice and wheat.

4.2.1 Improve C_3 Photosynthesis

C_3 Photosynthesis is compartmentalized within the chloroplasts of single cells. We can distinguish three steps where enzymes are involved which may be targets of genetic engineering, (*i*) diffusion of inorganic carbon (CO_2 or HCO_3^-) into the chloroplasts, (*ii*) CO_2 fixation by ribulose-bis-phosphate carboxylase/oxygenase (RubisCO) and (*iii*) the regeneration of ribulose-bis-phosphate as CO_2 acceptor of carboxylation by RubisCO.

(*i*) *Acquisition of inorganic carbon:* Concentration gradients of HCO_3^- and CO_2 for diffusion of inorganic carbon into the chloroplasts are maintained by pH effects (slightly alkaline pH in the cytosol and in the chloroplast stroma) and the pH-dependent reaction of carbonic anhydrase (CA) converting the forms of inorganic carbon $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. CO_2 is transported across the chloroplast envelope by CO_2-permeable aquaporins (Uehlein et al. 2008). Both enzymes but particularly CO_2-permeable aquaporins have been suggested to be promising targets for genetic modification (von Caemmerer and Evans 2010).

(*ii*) *CO_2 affinity of RubisCO*: Photosynthetic rates are highly correlated with levels of RubisCO. RubisCO is an ancient enzyme that has evolved in early geological time when the CO_2 concentration of the planet was very high and O_2 concentration was low. Therefore, extant RubisCOs have inherited a rather low affinity for the substrate CO_2 and also dual substrate affinity for both gases (hence the name carboxylase/oxygenase). Current environmental CO_2 concentrations are far below substrate saturation of the enzyme. The relative specificity of RubisCO for its two substrates is characterized by the so called specificity factor, Σ:

$$\Sigma = \frac{V_{max}^{CO2}}{V_{max}^{O2}} \cdot \frac{K_M^{O2}}{K_M^{CO2}}, \qquad [2]$$

where K_M are the Michaelis constants and V_{max} the maximal reaction rates with CO_2 and O_2, respectively, as indicated by the superscripts. The specificity factor is a constant for each individual RubisCO enzyme (Spreitzer and Salvucci 2002). When we compare Σ values obtained for extant photosynthetic organisms of different levels of organization or different evolutionary distances from early photosynthesis, we may note a certain improvement with increasing bias towards CO_2, because as shown in Table 4 Σ increased as higher organisms evolved. Evidently Σ is quite low in some prokaryotes and dinoflagellates. From the prokaryotic cyanobacterial subgroup of the eubacteria to the eukaryotic photosynthesising organisms there is a 1.5-fold increase to the green algae and a more than 2-fold jump to the Σ of angiosperms. The thermophilic red alga *Galdieria partita* is a special case. At 25 °C it has the highest Σ ever recorded for any RubisCO, but Σ is much lower at its natural growth conditions of 45 °C (Uemura et al. 1997). It is suggested to use the genes of such red alga RubisCOs to engineer crop species (Long et al. 2006, Ainsworth et al. 2008, von Caemmerer and Evans 2010, Long and Ort 2010). RubisCO is quantitatively by far the dominant protein in green photosynthesizing cells. With more efficient RubisCO lower amounts of the enzyme may be required also controlling the strong nitrogen demand of photosynthesis. The oxygenase activity of RubisCO leads into the pathway of photorespiration which is associated with the loss of carbon and energy, and hence, a loss of productivity. Its molecular manipulation, however, would be a multi-gene endeavor (Long et al. 2006).

(*iii*) *Calvin cycle enzymes:* The first stable product of CO_2 fixation by RubisCO is the C_3 compound phospho-glyceric acid, hence the name C_3 photosynthesis, which is reduced to triose-phosphate. The CO_2 acceptor ribulose-bis-phosphate is regenerated from triose-phosphate in the Calvin cycle. Key enzymes of the Calvin cycle involved in converting C_3, C_4, C_5 and C_7 sugar-phosphates into each other are transaldolase, transketolases and sedoheptulose-1,7-bis-phosphatase. Especially the overexpression of the latter enzyme increases both photosynthetic rate and productivity and is a very promising single gene manipulation for crop improvement (von Caemmerer and Evans 2010, Long and Ort 2010).

Tab. 4 Specificity factors, Σ, of extant photosynthetic organisms of increasing evolutionary level (major general reference: Spreitzer and Salvucci 2002)

Organisms	Σ	References
Prokaryotes Dinoflagellates	15	Jordan and Ogren 1981 Whitney and Andrews 1998
Cyanobacteria	40	Jordan and Ogren 1981, Spreitzer 1999
Galdieria partita, thermophilic red alga	240 at 25 °C 80 at 45 °C	Uemura et al. 1997
Green algae	60	Jordan and Ogren 1981, Spreitzer 1999
Land plants with C_3 photosynthesis	80–100 80–130	Jordan and Ogren 1981, Spreitzer 1999 Buchanan et al. 2000

4.2.2 Expand C_4 Photosynthesis

C_4 photosynthesis is a mode of photosynthesis with a loop of carbon flow through metabolites before CO_2 fixation by RubisCO. The green tissue of C_4 plants is separated in a peripheral mesophyll tissue and a central bundle sheath tissue. The loop starts in the mesophyll tissue where primary fixation of HCO_3^- (generated from CO_2 in the CA reaction, see Section 4.2.1 point [*i*]) occurs by phospho*enol*pyruvte carboxylase (PEPC). This enzyme has a 60-fold higher specificity to CO_2 than RubisCO and serves a CO_2-concentrating mechanism. The product, the C_4 acid malic acid (hence the name C_4 photosynthesis) is transported into the bundle sheath tissue, where it is decarboxylated. This results in an about 10-fold increase of the internal CO_2 concentration as compared to the ambient concentration, and thus RubisCO can operate at CO_2 substrate saturation, which also suppresses its oxygenase function and hence photorespiration. The average maximum rates of photosynthesis of C_4 plants are long known as being twice those of C_3 photosynthesis, i.e. 0.4 – 1.1 (C_3) and 1.1 – 2.2 (C_4) CO_2 m^{-2} leaf area s^{-1}, respectively (Black 1973).

The key enzymes which may be targets of genetic engineering are the following (Hibberd et al. 2008):

In the mesophyll:
- carbonic anhydrase;
- phospho*enol*pyruvate (PEP) carboxylase forming oxaloacetate by CO_2 fixation;
- NADP-dependent malate dehydrogenase (MDH) reducing oxaloacetate to malic acid;
- pyruvate-P_i-dikinase (PPDK) forming PEP from pyruvate.

In the bundle sheath:
- NADP-dependent malate decarboxylase (malic enzyme, ME) decarboxylating malic acid,
- RubisCO.

However, single gene modifications appear illusionary given the intricate complexity of C_4 reaction systems.

Of the world's 6 most important crop plants only two are C_4 species, maize and sorghum (Tab. 5). The genomes of both of them are known (Westhoff and Gowik 2010). Other important C_4 crops are *Panicum miliaceum* (millet) and *Saccharum officinarum* (sugar cane). In addition we should not forget the C_4 grasses, which are important species of pastures. Native African C_4 grasses were introduced for grazing purposes to South America.

Tab. 5 The world's most important crops in terms of yield in 2004 (data from Long et al. 2006). Note that according to Ainsworth and Ort (2010) the sequence is different, namely number 1 = rice, number 2 = wheat followed by maize and soybean. Another different matter is a listing in terms of value in US-dollars, where the C_4-crops maize and sugar cane are 4th and 6th, respectively (Sage and Zhu 2011).

Crop species	Mode of photosynthesis	Total yield (mega tons)
Zea mays (maize)	C4	823
Oryza sativa (rice)	C3	725
Triticum aestivum and *Triticum durum* (wheat)	C3	555
Glycine max (soybean)	C3	186
Hordeum vulgare (barley)	C3	142
Sorghum bicolor (sorghum)	C4	59

In Asia the largest human populations depend on rice. The global challenge of having to double agricultural production in the near future to avoid a human disaster also applies specifically to Asia, and hence, specifically to rice. More than half of the about 1 billion people of the global population already suffering famine are Asians. However, rice is a C_3 crop. Thus, the extraordinarily ambitious project came up to engineer rice to a C_4 crop (Surridge 2002, Mitchell and Sheehy 2006, Hibberd et al. 2008, von Caemmerer and Evans 2010, Westhoff and Gowik 2010, Sage and Zhu 2011: commentary on a special issue on the topic of *Journal of Experimental Botany*). To engineer rice and possibly also wheat (Reynolds et al. 2009) to become C_4 plants is vigorously pursued by several working groups but extremely difficult. A concerted team effort spanning many disciplines is needed and the time required may be two decades (Long et al. 2006, von Caemmerer and Evans 2010). Due to the high complexity of the C_4 photosynthesis machinery it may perhaps turn out eventually to be impossible to engineer C_4 crops from cultivated C_3 species. Nevertheless, Sage and Zhu (2011) express optimism, "that C_4 engineering will be feasible in the next few decades". If it succeeds it will be a very major step towards solving the problem of doubling global agricultural output, but not, of course, if societies continue to attach to ideology of GMO hostility.

As we have seen above C_4 photosynthesis is a complex of metabolic networks in different tissues. Although there are some Chenopodiaceae which do perform C_4 photosynthesis in single cells, these are plants adapted to stressful habitats in the Eurasian steppes (Edwards et al. 2004, Akhani et al. 2005, Park et al. 2009). C_4 crop plants require the complex compartmentation of photosynthetic metabolism in mesophyll and bundle sheath tissues. Attempts of a genetic change of single enzymes for C_4 photosynthesis have led to profound abnormalities or at least have not shown dramatic improvements in photosynthesis (Miyao et al. 2011). On the other hand the observation that C_4 photosynthesis has evolved polyphyletically at least 45-times (Hibberd et al. 2008) up to perhaps more than 62-times (Sage et al. 2011) among the angiosperms suggests that it should not have been such a complicated evolutionary innovation that one might not be able to engineer it molecularly. It would be effective if one were able to identify one or a very few master genes regulating the gene cascade in the whole genetic architecture of C_4 photosynthesis. Studies of the evolution of C_4 photosynthesis and parallel genome sequencing of phylogenetically related C_3 and C_4 species (von Caemmerer and Evans 2010, Bräutigam et al. 2011) as well as studying C_3-C_4 switches in C_3-C_4 intermediate species as they occur especially in the genus *Flaveria* (Westhoff and Gowik 2010) may help to identify the miraculous genetic master switch. Meanwhile in the endeavor to use C_4 photosynthesis for solving the world's food problems it is much more promising to use breeding and molecular engineering for improving the performance of existing C_4 crops under various stress conditions and thus extend their environmental range for new acquisition of arable land (Long et al. 2006).

5. Conclusions

The essay started from the population-political prediction of 9 billion people on Earth posing the question of whether the planet's biosphere or Gaia *sensu* Lovelock (1979, 2009, Matyssek and Lüttge 2013) can feed them. The question is evaluated in three following sections. There are two sections related to scientific observations. A section is placed in between asking socio-political questions of what kind of wishful thinking and ideology we can afford under the challenge of having to feed 9 billion people on the planet.

The scientific observations first cover an analysis with some selected examples of vanishing resources (Section 2). This had to be followed by the socio-political questions (Section 3) because it requires the examination of what is compatible with the observation of vanishing resources and what we can afford in terms of wishful illusions and factual politics based on them. Each of the questions asked in Section 3 needs to be examined thoroughly with quantitative evaluations completely unbiased by any ideology. This section had to precede the exploration of scientific contributions to measures (Section 4) because the answers to "will we afford?" determine if we can realize scientific and technological measures or if the current societies' attitudes unwilling to accept the challenge of the future and the related global politics unable to perform concerted action hinders this.

The anthropogenic increase of atmospheric CO_2 I have called above the tip of the iceberg. The half-hearted handling of this presently overt problem by national and global politics is alarming. Global politics is at the threshold of failing to counteract the already pertinent global change because it is dominated by tribal and national short sighted particular interests of acquisition rather than balanced allocation and partitioning. This raises strong skepticism if it will ever – let alone on time – handle over-population, related feeding and energy management. Politics so far has simply excluded thinking about the threatening problem of how to feed the growing human population on the planet. Urgent concerted action is required on interrelated levels of problems comprising almost all aspects of politics. In addition to promoting agricultural research this must involve politics of land use with spatial planning for controlling soil sealing due to settlement and urbanization, land ownership, energy, commerce, trade and finance etc. (see *Académie d'Agriculture de France* 2009). Globally the most critical political concern must be control of population growth and energy demands of yet barely assessable dimensions associated with it. Time now is at risk of running out. The plea of this essay is that socio-political ideologies need sober and critical re-thinking. The author of this essay will not witness 2050 but his grand children then shall be in the middle of their most active life. When societies and politics do not wish to see and to understand the realities associated with a population of 9 billion people on Earth, when we decide now to be able to afford socio-political ideologies, it may get too late when time teaches that we are not.

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Nova Acta Leopoldina NF *114*, Nr. 391, 365–369 (2013)

Synthesis of Section IV: The Systems: Holobionts and Hierarchy Theory

Ulrich LÜTTGE ML (Darmstadt)

With 2 Figures

Rays from the previous sections and groups of chapters in this book merge from the "objects", the "processes" and the "scales" towards the "systems", i.e., the holobionts and hierarchy. The key terms at the start are "trade-off" and "balance", the latter originating from the "Growth Differentiation Balance Theory" (GDB) (HERMS and MATTSON 1992). The two terms are coming close to being synonymous referring to a choice or balance, respectively.

At the outset with the original GDB this is the balance between the two options, growth and defense. However, in Section I of the Symposium it already became clear that more than the two players, growth and defense, are involved in the game, where the establishment of additional sinks extends the playground. This widens the view of complexity at the level of organismic systems.

However, complexity of systems is potentiated when we consider different scalar levels and hierarchies. In Section III of the Symposium it was already seen that a new view of "systems biology" develops when one fathoms the genetic and metabolic roots of spatio-temporal pattern formation (HÜTT 2013, this volume). The scope of scalar levels of systems from sub-atomic particles in the light reactions of photosynthesis to global functioning of the entire biosphere is overwhelmingly enormous. It spans 16 orders of magnitude in space and 32 orders of magnitude in time (LÜTTGE and HÜTT 2009, LÜTTGE 2013). Complexity is so high and emergence within the various levels of hierarchies of systems' components is so rich that outlooks must proceed further ahead from simple alternatives of trade-offs. Thus, the outcome of the synthesis considering the systems as done in Section IV of the Symposium shows that it is too simplistic to visualize trade-offs as exchanges in pairs, such as carbon versus nitrogen, parasitism versus mutualism, competition versus facilitation, growth versus defense.

The first contribution to Section IV of the Symposium by KÖRNER (2013, this volume) is setting this out underlining that the emerging "investment policy" of plants in hierarchically scaled systems demands abandoning a purely carbon centered view of the performance of natural as well as agro- and forest-ecosystems. Mineral nutrition is moved into the center of considerations particularly focusing on nitrogen where phosphorus might need to be developed to an additional and possibly in many cases even overriding issue. Nitrogen determines growth and with it the construction of sinks including stores. With this particular focus of attention it is conceived that mineral nutrition dependent growth drives photosynthesis and not *vice versa*. SAUGIER (2013, this volume) is picking this up with quantitative evaluations on a global perspective. This contribution also highlights the different performance of trees and forests in comparison to herbaceous cultivated plants and crops comprising the management of resources with the major elements C, N and P. With higher longevity of leaves and lower rates of photosynthesis trees have similar productivity and larger sinks. This overall makes the process of "storage and remobilization" an additionally emerging important player essential for the spatiotemporal dynamics extending the GDP concept. Storage comes in as a new dynamic pool allocating material together with growth and defense, and thus, requires an extrapolation of GDB. (Fig. 1)

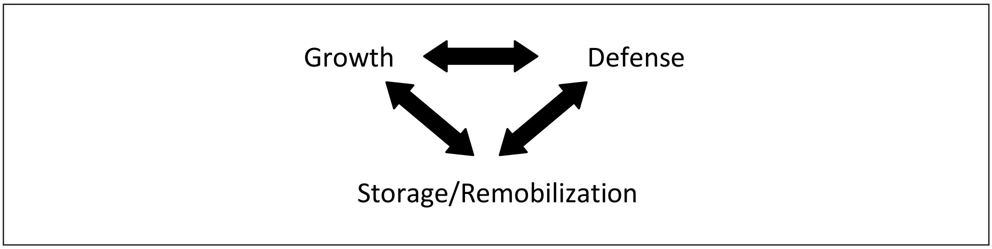

Fig. 1 Extension of GDB

As already illustrated in Section II of the Symposium by Lehmeier (2013, this volume) storage and remobilization can function as a buffer in the trade-off or balance of growth and defense. Storage also allows continuation of photosynthesis until stores flow over, and thus, storage modulates the encounter of C–N. Furthermore, in this context photoinhibition also has to be born in mind as an additional threatening when nutrition limits energy dissipation via carbon assimilation.

The emergent complexities on the hierarchic levels of systems can be symbolized by ladders of scalar levels, by Russian dolls with one inside the other or by networks. When networks at a given scalar level are compressed into nodes building up other networks on increasingly higher scalar levels of integration this gives mega- and supra-networks. It is impossible to deal with this without mathematical network theory as already unraveled in Section III of the Symposium (Hütt 2013, this volume). Both mathematical courses of thought and information-theory as explored in Section IV by zu Castell (2013, this volume) and Hamacher (2013, this volume) develop essential tools in biology. This must rest on three legs reminding to the magic tripod on which the goddess Pythia sat uttering the Delphi oracles of ancient Greece or on which the witches of the Middle Ages cooked their magic broths. Here the three legs are the essential components of a network of approaches, i.e. theory, model/simulation and experiment/observation or as given in Figure 2 with the words of zu Castell (Fig. 2: roman letters) and Hamacher (Fig. 2: italics).

The power of model simulations has already been demonstrated in contributions of Section II (Gayler, Lehmeier, Pretzsch) and III (Rötzer, all 2013, this volume). Zu Castell (2013, this volume) now focused on the theoretical aspects of knowledge gathering approaches. He distinguishes on the one side simulation by statistical analysis and probabilistic modeling which allows deriving tests and corresponding quality criteria, and on the other side mathematical modeling using mathematical operators for comprehending dynamics. He advocates that progress in biology will only be possible combining both approaches in strengthening the legs of the magic tripod. Hamacher (2013, this volume) abandons

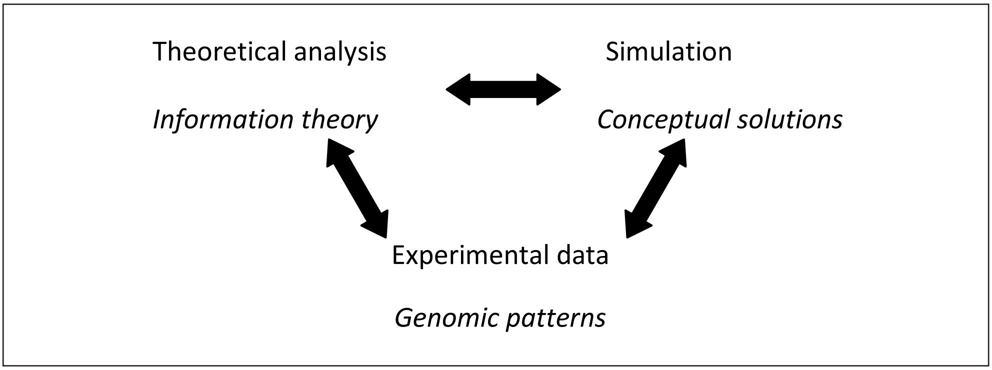

Fig. 2 Magic tripod of approaches

simplistic alignments for understanding genomic patterns, which even now might still be better performed by eye than by computer. He moves far ahead extending information theory towards new ways of effectively getting hold of the emergence of complex patterns out of the genomic information. The ball was already played in Section III of the Symposium, where Hütt (2013, this volume) showed a network of dynamic processes sitting on top of genes to be regulated. Evidently this requires reassessing genomic trees and associated conclusions of relatedness of organisms. We already note that biology must abandon its genome centered bias when we recall genome sizes of organisms of vastly different complexity:

- man 25 000; about 300 genes different from the Chimpanzee (1.3 %);
- a little nematode and the fruit fly 15 –20 000;
- the weed *Arabidopsis thaliana* 27 000.

This cannot be all the information needed for emerging complexity. It is hard or perhaps even impossible to find algorithms for quantifying complexity. Epigenetic gene regulation and epigenomic plasticity must be essential features for handling the outcome from genome information with much further generation of information on the various levels of emergence of complexity.

Epigenetic mechanisms are also involved in the functioning of the stress memory in plants (Chinnusamy and Zhu 2009, Verhoeven et al. 2010). The introductory talk of the Symposium by M. Thellier has shown that memory processes in the control of plant growth and morphogenesis (Thellier et al. 2013, this volume) comprise a form of habituation where after exposure to a first stimulus subsequent responses to a second stimulus of the same type are modified. Most importantly there is a second form of memory which allows storage of information and recall of that information and is therefore termed STO/RCL. Storage of information can occur for various kinds of stress, such as manipulation of plants, drought, wind, cold shock and even low intense electromagnetic radiation. At the molecular level proteins are involved in the STO/RCL functions. Possibly small RNAs are participating in the signaling cascades because in epigenetic modifications of DNA and nucleosomal histones by methylation small RNAs are involved (Chinnusamy and Zhu 2009). The physiological experiments show that storage of information is robust and it can be recalled after many days and weeks (Thellier et al. 2013, this volume). Studies of epigenetics demonstrate that information of stress received by plants can even be transferred to subsequent generations (Verhoeven et al. 2010).

Considering emergence of complexity we move on the paths from "plant to planet" (SCARANO and GARBIN 2013, this volume) or "holobiont to Gaia" (MATYSSEK and LÜTTGE 2013, this volume). This was already marked by SAUGIER (2013, this volume) in his earlier contribution to Section IV of the Symposium when he spoke of the global challenges of food supply for a world population growing from current 6.8 to future more than 9 billions of people and when he was advocating an "ecologically-intensive agriculture" (see also LÜTTGE 2013, this volume).

Looking at the planetary crisis, SCARANO arrives at a somewhat optimistic view by his discovery of highly unexpected plasticity illustrated by two fascinating examples of plants in Brazil, i.e. a hemi-epiphytic strangler of wet tropical rainforest colonizing dry and bare coastal sand plains, and a nitrogen-fixing species of tropical flood-forests rehabilitating solid industrial bauxite tailings burying a former Amazonian lake. Clearly such ecological plasticity is a result of epigenetics. However, it operates not only in times of development ("devo") and ecology ("eco") but also evolution ("evo"). In evolutionary terms this brings into play what GOULD (2002) calls "exaptation" supporting the GOULD/ELDREDGE concept of evolutionary punctualism. There are non-adaptive, i.e. effectively or nearly neutral features of organisms not directly built as adaptations for a current function, which were persisting the pressures of natural selection. Such features can have the quality of dormant co-options for utility. They can suddenly reveal evolutionary importance in occupying new niches or spandrels in GOULD's metaphor (see also MATYSSEK and LÜTTGE 2013, this volume). This widens the view of the planetary crisis to a more optimistic outlook, where SCARANO proposes the exciting new concept of "stem species" in analogy to "stem cells" as essential elements of effective ecological repair systems. With the stem species we can extrapolate the established concept of "evo-devo" with its step from genomics to epigenomics to an "evo-devo-eco" concept with a further step including environmental influences.

The vein of the subsequent contribution by MATYSSEK and LÜTTGE (2013, this volume) gives a less optimistic outlook. Considering geological times with environmental fluctuations leading to several waves of mass extinctions of organisms they do not find themselves in a position to exclude that the current wave of man-made accelerated mass extinction of species may take man himself away with it. The entire biosphere, as host also of man as one of its parts or guests and not its master, can be considered as a mega-holobiont (definitions see MATYSSEK and LÜTTGE 2013, this volume) or actually the supra-organism Gaia. Can Gaia as a self-organizing and self-sustaining mega-system still regulate and stabilize conditions where life is maintained without or perhaps also with man present in the future? This is the critical and somewhat pessimistic question asked. Is man given still a chance and is he going to make the right use of it?

With the new view of systems biology developed by the Symposium looking at growth and defense of plants with resource allocation at different scales we may have learned to make a comparison: What is the trade-off growth-defense for the plants appears to be the trade-off growth-survival for mankind. There is a lot of wishful thinking with a plethora of socio-political ideologies worldwide. We cannot afford this much longer with the challenge to avoid an apocalypse and to sustain the capability of Gaia to feed more than 9 billion people and support human life on the planet Earth (SAUGIER 2013, LÜTTGE 2013, MATYSSEK and LÜTTGE 2013, all this volume). Science and engineering provide far reaching capabilities for imaginative innovations. They also have the enormous responsibility to speak out and to severely press tribal, national and global politics to move away from the short-term return-based egoistic paradigm of acquisition to balanced allocation and partitioning using the terminology of the Symposium. The two institutions which have jointly supported this Symposium are strongly dedicated to this. The German Academy of Sciences Leopoldina in its foundation in the year 1652 has written in the demand of "naturae curiosorum" and is institutionally occupied with policy advice, public debate, and editing statements and recommendations. The Technical University of Munich (TUM) excels in technology motivated applied research on foundations of basic knowledge and understanding. Both cast bridges between scientific curiosity and existential requirements of man.

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Durch Lebensereignisse verbunden

Festgabe für Dorothea Kuhn zum 90. Geburtstag am 11. März 2013

Acta Historica Leopoldina Nr. 62
Herausgegeben von Jutta Eckle (Weimar) und Dietrich von Engelhardt (Karlsruhe)
(2013, 440 Seiten, 84 Abbildungen, 4 Tabellen, 26,95 Euro,
ISBN: 978-3-8047-3159-2)

Zum 90. Geburtstag von Dorothea Kuhn, der langjährigen Herausgeberin der Leopoldina-Ausgabe von Goethes Schriften zur Naturwissenschaft, legt die Leopoldina eine wissenschaftshistorische Festschrift vor. Neben dem Leben und Wirken der Jubilarin, das u. a. mit einer vollständigen Bibliographie gewürdigt wird, sind Beiträge namhafter Wissenschaftshistoriker und Germanisten aus den verschiedenen Interessengebieten der Geehrten versammelt: zu Naturwissenschaft und Medizin, Kunst und Philosophie um 1800, zu Goethes naturwissenschaftlichen Forschungen, zu Italienerlebnissen reisender Naturforscher, zur Verlagsgeschichte, vor allem des Cotta-Verlages, zur Editions- und Buchgeschichte sowie zur Akademiegeschichte.

Wissenschaftliche Verlagsgesellschaft Stuttgart

Ergebnisse des Leopoldina-Förderprogramms VII

Nova Acta Leopoldina N. F., Supplementum 26
Herausgegeben von Gunnar Berg (Halle/Saale), Andreas Clausing (Halle/Saale)
und Jörg Hacker (Halle/Saale – Berlin)
(2012, 184 Seiten, 95 Abbildungen, 21,80 Euro, ISBN: 978-3-8047-3061-8)

Die Deutsche Akademie der Naturforscher Leopoldina – Nationale Akademie der Wissenschaften bemüht sich mit ihrem Leopoldina-Förderprogramm um die Unterstützung von jungen herausragenden Wissenschaftlerinnen und Wissenschaftlern in ihrer beruflichen Weiterentwicklung durch einen Auslandsaufenthalt an einer weltweit führenden Forschungseinrichtung. Im Jahre 1996 als Projekt durch das Bundesministerium für Bildung und Forschung (BMBF) in Trägerschaft der Leopoldina eingerichtet und jährlich fortgeschrieben, wurde es 2009 Bestandteil des vom BMBF und vom Land Sachsen-Anhalt getragenen Haushalts der Akademie. Der vorliegende Band gibt einen Einblick in die Vielfalt und liefert Beispiele für die Projekte und erreichten Ergebnisse in den Jahren 2009 bis 2011. Damit werden Chancen und Ansprüche des Förderprogramms für künftige Bewerber deutlich.

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Altern in Deutschland

Die Deutsche Akademie der Naturforscher Leopoldina und die Deutsche Akademie für Technikwissenschaften acatech gründeten im Mai 2005 eine gemeinsame interdisziplinäre Akademiengruppe „Altern in Deutschland“, die auf der Grundlage der besten verfügbaren wissenschaftlichen Evidenz öffentliche Empfehlungen erarbeitete, um die Chancen der im letzten Jahrhundert erheblich gestiegenen Lebenserwartung – die „gewonnenen Jahre“ – vernünftig zu nutzen und mit den Herausforderungen des demographischen Alterns klug umzugehen.

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