Nova Acta Leopoldina NF *96*, Nr. 357, 7–13 (2009)

Imaging and Integrating Heterogeneity of Plant Functions: Functional Biodiversity from Cells to Biosphere – A Synopsis

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With 1 Table

Abstract

Spatial heterogeneity and temporal dynamics of structures and functions are essential to understand plants and their interaction with soils and atmospheric processes. Quantitative analysis plays an essential role for the generation of basic knowledge as well as for the development of innovative applications in plant production. Thus, novel imaging methods and quantitative image (sequence) analysis that are presently developed provide the basis for an entirely new understanding of the role of heterogeneity and dynamic in plants and their environment on all scales from the cell to the ecosystem. It is only now that we can see the real dynamic nature of growth, photosynthesis and transport and the relevance of spatial and temporal patterns for plants. Research on these topics has only just begun; it requires a very high degree of interdisciplinarity and communication between technology developers, researchers in plant and environmental sciences, modeling and theory. The Leopoldina workshop "***Imaging and Integrating Heterogeneity of Plant Functions: Functional Biodiversity from Cells to Biosphere***" has clearly indicated that the integration of modern methods, innovative experiments and theory will revolutionize our understanding of plants in their ever changing environment.

Zusammenfassung

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. Erst jetzt wird deutlich, wie dynamisch Wachstum, Photosynthese und Transport wirklich sind und welche Bedeutung räumliche und zeitliche Muster für Pflanzen haben. Die Forschung hierzu hat erst angefangen; sie benötigt ein hohes Maß an Interdisziplinarität und Kommunikation zwischen Entwicklern von Verfahren, Anwendern aus den Pflanzen- und Umweltwissenschaften sowie Modellierern und Theoretikern. Der Leopoldina-Workshop „***Imaging and Integrating Heterogeneity of Plant Functions: Functional Biodiversity from Cells to Biosphere***" hat gezeigt, 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.

1. Heterogeneity and Dynamics in Plants and their Environment

Heterogeneity and dynamic changes are the normal case and certainly not the exception for plants growing in natural ecosystems and even in managed agricultural environments. With their sessile mode of life, plants are exposed to ever changing weather conditions during all seasons. They are exposed to fluctuating biotic and abiotic stressors, as they simultaneously cope with greatly contrasting environmental conditions belowground and aboveground

(Tab. 1). Having no central control unit ("brain"), the organization of plants makes heterogeneous and distributed signal perception and processing a fundamental strategy. However, it is not only a matter of survival in a dynamically changing environment: proper mechanisms for utilization and acquisition of heterogeneously available resources from the environment are of central importance for the efficiency of plants and an important determinant of their fitness (Schurr et al. 2006). Thus, heterogeneity in space and time is a key to understanding plant structure and function (Walter et al. 2009).

Tab. 1 Characteristic features of leaf and root environment

	Leaf environment	Root environment
Resources	Light, CO_2, O_2	Nutrients, water
Physical characteristics	Low density Mechanical conditions mainly determined by plant characteristics	High density (often strong mechanical impedance) Strong external mechanical constraints
Chemical characteristics	Gas phase, thus well mixed phases; colloidal conditions	Mixture of gas, aqueous and solid
Biological conditions (biological interaction)	Exchange between biota over long distances possible	Biotic interactions often confined in externally determined spaces
Characteristics of dynamic and heterogeneity	No simple, predictable spatial structure of resources CO_2, O_2: well mixed Light: often highly fluctuating in time and space within natural canopies (e.g. light flecks)	Patchy, determined by soil characteristics Strong spatial heterogeneity of major resources is common

Up to now experiments have often aimed to maintain environmental conditions as constant as possible in space and time. However, novel experimental approaches and theoretical concepts are required in plant sciences to identify and observe heterogeneity and dynamics of structure and functions at the cell, tissue, organism and ecosystem levels, and to integrate and interpret these functions in the complex interaction with the spatial and temporal heterogeneity of the environment (Walter and Schurr 2006). Only if this is achieved, it is possible to understand and predict the feedbacks of living plant biota on the Earth system and to use the potential of plants for sustained production of the food, fuel and fiber needed by humankind in an optimal and efficient manner. Eventually, we will have to incorporate these insights into the design of plants through breeding or transgenic routes to obtain plant varieties better suited to the changed climate and shifting economic realities.

2. Imaging Technologies Promote Research on Heterogeneity and Dynamics of Plants

The general character of an image is that quantitative information of a specific physical parameter is assigned to a specific spatial position. Image sequences demonstrate the change of such assignments with time. Thus imaging and especially image sequences are tools of choice to evaluate heterogeneity, dynamic processes and patterns. Images can either be taken by arrays of sensors (e.g. the light sensitive elements of a CCD chip) or can be built up through a sequence of analyzing processes, if the structure or function that is determined is changing slower than the measurement process. Development of imaging techniques has a proven record of accelerating progress in botany and plant sciences from early times on, when plant images were used to illustrate plant structures, e.g. for purposes of plant systematic or for the analysis of cellular and subcellular processes associated with the various jumps in microscopy techniques from light and electron microscopy to confocal laser scanning and analytical microscopy. Historically, there have been many examples of image sequences that have become critically important for functional analysis in the plant sciences. However, the outburst of imaging technologies in recent years that have originated from the medical field and from monitoring of the environment, e.g. in remote sensing from satellites and airplanes (Pieruschka et al.: Optical Remote Sensing and Laser Induced Fluorescence Transients [LIFT] to Quantify the Spatio-Temporal Functionality of Plant Canopies) has inspired the development of novel imaging systems in plant sciences, opening new routes for insight into plant characteristics and their dynamic responses.

For example, **tomographic imaging** using differences in magnetic resonance (mainly of protons) to give contrast and thus deliver non-invasive 3D images and -sequences has only recently been assimilated in the plant sciences and promises a wide range of applications in the future. The paper by Blümler et al. (Magnetic Resonance of Plants) provides a sound basis for understanding opportunities and limitations of this technology as well as some impressive examples of what can already be achieved. At a different scale, **quantitative 3D imaging** provides a tool to measure plant and stand geometrical characteristics (Biskup et al.: Quantification of Plant Surface Structures from Small Baseline Stereo Images to Measure the Three-Dimensional Surface from the Leaf to the Canopy Scale) and illustrates the approach that quantitative image analysis goes beyond providing “nice pictures”. Given that the correct mathematical tools are combined with proper image acquisition and analysis in measuring devices, it becomes possible to span a wide range of scales in space and time.

Recording chlorophyll fluorescence has become one of the most powerful tools in studying heterogeneity and dynamics of photosynthesis. Most interestingly in the context of this issue of *Nova Acta Leopoldina* the greatest information content is gained from analyzing the change of chlorophyll fluorescence over time – an approach, which became generally feasible with the development of the Pulse Amplitude Modulated Fluorometry as a non-invasive quantitative measure that has been extended into an imaging technology (Schreiber et al. 1995, Siebke and Weis 1995). Further developments extending the application of chlorophyll fluorescence to the scales of canopies and beyond are on their way (Pieruschka et al.: Optical Remote Sensing and Laser Induced Fluorescence Transients [LIFT] to Quantify the Spatio-Temporal Functionality of Plant Canopies). These technological developments push for the analysis of photosynthesis dynamics and heterogeneity under field conditions and at the stand level. Here additional mechanisms come into play and determine light use efficiency of canopy photosynthesis. Moreover through the technological approach of analyzing chlorophyll fluorescence from above even previously not accessible locations (and thus functions) can be explored.

3. Heterogeneity of Photosynthesis – a Prototypic Example of the Role of Spatial and Temporal Organization in Plants

Photosynthesis is one of the most central processes for all life on earth and one of the most well studied plant functions. Characteristically the three known modes of photosynthesis are different in their spatial and temporal organization. The most common C_3 photosynthesis is located in all cells of the palisade and spongy parenchyma of the plants expressing this form of photosynthesis. In contrast, plants running CAM photosynthesis are characterized by the defined temporal sequence of storage and release of carbon intermediates throughout the diel cycle. However, heterogeneity of CAM photosynthesis also has a whole plant aspect that is dependent on the developmental stage and linked all the way back to the evolutionary position of the species. Thus, CAM photosynthesis has been developed in recent years into one of the most prominent examples, in which the combination of chlorophyll fluorescence imaging, and theoretical modeling guided by destructive sampling leads to a mechanistic understanding of the role of functional heterogeneity in these plants (Lüttge: Crassulacean Acid Metabolism a Natural Tool to Study Photosynthetic Heterogeneity in Leaves).

The biochemical heterogeneity and spatial separation of the components of the CO_2-concentrating mechanisms of C_4 photosynthesis has long challenged our ability to offer tight interpretations of the evolution of this functional biodiversity in the face of selective pressures in different environments. Imaging in combination with growth of plants in controlled environmental conditions has offered yet another scale of opportunities for analysis of photosynthetic heterogeneity at the cellular and biochemical level in relation to optimization of light and nitrogen use efficiency (von Caemmerer and Osmond: Testing the Functional Implications of Photosynthetic Heterogeneity in Leaves of C_4 plants: Reductionism during Scale Expansion). These studies confirm that anatomical and biochemical heterogeneity among C_4 types seem to deliver an array of compensating responses that confer a similar and robust, leaf-level advantage with respect to water and nutrient economy. The heterogeneity presumably reflects the independent origins of the C_4 pathway in different plant taxa. Other processes, unrelated to the pathway itself, may be responsible for distinctive patterns of distribution of the differing biochemical C_4 type grasses in relation to precipitation. Thus heterogeneity of biochemical characteristics in plants can deliver common outcomes that contribute to evolutionary fitness in broad terms, whereas other “higher order” structural and developmental properties might determine plant distribution and abundance in vegetation.

Homogenization of spatially distinct responses of photosynthesis could happen through the lateral diffusion of carbon dioxide within open gas spaces inside of leaves. However, such lateral transport may be strongly restricted and has most prominent effects, when stomata are closed and thus internal transport in leaves can play a significant role over the exchange through stomata (Jahnke and Pieruschka: Lateral Gas Diffusion inside Leaves: a Long Neglected Topic in Plant Physiology). Nevertheless, in homobaric leaves local differences in photosynthesis and respiratory activity due to localized metabolic action driven by environmental (e.g. light gradients) or plant-internal heterogeneity can cause carbon dioxide fluxes that may contribute to fitness under transitory drought or variable light regimes.

In nature biological fitness is undoubtedly related to the ability of plants to defend them against biotic attack. While plants defense has a local character to restrict negative impact to as little area as possible, other mechanisms alert other regions to prepare for potential attack (Conrad et al. 2002). This example illustrates that communication between heterogeneously responding parts of tissues and plants is essential to optimize plant performance when challenged by spatially and temporally variable environmental cues.

4. Dynamics and Heterogeneity of Plant Growth and Transport

Sensing of heterogeneous environmental conditions requires highly responsive sensory elements. Within the highly spatially organized soil environment (Tab. 1) the root apex can be shown to be very receptive to environmental variation. The root apex hairs can be used as a model system to understand the interaction between plant polarity, cell-cell communication, and sensory plant biology (Baluška et al.: Intracellular Domains and Polarity in Root Apices: from Synaptic Domains to Plant Neurobiology). Clearly the polarity and the dynamics of elements of the cytoskeleton in the root apex – as integral parts of the sensory system of cells – are linked to identifying and communicating environmental changes to the plant. Different parts of the root growth zone show distinct sensitivity to environmental impact and these functions correlate with the cellular and tissue configuration and also resemble their exchange activity with the surrounding soil. It is tempting to compare these sensory functions of the different parts of growing tissues with sensory elements in animals, even though the basic mechanisms are quite distinct and include oriented transport of plant signaling substances.

Finely tuned signaling is also required for the integration of various environmental factors and resource availability with the plant-internal organization of the dynamic of growth. Growth itself is a highly dynamic process and is organized in characteristic spatial and temporal modi (Walter et al. 2009). As growth is the endpoint of a sequence of processes and dependent on the availability of many resources it integrates external impact as well as internal mechanisms (Walter: Leaf Growth Dynamics). The results clearly identify endogenously triggered temporal changes – so-called “endogenous rhythms” – as important nominators of aboveground growth control in dicots to override fast changes in aboveground environmental cues. Externally triggered alterations of growth, e.g. via changing light intensity, show a characteristic, non-linear relation between effector and reaction. This is contrasted by the root growth control, which is governed strongly by long-distance transport of carbon and which is linked more directly to environmental patterns. Such progress has only been possible through the development of suitable imaging tools to analyze growth of leaves and roots with high spatial and temporal accuracy. The heterogeneity of resource availability to growing tissues can even be traced back months after growth has ceased (Gloser et al.: Shoot Heterogeneity in Trees: Consequences of Patchy N Availability and Vascular Transport). It is obvious that even long-lived trees do not have mechanisms to homogenize imbalances of nitrogen distributions that have been laid down during growth. This links the patchy distribution of nutrients in the soil with the heterogeneity of aboveground composition.

5. Modeling and Theory – Towards Understanding of the Role of Spatial and Temporal Heterogeneity

The role of heterogeneities at various levels has been a major focus and challenge of the workshop covered by this issue of *Nova Acta Leopoldina*. Aspects of non-linearities and scaling problems gain a special importance in the context of heterogeneities. Here significant input from theory is required in order to allow adequate up- and downscaling of heterogeneous processes and structures. This will be crucial for understanding the role of heterogeneity at larger scales (upscaling) and the composition of effective quantities at larger scales from local and temporally variable parameters.

Multilevel and integrated modeling is necessary to obtain a set of conclusive interpretations of ecophysiological data and interpretative models (Bohn: Integrative Computational Approaches to Complex Ecophysiological Systems). Adequate interaction between experimentalists and modelers – as illustrated in the successful analysis of CAM photosynthesis as well as of heterogeneity in biofilms – is the basis for rapid and consistent progress on sound experimental basis and on valid models. This challenge goes beyond interpretation of heterogeneity and dynamics in plants, but is a general requirement for systems biology, in which complex and huge datasets need to be extracted to gain relevant information.

The many examples of heterogeneity in plant structure and function demonstrated in the workshop and this issue of *Nova Acta Leopoldina* indicates patterns rather than stochastic fluctuations. Self-organization and decentralized signal perception and processing are crucial mechanisms in plants. Local neighborhood relations and regulatory loops play an important role in establishing such patterns. Through advancements in the theory of pattern recognition and the integration of self-organization spatio-temporal patterns in biological systems in general can be approached by pattern analysis (Geberth et al.: Systematics of Spatiotemporal Heterogeneity – Regulation of Large-scale Patterns by Biological Variability). Biological mechanism form deterministic structures that distinguish pattern formation in plants from pure physical processes – a feature that can be exploited to collect additional information about the biological system.

While heterogeneity is a common feature from the molecular to the ecosystem level, the individual structural and functional elements are often not randomly organized but rather in patterns. Lüttge and Hütt (Talking Patterns: Communication of Organisms at Different Levels of Organization – an Alternative View on Systems Biology) feature the role of communication and signaling at all scales for the development of such patterns. The identification of universal rules integrating heterogeneity across many scales is one of the most outstanding roles of theory for the biological sciences, as it identifies common principles in living systems and (eco-)systems shaped by them.

6. Future Prospects of Imaging and Integrating Heterogeneity of Plant Structure and Function

Already in May 2002 the Leopoldina had addressed this important topic in a workshop at Darmstadt on „Nonlinear Dynamics and the Spatiotemporal Principles of Biology“ (*Nova Acta Leopoldina* NF *88*, No. 332). The Jülich workshop (July 2007) has indicated the significant development that this field has taken already within this relatively short period of time. This workshop, as illustrated by the diverse contributions to this issue of *Nova Acta Leopoldina*, made clear that different communities have used different approaches to analyze and gain new insight from imaging approaches. Still there are major gaps in communication between disciplines like remote sensing and plant physiology, but concepts and approaches from each discipline will proof to be valuable in the future in other field and scales. A major challenge remains the lack of communication and common language between disciplines. Here the workshop provided a first step and a strong need was seen to continue trans disciplinary approaches in research but also in education. On such a basis significant progress will be made in the future in the quantification of a large variety of key processes in plant sciences at various scales and temporal frequencies – illustrating the importance of spatial and temporal heterogeneity for plant performance in a changing environment as well as being an important characteristic of the strategy of plants to establish efficient mechanisms due to their sessile life form. The increasingly available technologies and the development of proper concepts will allow taking the next steps – namely the linking of processes and scales to understand plant behavior at a holistic level. This will allow better understanding of plants in the environment for urgent problems in general and for processes that explicitly are characterized by environmental cues changing in space and time like climate change. In addition this will help open new routes for application in breeding (phenotyping) as well as in agricultural practice and land management (precision agriculture and remote sensing).

References

Conrad, U., Pieterse, C. M. J., and Mauch-Mani, B.: Priming in plant-pathogen interactions. Trends Plant Sci. *7*, 210–216 (2002)

Schreiber, U., Bilger, W., and Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.-D., and Cadwell, M. M. (Eds.): Ecophysiology of Photosynthesis; pp. 49–70. Berlin, Heidelberg, New York: Springer 1995

Schurr, U., Walter, A., and Rascher, U.: Functional dynamics of plant growth and photosynthesis – from steady-state to dynamics – from homogeneity to heterogeneity. Plant Cell Environ. *29*, 340–352 (2006)

Siebke, K., and Weis, E.: ‘Assimilation images’ of leaves of *Glechoma hederacea*; analysis of non-synchronous stomata related oscillations. Planta *196*, 155–165 (1995)

Walter, A., Silk, W. K., and Schurr, U.: Environmental effects on spatial and temporal patterns of leaf and root growth. Annu. Rev. Plant Biol. *60*, 279–304 (2009)

Walter, A., and Schurr, U.: Botanical Briefing: Dynamics of leaf and root growth: endogenous control versus environmental impact. Ann. Bot. *95*, 891–900 (2005)

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