

Editorial

Cutting-edge international tree research in *New Phytologist*

'A tree is a tree – how many more do you need to look at?' (R. Reagan, 1966)

New Phytologist publishes top-quality plant science of international relevance and significance. The broad scope of the journal is made more manageable by classifying four sections of research – Environment, Interaction, Evolution and the newly launched Physiology & Development (Hetherington & Slater, 2007). These sections provide a familiar order of research articles in each issue. However, it is always worth taking some time to scan through a few issues to look at other ways that the science we publish can be classified. For example, while working through the issues of 2006, a clear natural classification emerged in terms of research on trees. The research is international and global in extent, as shown on the accompanying map (Fig. 1), bringing many different tools and approaches to organisms that play a major role in global cycles of carbon and water. It is interesting to look at these approaches and the questions posed by researchers.

The quote by Reagan describes the undoubted truth that a tree is instantly recognizable, with a woody trunk branching out to carry many leaves. This hides a whole suite of varying processes by which trees grow, develop and accumulate resources. Best hidden are the below-ground mycorrhizal associations. Mycorrhizal uptake of phosphorus and nitrogen complements the tree's more limited capacity for uptake on nutrient-limited soils. This capacity distinguishes life and death for young tree seedlings on Mount Fuji's 'sea of volcanic desert' in Japan (Nara, 2006; map reference 8). In established woodland, nutrients may be transferred between different tree species by the mycorrhizal network (X. H. He *et al.*, 2006; map reference 3). The composition of the mycorrhizal fungal community also changes in response to variations in soil nutrients, even over small distances (Toljander *et al.*, 2006; map reference 15), indicating a complex and dynamic interplay with the tree host and soil. The masting habit in certain

Cameroonian rainforest trees is strongly correlated with the ectomycorrhizal habit and probably associated with the resulting enhanced phosphorus supply (Newbery *et al.*, 2006; map reference 10). Ectomycorrhizas are characteristic of cool forests, while arbuscular mycorrhizas are more abundant in tropical forests (Alexander, 2006). However, this is not exclusively the case, as in the Cameroonian example and, as reported for the first time, in the Dipterocarpaceae of Venezuela (Moyersoen, 2006; map reference 2). Ectomycorrhizas are considered younger in evolutionary terms than arbuscular mycorrhizas, with the oldest fossil ectomycorrhizas only dating from 50 million years ago (Alexander, 2006). Moyersoen (2006) argues that the occurrence of ectomycorrhizas on the Dipterocarpaceae of Venezuela indicates an origin before the break-up of Gondwana and the separation of South America and Africa, about 135 million years ago.

The activity of the tree root may be seen as rather secondary to that of mycorrhiza, particularly when considering nutrient acquisition on poor soils. However, plant survival in saline soils is heavily dependent on the capacity of roots either to exclude or to regulate sodium and chloride ion uptake. The significant risk of seawater intrusion into coastal areas of southwest Australia led to experiments to test for the capacity of native plant species to tolerate the joint environmental problems of salinity and waterlogging (Carter *et al.*, 2006; map reference 12). Large differences in sodium and chloride ion exclusion were observed between the saline and waterlogging-tolerant *Melaleuca cuticularis* and the intolerant *Banksia attenuate*, indicating the potential for significant change in community structure with seawater intrusion. Genetic differences in performance are also found within tree species and such differences are at the forefront of identifying new genotypes that are productive under changing climatic conditions, in particular enhanced frequencies of drought. Species of *Populus* are very fast-growing and suitable for the industrial production of biofuels and biomass. Monclus *et al.* (2006; map reference 14) demonstrated that the most productive genotypes of *Populus* hybrids were the most drought-susceptible, and vice versa. So a future of increasing drought would have a negative impact on nonirrigated productivity. However, one out of 29 different genotypes retained high productivity with drought tolerance and provides a model for investigating the different attributes that maintain productivity and tolerance.

Although the majority of tree biomass is in the trunk and branches, it is the low-biomass, short-lived leaves and roots that determine productivity and environmental tolerance. This was clearly seen for the different *Populus* genotypes. The recognition that particular leaf traits have characteristic

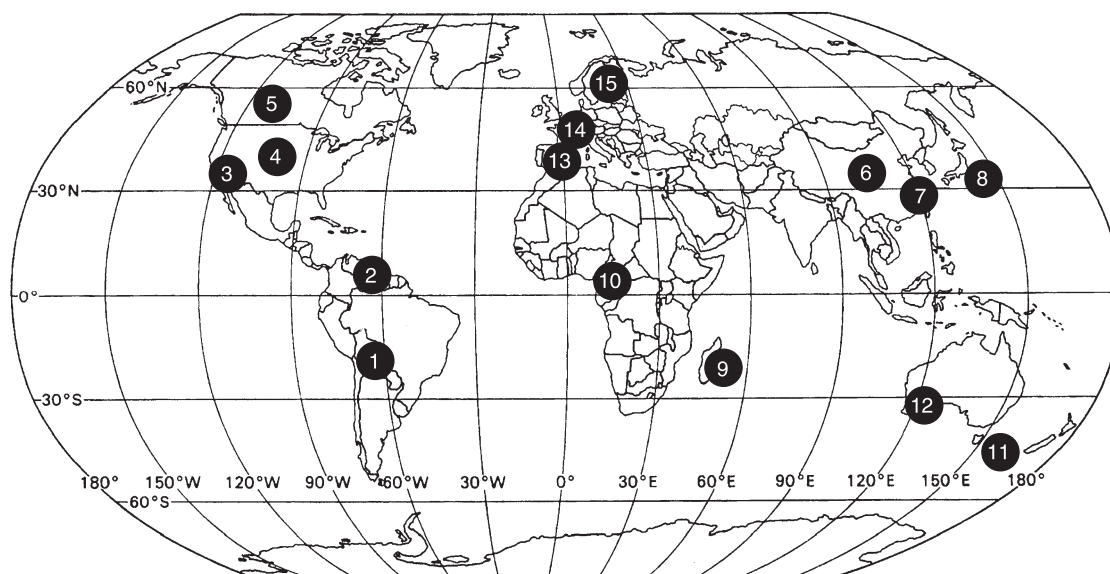


Fig. 1 Locations of some of the research published on trees in *New Phytologist* in 2006. Numbers refer to publications as follows: 1, van Gelder *et al.*; 2, Moyersoen; 3, X. H. He *et al.*; 4, Zarter *et al.*; 5, Michaletz & Johnson; 6, J. S. He *et al.*; 7, Chen & Xu; 8, Nara; 9, Chapotin *et al.*; 10, Newbery *et al.*; 11, Hovenden & Vander Schoor; 12, Carter *et al.*; 13, Filella *et al.*; 14, Monclus *et al.*; 15, Toljander *et al.*

correlations with the environment (Wright *et al.*, 2005) has encouraged field surveys to quantify and search for generalities in correlations between the environment and traits such as specific leaf area, nitrogen concentration and stomatal density. A very extensive survey of trees and herbs from the Tibetan Plateau (J. S. He *et al.*, 2006; map reference 6) confirmed previous observations, such as increasing photosynthetic rate with nitrogen concentration and low nitrogen concentrations with high leaf mass per area (low specific leaf area) as found in other parts of the world (Wright *et al.*, 2005). However, over a study area of about 0.5×10^6 km², the modulation of these characteristics by climate was unusually weak. By contrast, leaves of *Nothofagus cunninghamii* respond strongly to the changes in climate with altitude (Hovenden & Vander Schoor, 2006; map reference 11), in particular with changes in specific leaf area and stomatal density. It was notable that the altitude of origin of plant material also exerted a marked influence on leaf responses under controlled-environment experiments.

The general nature of leaf trait relationships hides the capacity of the photosynthetic system to respond markedly to changing environments during the life of a leaf. Subalpine conifers completely down-regulate photosynthesis during winter (Zarter *et al.*, 2006; map reference 4). The complex structure of conifer foliage also leads to leaves and buds experiencing temperatures very different from air temperature and standard engineering theory, and with great sensitivity to irradiance and wind (Michaletz & Johnson, 2006; map reference 5). Spring then becomes a crucial time for up-regulation of photosynthesis in the overwintered conifer leaves. The tree is then responding to multiple signals from a

thawing soil, increasing irradiance and temperature, but with the threat of frost present well into the growing season. Observations on fir, spruce and pine indicated that trees respond quickly to day-to-day variations in the environment, integrating responses to multiple environmental signals. Species differences in photosynthetic responses to rapid environmental change have been observed in mulberry and ginkgo (Chen & Xu, 2006; map reference 7). In mulberry a transition from saturating to light-limited photosynthesis leads to some dissociation, which is slowly reversible, of the light-harvesting antenna from photosystem II, while in ginkgo no such effect was observed. These differences will prove critical in natural environments with widely varying irradiance, such as under tree canopies. Leaves are subjected to herbivory and environmental changes, such as fluctuating ozone concentrations and reduced water supply from leaves during drought. Trees integrate their responses to these challenges by changing the production and movement of signalling molecules such as jasmonic acid (JA). Spraying *Quercus ilex* trees with JA (Filella *et al.*, 2006; map reference 13) induced reductions in photosynthesis and stomatal conductance within 24 h, in addition to stimulating the emission of volatile organic compounds.

Trees demonstrate many different responses to changing environments, but one of the strangest is seen in many tropical trees from seasonally dry environments. In these environments a new flush of leaves can occur before the end of the dry season and before the onset of the rains. In Madagascar, the baobab tree has a full crown of leaves several weeks before the rainy season (Chapotin *et al.*, 2006; map reference 9). The baobab tree is famous for its huge girth and

stem succulence and so it has been suggested that precocious leaf flushing could occur using water reserves from the stem and allow the plant to be photosynthetically active before the rains arrive, providing a potential competitive advantage over other species. Chapotin *et al.* (2006) demonstrated quite clearly that stored water reserves are used to support the growth of new leaves but only the inevitable and small losses of water through the leaf cuticle. Stomatal opening and significant photosynthesis only occur after significant rainfall.

Looking at a baobab and its response to the environment clearly indicates that one tree does not reveal all that is needed to be known for all trees. A walk in a tropical rainforest demonstrates that even looking at the shape of different trees can indicate much about their engineering properties (van Gelder *et al.*, 2006; map reference 1). Tall trees have lower safety factors for bucking and bending, while the opposite is the case for trees with large diameters. Taking a sample of wood to determine density provides a correlate of shade tolerance and life history. Dense wood is characteristic of shade-tolerant late successional species. Weak wood is characteristic of shorter-lived pioneer species.

The map in Fig. 1 indicates just a selection of the research on trees that has been published in *New Phytologist*. A global view has been necessary both to include features that could not be observed at one location and to provide an increasingly complex view and understanding of the ways in which trees accommodate to their environment and their structures.

We are providing open access to the 15 papers that have been highlighted in this editorial, so that you can enjoy the breadth and depth of just some of the research on trees that was published in 2006.

F. I. Woodward
Editor-in-Chief
H. Slater
Managing Editor

References

- Alexander IJ. 2006. Ectomycorrhizas – out of Africa? *New Phytologist* 172: 589–591.
- Carter JL, Colmer TD, Veneklaas EJ. 2006. Variable tolerance of wetland tree species to combined salinity and waterlogging is related to regulation of ion uptake and production of organic solutes. *New Phytologist* 169: 123–133.
- Chapotin SM, Razanameharizaka JH, Holbrook NM. 2006. Baobab trees (Adansonia) in Madagascar use stored water to flush new leaves but not to support stomatal opening before the rainy season. *New Phytologist* 169: 549–559.
- Chen Y, Xu DQ. 2006. Patterns of leaf photosynthetic response to irradiance transition from saturating to limiting one in some plant species. *New Phytologist* 169: 789–797.
- Filella I, Penuelas J, Llusia J. 2006. Dynamics of the enhanced emissions of monoterpenes and methyl salicylate, and decreased uptake of formaldehyde, by *Quercus ilex* leaves after application of jasmonic acid. *New Phytologist* 169: 135–144.
- van Gelder HA, Poorter L, Sterck FJ. 2006. Wood mechanics, allometry, and life-history variation in a tropical rain forest tree community. *New Phytologist* 171: 367–378.
- He XH, Bledsoe CS, Zasoski RJ, Southworth D, Horwath WR. 2006. Rapid nitrogen transfer from ectomycorrhizal pines to adjacent ectomycorrhizal and arbuscular mycorrhizal plants in a California oak woodland. *New Phytologist* 170: 143–151.
- He JS, Wang ZH, Wang XP, Schmid B, Zuo WY, Zhou M, Zheng CY, Wang MF, Fang JY. 2006. A test of the generality of leaf trait relationships on the Tibetan Plateau. *New Phytologist* 170: 835–848.
- Hetherington AM, Slater H. 2007. *New Phytologist* on 'Physiology & Development'. *New Phytologist* 173: 1–2.
- Hovenden MJ, Vander Schoor JK. 2006. The response of leaf morphology to irradiance depends on altitude of origin in *Nothofagus cunninghamii*. *New Phytologist* 169: 291–297.
- Michaletz ST, Johnson EA. 2006. Foliage influences forced convection heat transfer in conifer branches and buds. *New Phytologist* 170: 87–98.
- Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Thiec D, Brechet C, Brignolas F. 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. *New Phytologist* 169: 765–777.
- Moyersoen B. 2006. *Pakaraimaea dipterocarpacea* is ectomycorrhizal, indicating an ancient Gondwanaland origin for the ectomycorrhizal habit in Dipterocarpaceae. *New Phytologist* 172: 753–762.
- Nara K. 2006. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist* 169: 169–178.
- Newbery DM, Chuyong GB, Zimmermann L. 2006. Mast fruiting of large ectomycorrhizal African rain forest trees: importance of dry season intensity, and the resource-limitation hypothesis. *New Phytologist* 170: 561–579.
- Toljander JF, Eberhardt U, Toljander YK, Paul LR, Taylor AFS. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytologist* 170: 873–883.
- Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Garnier E, Hikosaka K, Lamont BB, Lee W, Oleksyn J, Osada N, Poorter H, Villar R, Warton DI, Westoby M. 2005. Assessing the generality of global leaf trait relationships. *New Phytologist* 166: 485–496.
- Zarter CR, Demmig-Adams B, Ebbert V, Adamska I, Adams WW III. 2006. Photosynthetic capacity and light harvesting efficiency during the winter-to-spring transition in subalpine conifers. *New Phytologist* 172: 283–292.

Key words: environmental change, evolution, leaf trait, mycorrhiza, photosynthesis, physiology and development, poplar, tree research.

Commentary

Towards a rhizo-centric view of plant-microbial feedbacks under elevated atmospheric CO₂

The stimulatory effects of elevated CO₂ on plant productivity have been reported for many ecosystems (Ainsworth & Long, 2005), but whether such effects will persist in the face of increasing nutrient limitation is unclear. In nitrogen (N)-limited ecosystems, elevated CO₂ has been hypothesized to decrease nutrient availability as the N becomes sequestered in plant and soil pools with slow turnover rates. Alternatively, an elevated CO₂ may increase nutrient availability by stimulating N release from soil organic matter (SOM), resulting in positive feedbacks to primary production. Previous reports on the effects of elevated CO₂ on N cycling have been variable, with reports of increases, decreases or no change in soil N dynamics under elevated CO₂ (Zak *et al.*, 2000). One major source of uncertainty is the degree to which potential changes in root-derived C affect the microbial regulation of soil N availability. In this issue of *New Phytologist* (pp. 778–786), de Graaf *et al.* describe a novel approach to examining the effects of elevated CO₂ on root-derived inputs of N to soil. Their results support an emerging ‘rhizo-centric’ view, whereby root–microbial interactions may be the central processes in controlling the magnitude and duration of plant productivity responses under elevated CO₂.

‘... roots and rhizosphere microbes play a more important role than has been previously considered in mediating soil N availability under elevated CO₂.’

By labeling plants with ¹⁵N via foliar uptake, de Graaf *et al.* quantified the magnitude and fate of N from rhizodeposition in wild and cultivated genotypes of wheat and maize exposed to ambient and elevated levels of CO₂. Their study reported that rhizodeposition was a strong sink for

foliar-applied N in all plants (5–10% of the total uptake from leaves), and that elevated CO₂ increased this flux in those plants that also increased in total biomass (e.g. wheat but not maize). Moreover, this study reports that CO₂-induced increases in rhizodeposition decreased soil N availability, as greater amounts of root-derived N were immobilized in the rhizosphere of the labeled plants and lesser amounts were taken up by unlabeled, ‘receiver’ plants growing in the same pots.

Increased C fluxes from roots to soil under elevated CO₂ have been reported previously but the consequences of such changes for soil N cycling are unclear (Cheng, 1999). de Graaf *et al.* suggest that greater N immobilization by rhizosphere microbes and decreased N uptake by receiver plants are evidence of enhanced N limitation. However, an alternative interpretation of this is that plants grown under elevated CO₂ retain a greater proportion of rhizodeposited N within their rhizosphere. This could be accomplished through CO₂-induced increases in rhizosphere microbial biomass as a result of increased root exudation. Such immobilization would not necessarily represent a major loss of N from an individual plant because much of the N would be available for subsequent uptake due to the rapid turnover of the rhizosphere microbial biomass (Paterson, 2003). Furthermore, the loss of root-derived N would likely be minor relative to potential N gains from increased root growth and/or root-induced stimulation of decomposition (Cheng & Kuzyakov, 2005). Both hypotheses are consistent with the data, and suggest that roots and rhizosphere microbes play a more important role than had previously been thought in mediating soil N availability under elevated CO₂.

Rhizosphere feedbacks under elevated CO₂

Previous conceptual models of plant–microbial interactions under elevated CO₂ have focused on bulk soil processes (Fig. 1a). Although such models are appropriate for understanding ecosystem responses in the long term, they do not consider how spatially and temporally dynamic processes occurring in the rhizosphere can influence ecosystem response to elevated CO₂. This may explain, in part, why several studies have been unable to account for CO₂-induced increases in N in ecosystem budgets (Johnson, 2006). At the Duke Forest free-air carbon dioxide enrichment (FACE) site (North Carolina, USA), the N content of the canopy trees has increased in response to elevated CO₂ despite there being no evidence of increased net N mineralization rates in the soil (Finzi *et al.*, 2006). Because net N mineralization is measured in the absence

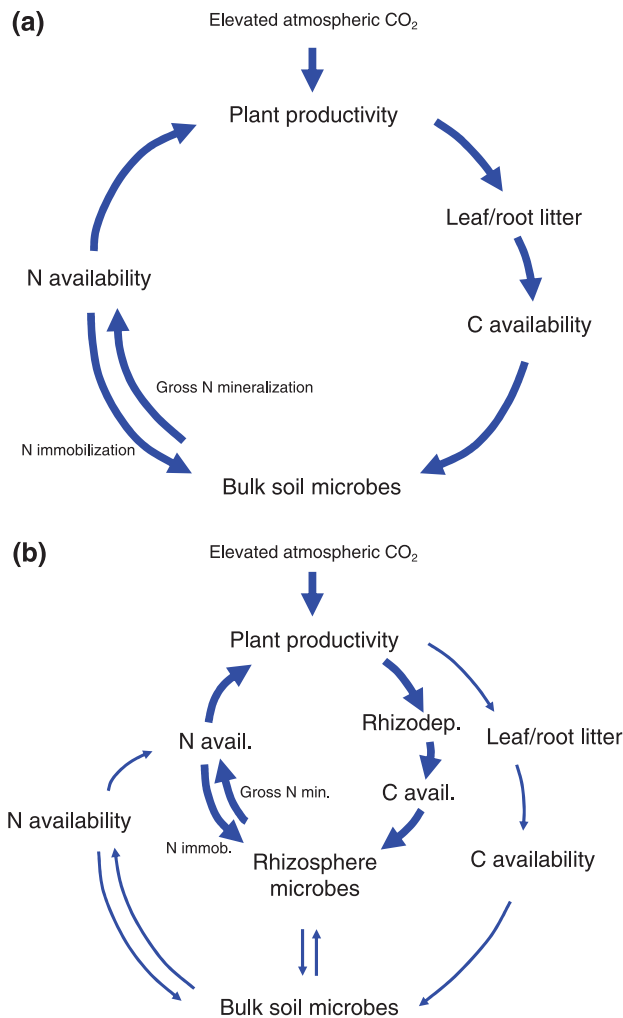


Fig. 1 Conceptual models of plant-microbial feedbacks under elevated CO₂. Previous conceptual models (a), emphasize bulk soil processes (after Zak *et al.*, 2000). The newly proposed rhizo-centric model (b), considers the importance of rhizosphere processes.

of roots, it is plausible that the unaccounted-for canopy N is derived from enhanced N availability due to rhizosphere processes. A more rhizo-centric view would account for how changes in the intensity of root–microbial interactions under elevated CO₂ affect soil N availability and feedbacks to plant productivity (Fig. 1b).

The increased importance of rhizosphere processes under elevated CO₂ results from both increased rhizodeposition and changes in rhizosphere N availability. Elevated CO₂ can increase rhizosphere C flux through increases in fine root biomass (Norby *et al.*, 1987; Uselman *et al.*, 2000) and/or increases in mass-specific exudation (Phillips *et al.*, 2006). Moreover, elevated CO₂ may also induce changes in the chemical composition of exudates (Hodge & Millard, 1998; Phillips *et al.*, 2006). Will such CO₂-induced changes in the quantity and chemical quality of exudates influence the microbial processing of soil N? Most root exudates are low molecular weight organic compounds (sugars, amino acids, organic acids) that have traditionally been viewed in light of their effects on P and Fe availability (Marschner, 1995). However, exudates are also the preferred substrates for the rhizosphere microflora (Cheng, 1999), and the rapid assimilation of exudates creates a ‘rhizosphere effect’ around roots where the tight coupling between substrate availability and soil microbial activity is likely to influence soil N availability (Paterson, 2003).

Rhizosphere effects on soil N availability

There are several ways in which roots may increase soil N availability under elevated CO₂ (Table 1). First, rhizodeposition may stimulate decomposition through priming effects (Cheng & Kuzyakov, 2005). This has the potential to dramatically increase soil N availability because of the large size of the N pool in SOM. Second, increased C allocation to roots and mycorrhizal fungi under elevated CO₂ could increase N availability through increased foraging in soil. Moreover, mycorrhizal fungi (and some plants) may increase N availability through the uptake of organic N (Jones *et al.*, 2005). A third rhizosphere process which may provide a

Table 1 Potential rhizosphere effects on soil N availability under elevated CO₂

Rhizosphere process	Mechanism	Effects on N*
Exudate-induced decomposition	Increased SOM decomposition due to priming of rhizosphere microbes	++
Mycorrhizal growth	Increased soil exploration, exo-enzyme activity, organic N acquisition	++
Fine root growth	Increased soil exploration; expansion of rhizosphere extent	+
Associative N fixation	Increased fixation due to high C availability and low O ₂ potentials	+
Grazing of rhizosphere microbes	Increased release of NH ₄ via the microbial loop	+
Release of novel compounds	Increased/decreased N due to exudate effects on specific microbial taxa; decreased root competition from allelochemical release	+ or –
Root allocation of metabolites	Increased N immobilization due to higher C : N of root exudates	–
Rhizosphere denitrification	Increased N loss due to high C availability and low O ₂ potentials	–

*Indicates the magnitude and direction of change (increase +; decrease –) in soil N availability resulting from each process.

new source of N under elevated CO₂ is associative N fixation. Although there have been few reports of increased fixation under elevated CO₂, increased rates may occur in the rhizosphere where enhanced substrate availability and low O₂ potentials may provide more favorable conditions than in the bulk soil (Dakora & Drake, 2000).

In addition to accessing new sources of N, rhizosphere processes may accelerate or slow-down N turnover under elevated CO₂ (Table 1). The grazing of rhizosphere microflora by soil fauna may accelerate N turnover if increased rhizodeposition stimulates rhizosphere microbes and NH₄ release through the microbial loop (Bonkowski, 2004). Second, the production of novel compounds under elevated CO₂ could increase or decrease N availability if certain microbial taxa with specific enzymatic capabilities are affected. Finally, CO₂-induced increases in the C : N of rhizodeposits may decrease N availability if the roots allocate more C-rich or less N-rich metabolites to root secretions (Paterson, 2003).

Unresolved questions and future research needs

Our present understanding of how rhizosphere processes will affect feedbacks to plant productivity under elevated CO₂ is constrained by the lack of appropriate methods. Most studies of CO₂ effects on root-derived C have been conducted with small plants growing in an artificial medium (Grayston *et al.*, 1996), and most studies of CO₂ effects on N mineralization have been conducted in soil cores from which the roots have been excluded (Zak *et al.*, 2000). Thus, a fundamental challenge in adopting a more rhizo-centric view is that there are few good methods for quantifying rhizosphere processes in intact root–soil systems. This is especially true in the case of root exudation, which has rarely been measured *in situ*. Such a knowledge gap has limited our understanding of some very basic questions. For example, what are the effects of a root's age, diameter, order or mycorrhizal status on the quantity and chemical quality of exudates? How do abiotic factors such as soil temperature, moisture and fertility affect exudation? Although recent reviews have highlighted our progress in understanding controls on exudation (Jones *et al.*, 2004), more efforts are needed to develop field-based approaches, with the larger goal of integrating the results from field observations with those from highly controlled experimental systems (e.g. growth chambers, FACE sites).

Similarly, relatively few studies have examined the effects of exudate chemistry on soil N transformation rates under rhizosphere-relevant conditions. For example, how much C is needed (and in what chemical form) to stimulate net N mineralization? Does the response depend on the physical, chemical, or biotic properties of the soil? A future research priority should be to develop methods which can better simulate the rhizosphere environment in order to provide a more mechanistic understanding of the fate of root exudates in soil under realistic conditions.

An emerging view in elevated CO₂ research is that root–microbial interactions are likely to play an increasingly important role in controlling ecosystem-scale responses to global change. This would argue for a more rhizo-centric view of the interactions between plants and soil microbes, and a better understanding of how roots influence soil N availability. Because today's rhizosphere is yesterday's (and tomorrow's) bulk soil it is critical to integrate rhizosphere mechanisms into models of bulk soil processes in order to better understand the long-term response of ecosystems to global change.

Richard P. Phillips

Department of Biology, Box 90340, Duke University,
Durham, NC 27705, USA
(tel +1 919 660 7262; fax +1 919 660 7425;
email richard.phillips@duke.edu)

References

- Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist* **165**: 351–372.
- Bonkowski M. 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* **162**: 617–631.
- Cheng WX. 1999. Rhizosphere feedbacks in elevated CO₂. *Tree Physiology* **19**: 313–320.
- Cheng W, Kuzyakov Y. 2005. Root Effects on Soil Organic Matter Decomposition. In: Royal Zobe IS Wright, eds. *Roots and Soil Management: Interactions Between Roots and the Soil*. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. 119–143.
- Dakora FD, Drake BG. 2000. Elevated CO₂ stimulates associative N-2 fixation in a C-3 plant of the Chesapeake Bay wetland. *Plant, Cell & Environment* **23**: 943–953.
- Finzi AC, Moore DJP, DeLucia EH, Lichter J, Hofmockel KS, Jackson RB, Kim HS, Matamala R, McCarthy HR, Oren R, Pippin JS, Schlesinger WH. 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO₂ in a warm-temperate forest. *Ecology* **87**: 15–25.
- de Graaff M-A, Six J, van Kessel C. 2007. Elevated CO₂ increases nitrogen rhizodeposition and microbial immobilization of root-derived nitrogen. *New Phytologist* **173**: 778–786.
- Grayston SJ, Vaughan D, Jones D. 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology* **5**: 29–56.
- Hodge A, Millard P. 1998. Effect of elevated CO₂ on carbon partitioning and exudate release from *Plantago lanceolata* seedlings. *Physiologia Plantarum* **103**: 280–286.
- Johnson DW. 2006. Progressive N limitation in forests: Review and implications for long-term responses to elevated CO₂. *Ecology* **87**: 64–75.
- Jones DL, Healey JR, Willett YB, Farrar JF, Hodge A. 2005. Dissolved organic nitrogen uptake by plants: an important N uptake pathway? *Soil Biology and Biochemistry* **37**: 413–423.
- Jones DL, Hodge A, Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* **163**: 459–480.
- Marschner H. 1995. *Mineral Nutrition of Higher Plants*. London: Academic Press.
- Norby RJ, O'Neill EG, Hood WG, Luxmoore RJ. 1987. Carbon allocation, root exudation, and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO₂ enrichment. *Tree Physiology* **3**: 203–210.

- Paterson E. 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. *European Journal of Soil Science* 54: 741–750.
- Phillips DA, Fox TC, Six J. 2006. Root exudation (net efflux of amino acids) may increase rhizodeposition under elevated CO₂. *Global Change Biology* 12: 561–567.
- Uselman SM, Qualls RG, Thomas RB. 2000. Effects of increased atmospheric CO₂, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). *Plant and Soil* 222: 191–202.
- Zak DR, Pregitzer KS, King JS, Holmes WE. 2000. Elevated atmospheric CO₂, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist* 147: 201.

Key words: elevated CO₂, fine roots, global change, N cycling, plant–microbial interactions, progressive N limitation, rhizodeposition, rhizosphere effects

From the inside out: fungal endophyte–grass associations and grassland communities

Fungal endophytes and other clandestine citizens that reside within plants are increasingly appreciated for the role they play in community ecology. In a well designed study examining the interaction between the fungal endophyte *Neotyphodium lolii* and perennial ryegrass, *Lolium perenne*, Rasmussen *et al.* (this issue; pp. 787–797) address the question of whether the fungal alkaloid content of the host plant is a function of enhanced alkaloid biosynthetic rates within the endophyte, or of increased endophyte populations in the plant. The authors' investigations provide much-needed insight into how genetic and abiotic interactions affect the fungal endophyte–grass host relationship, and how these in turn could influence multidirectional biotic interactions within an agronomic grassland community.

'This would suggest that the host plant's C and N metabolite pool status conditions its ability to effectively limit fungal colonization.'

A clearer view through the looking-glass at interacting genomes

Plants respond to a myriad of endogenous and environmental cues by modulating their utilization of available

carbon and nitrogen resources, which serve to accommodate the resource demands of growth, development and reproduction, as well as interactions with other organisms. The metabolic networks that promote allocation of C and N resources to different plant parts, and partitioning of those resources into different biosynthetic pathways, not only are under exquisite genetic control, but also are incredibly responsive to cues such as C and N resource status. For example, genome-wide analyses of gene expression have demonstrated that extensive reconfiguration of the transcriptome occurs in response to differential N availability, which effects dramatic changes in primary and secondary metabolism as well as growth and developmental processes (e.g. Scheible *et al.*, 2004). But how do changes to a plant's resource status affect the association of the plant with interacting organisms such as endophytic fungi?

Rasmussen *et al.* investigated how the resource status of *L. perenne* affects communications between the host plant and its fungal endophyte, *N. lolii*. To this end, the authors manipulated the resource status of *L. perenne* through increasing N availability as well as by use of cultivars exhibiting contrasting levels of soluble carbohydrate.

Two manifestations of resource-mediated interactions between the host plant and its fungal endophyte may be altered colonization of the host plant by *N. lolii*, and/or a change in the fungal endophyte's partitioning of resources to alkaloid biosynthesis. Without a reliable means of quantifying fungal biomass, these two scenarios remain confounded. Rasmussen *et al.* shed light on these questions by using quantitative PCR as a means to estimate *N. lolii* fungal biomass within the host, *L. perenne*. The sensitivity and specificity of quantitative PCR is having a transformative effect on the study of interacting genomes. The ability to quantify the extent of colonization of a plant host by microbial associates provides a powerful lens through which to view these interacting species (Schena *et al.*, 2004; Mumford *et al.*, 2006). The addition of spatial or temporal dimensions to these analyses allows a means of exploring the dynamics of plant–microbe interactions. The utility of quantitative PCR is nicely illustrated by the authors' investigations of the *N. lolii*–*L. perenne* symbiotum.

Rasmussen *et al.*'s quantitative PCR analyses revealed that fungal endophyte biomass was negatively correlated with increasing plant-soluble resource pools, measured either as soluble carbohydrates or N sources (amino acids and proteins). In the case of the soluble carbohydrate pools these differences are a function of host plant differences at the genetic level, whereas in the case of the soluble N pools these differences result from N fertilization. Thus fungal endophyte population levels can be influenced by both the host's genotype and the environment. The authors present a plausible argument that the difference in fungal DNA levels in these high soluble-N or high soluble-C plants is not a consequence of a 'dilution effect', that is, caused by

increased N availability stimulating growth of the plant more than the endophyte (*sensu* Lane *et al.*, 1997). This would suggest that the host plant's C and N metabolite pool status conditions its ability to limit fungal colonization effectively. How does this happen? Does the plant employ strategies akin to the defence mechanisms invoked in response to pathogen attack? Or do other mechanisms come into play? An understanding of the mechanisms that the plant uses to regulate fungal growth is surely one key to unlocking the mystery of fungal endophyte symbioses. As one step towards elucidating this relationship, two outstanding studies recently revealed that reactive oxygen species produced by the NADPH oxidase NoxA act to regulate hyphal branching in the fungal endophyte *Epichloë festucae*, and identified regulators of NoxA (Takemoto *et al.*, 2006; Tanaka *et al.*, 2006).

The authors also used an elegant regression method to demonstrate that concentrations of fungal alkaloids are proportional to fungal DNA concentrations. In other words, plant resource availability does not influence the proportion of alkaloids synthesized per unit fungi. Interestingly, the fungal endophyte increased soluble pools of low molecular-weight carbohydrates in the host plant, but only for the plant cultivar with standard carbohydrate levels. Thus, at least in this study, the endophyte is capable of altering partitioning of C resources in the host plant, but the host plant does not appear to alter partitioning of C resources towards alkaloid biosynthesis in the endophyte associate.

Grass–fungal endophyte interactions in the context of grassland communities

Bioactive alkaloids synthesized by fungal endophytes have been well characterized as antiherbivory agents (summarized by Schardl *et al.*, 2004). Tanaka *et al.* (2005) recently provided genetic evidence that peramine, an alkaloid synthesized by *N. lolii*, provides the host plant with protection against insect herbivory. Considerable evidence exists that colonization of grass species with fungal endophytes has an impact on grass–herbivore interactions, and that these interactions, in turn, affect other species within the grassland community (e.g. de Sassi *et al.*, 2006).

Rasmussen *et al.* present compelling evidence that N availability affects fungal endophyte levels and, by extension, concentrations of three of the four fungal alkaloids that they examined. The finding that alkaloid concentrations decreased with increased N availability in endophyte-infected perennial ryegrass is consistent with the authors' earlier report (Hunt *et al.*, 2005), but contrasts with the reports of others (Belesky *et al.*, 1988; Arachevaleta *et al.*, 1992). On first glance, these results appear to be irreconcilable. However, Rasmussen *et al.* provide fresh insight that may help resolve this apparent conundrum. First, these various studies have often employed grass species and fungal endophytes of different genetic backgrounds (different

species or genotypes). As the authors demonstrate, the genotype of both host plant and fungal endophyte may influence the degree of fungal colonization, and hence alkaloid accumulation in the plant. These observations lend credence to the notion that symbiota comprising different species may well respond differently to environmental variables such as N availability. Second, these published studies measure a range of alkaloids that, as Rasmussen *et al.* show, can exhibit distinct responses to N fertilization. Third, obtaining comparable levels of endophyte colonization between research groups – indeed, even from one experiment to the next – is challenging. The results of Rasmussen *et al.* suggest that this variability will affect alkaloid accumulation, which may have a strong impact on the outcome of the study. The issue is compounded by the practice of inducing alkaloid accumulation by clipping leaf blades of the host plant, which may introduce even more variability. Future efforts that quantify alkaloid concentrations as a function of both plant dry weight and fungal biomass, as estimated by quantitative PCR, should help to delineate relationships between N availability, fungal endophyte biomass, alkaloid concentrations and herbivory.

As mentioned above, Rasmussen *et al.* showed that the genotype of the host plant influences fungal endophyte colonization levels. Because endophyte cell numbers were found to be correlated with fungal alkaloid concentrations, the plant genotype also potentially influences herbivores through its effect on endophyte levels. In a similar vein, Bailey *et al.* (2005) reported that the genotype of two *Populus* species and hybrids of these species conditions the tree's relationship with fungal endophytes. In this study, fungal endophyte infection of twigs from *P. fremontii*, *P. angustifolia* and their hybrids was negatively correlated with the concentration of condensed tannins in twig bark. Condensed tannin concentration, in turn, is a trait that is under strong genetic control in these poplars. We can thus begin to investigate fungal endophyte–host plant interactions within the context of community genetics; that is, genetic interactions between species in communities, and the influence of the abiotic environment on those interactions (Whitham *et al.*, 2006). This may be especially true where the host is a foundational species within either a pasture or natural grassland community, as we might predict that a gene exerting large phenotypic effects in a foundational species will have a proportionately greater effect within its community. These fungal endophyte–host grass interactions, and the interactions of the symbiotum with other organisms in the grassland community, provide an exciting system with which to explore emerging concepts such as inter-specific indirect genetic effects and community phenotypes.

Perspectives

Innovations in the ways in which we explore the interactions between plants and their microbial colonizers such as fungal

endophytes are instrumental in building a comprehensive model of plant host–microbial associate dynamics. The study by Rasmussen *et al.* provides a fine example where the insight that has been gained would not have been possible using conventional approaches to assess the fungal endophyte partner. Genomics resources are becoming available for an ever-expanding range of plant species and their microbial partners, and these resources are likely to form part of the toolbox for investigating grass–fungal endophyte associations. Assuredly, the power of molecular and genomics approaches will be indispensable in our quest to understand not only the mechanisms that condition fungal endophyte–host plant interactions, but also how these interactions affect community-level processes.

Janice E. K. Cooke

Department of Biological Sciences, University of Alberta,
Edmonton, AB, Canada T6G 2E9
(tel +1 780 492 0412; fax +1 780 402 9234;
email janice.cooke@ualberta.ca)

References

- Arachevaleta M, Bacon CW, Plattner RD, Hoveland CS, Radcliffe DE. 1992. Accumulation of ergopeptide alkaloids in symbiotic tall fescue grown under deficits of soil water and nitrogen fertilizer. *Applied Environmental Microbiology* 58: 857–861.
- Bailey JK, Deckert R, Schweitzer JA, Rehill BJ, Lindroth RL, Gehring C, Whitham TG. 2005. Host plant genetics affect hidden ecological players: links among *Populus*, condensed tannins, and fungal endophyte infection. *Canadian Journal of Botany* 83: 356–361.
- Belesky DP, Stuedemann JA, Plattner RD, Wilkinson SR. 1988. Ergopeptide alkaloids in grazed tall fescue. *Agronomy Journal* 79: 217–220.
- Hunt MG, Rasmussen S, Newton PCD, Parsons AJ, Newman JA. 2005. Near-term impacts of elevated CO₂, nitrogen and fungal endophyte-infection on *Lolium perenne* L. growth, chemical composition and alkaloid production. *Plant, Cell & Environment* 28: 1345–1354.
- Lane GA, Tapper BA, Davies E, Hume DE, Latch GCM, Barker DJ, Eason HS, Rolston MP. 1997. Effect of growth conditions on alkaloid concentrations in perennial ryegrass naturally infected with endophyte. In: Bacon CW, Hill AC, eds. *Neotyphodium/grass interactions*. New York: Plenum Press, 179–182.
- Mumford R, Boonham N, Tomlinson J, Barker I. 2006. Advances in molecular phytodiagnostics – new solutions for old problems. *European Journal of Plant Pathology* 116: 1–19.
- Rasmussen S, Parsons AJ, Bassett S, Christensen MJ, Hume DE, Johnson LJ, Johnson RD, Simpson WR, Stacke C, Voisey CR, Xue X, Newman JA. 2007. High nitrogen supply and carbohydrate content reduce fungal endophyte and alkaloid concentration in *Lolium perenne*. *New Phytologist* 173: 787–797.
- de Sassi C, Müller CB, Krauss J. 2006. Fungal plant endosymbionts alter life history and reproductive success of aphid predators. *Proceedings of the Royal Society of London B* 273: 1301–1306.
- Schardl CL, Leuchtman A, Spiering MJ. 2004. Symbioses of grasses with seedborne fungal endophytes. *Annual Reviews of Plant Biology* 55: 315–340.
- Scheible W-R, Morcuende R, Czechowski T, Fritz C, Osuna D, Palacios-Rojas N, Schinelasch D, Thimm O, Udvardi MK, Stitt M. 2004. Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of *Arabidopsis* in response to nitrogen. *Plant Physiology* 136: 2483–2499.
- Schena L, Nigro F, Ippolito A, Gallitelli D. 2004. Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi. *European Journal of Plant Pathology* 110: 893–908.
- Takemoto D, Tanaka A, Scott B. 2006. A p67^{Phox}-like regulator is recruited to control hyphal branching in a fungal–grass mutualistic symbiosis. *Plant Cell* 18: 2807–2821.
- Tanaka A, Tapper BA, Popay A, Parker EJ, Scott B. 2005. A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiont from insect herbivory. *Molecular Microbiology* 57: 1036–1050.
- Tanaka A, Christensen MJ, Takemoto D, Park P, Scott B. 2006. Reactive oxygen species play a role in regulating a fungus–perennial ryegrass mutualistic interaction. *Plant Cell* 18: 1052–1066.
- Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf EV, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks JC, Hart SC, Wimp GM, Wooley SC. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* 7: 510–523.

Key words: alkaloids, community genetics, fungal endophytes, interacting genomes, nitrogen, perennial ryegrass, quantitative PCR.

Meetings

A diversity of scales

The Biology of Transpiration: From Guard Cells to Globe, Snowbird Mountain Resort, Utah, USA, 10–14 October 2006. An American Society of Plant Biologists sponsored meeting

This was the sixth conference in a series first initiated in 1979 focusing on the biology of stomata; in this case emphasizing the role of transpiration. This meeting showcased many of the recent advances in the understanding and measurement of transpiration at diverse scales from the gene to the globe. An impressive array of techniques were clearly described and independently employed to identify those fundamental processes that control stomatal development, activity and transpiration at these scales of activity.

'stomata are the most significant biological regulatory system on the planet'

The cellular level: incorporating guard cell development and guard cell signalling

Over recent years, tremendous advances have been made in our understanding of the factors controlling guard cell development. The use of genetic techniques has led to the characterization of several negative regulators of stomatal development, but whether these act in a single pathway remains unclear (Bergmann, 2006). Two speakers described various components of the pathway which involves a succession of related transcription factors necessary for the division and differentiation of stomatal cells. The role of the putative transcription factor FOUR LIPS (FLP) (Lai *et al.*, 2005) in controlling the division of the guard mother cell was explored by Fred Sack (University of British Columbia, Vancouver, Canada). From comparison of the transcriptional profiles of *YODA* mutants (which have mutations in the gene encoding *YODA*, a mitogen-activated protein kinase kinase kinase) with altered numbers of stomata, Dominique Bergmann (Stanford University, CA, USA) described the identification

of *FAMA*, another putative transcription factor expressed specifically in cells of the stomatal lineage (Bergmann *et al.*, 2004). Like FLP, *FAMA* also appears to control progression through the stomatal development pathway. *fama-1* mutants have no recognizable stomata, whereas misexpression of *FAMA* leads to an epidermis consisting only of guard cells with a striking 'fish-scale' appearance (Ohashi-Ito & Bergmann, 2006). The importance of understanding the factors regulating stomatal development was underlined by Josette Masle (Australian National University, Canberra, Australia), who reported the first identification of a gene regulating transpiration efficiency from a screen using carbon isotopic discrimination. The identification of *ERECTA*, a putative receptor-like protein kinase involved in controlling stomatal density and other traits, raises the exciting possibility of designing strategies for improving water use efficiency or yield potential in crops (Masle *et al.*, 2005).

Many factors impact on the control of stomatal aperture and it is now accepted that this process is controlled by a web of interacting biochemical signalling pathways. The complex nature of these interactions has led to the interpretation of guard cell signalling pathways as a network (Hetherington, 2001). Reka Albert (Pennsylvania State University, USA) presented a dynamic mathematical model, incorporating over 40 components, exploring the topology of the abscisic acid (ABA)-induced stomatal closure network. This interpretation allows for complimentary overlap in the event of a partial systems failure (Li *et al.*, 2006), and aims to suggest candidates for manipulation to improve drought tolerance.

A number of speakers focused on the characterization of individual signalling components that contribute to the regulation of stomatal aperture. The role of guard cell cytosolic calcium elevations in stomatal aperture regulation was revisited by Rainer Hedrich (Julius-von-Sachs-Institut für Biophysik, Würzburg, Germany) and Julian Schroeder (University of California, San Diego, USA). From *in vivo* imaging studies using calcium-sensitive chameleon indicators, Schroeder proposed a new model to explain how elevations in guard cell calcium ion concentration could bring about differing aperture responses. In this model it is proposed that calcium sensors are primed (turned on) or deprimed (turned off) by previous stimuli (Israelsson *et al.*, 2006). Using a novel method to simultaneously measure cytosolic calcium concentrations and anion channel activation in guard cells of intact plants, Hedrich presented results suggesting that, in some species, ABA is able to activate ion channels by either calcium-dependent or calcium-independent routes

(Marten *et al.*, 2007). Both Alistair Hetherington (University of Bristol, UK) and Sally Assmann (Penn State University, Pennsylvania, USA) discussed the role of sphingolipids in calcium release (Ng *et al.*, 2001), and their interaction with G-proteins during ABA-induced reductions in stomatal aperture (Coursol *et al.*, 2003). Mike Blatt (University of Glasgow, UK) presented evidence indicating that plasma membrane vesicle trafficking and anchoring of K⁺ ion channels within complexes is important in ABA responses (Sutter *et al.*, 2006).

Through the identification of mutants with defects in ozone-induced stomatal closure, Triin Kollist (University of Helsinki, Finland) has identified a novel component in aperture control. Radical-induced cell death 3 (RCD3) is a putative chloroplast membrane-associated protein specific to guard cells, reviving the question as to why guard cells are green. This question was also considered by Susanne von Caemmerer (Australian National University, Canberra, Australia), who presented her recent investigations into stomatal behaviour in photosynthetic mutants, indicating that plants with impaired photosynthesis do not differ from plants with normal photosynthesis in their control of stomatal aperture (von Caemmerer *et al.*, 2004). In contrast, Julie Gray (University of Sheffield, UK) presented experiments suggesting that synthesis and metabolism of malate play a role in stomatal opening and closing, respectively.

Working with blue light photoreceptor mutants, Ken-ichiro Shimazaki (Kyushu University, Fukuoka, Japan) discussed the evidence for the activity of a protein phosphatase that may mediate signalling between phototropins and activation of the plasma membrane H⁺-ATPase which drives stomatal opening (Takemiya *et al.*, 2006). The same group, intriguingly, showed that the fern *Adiantum capillus-veneris* lacks the stomatal blue light response although it possesses functional phototropins and H⁺-ATPase (Doi *et al.*, 2006).

The whole-plant level: incorporating systemic signalling

Research at the plant level remains largely physiological, with mechanical considerations still dominating the integration of transpiration throughout the whole plant. A synthetic physical model of leaf transpiration, designed to mimic and allow for accurate measurement of hydration and equilibration kinetics, was presented by N. Michelle Holbrook (Harvard University, MA, USA; Zwieniecki *et al.*, 2004). This model suggests two spatially and temporally separate reservoirs, each fulfilling a different function within a leaf, but connecting the vascular system to other functional tissues. Use of this unique approach has provided a novel basis for defining and measuring the physiologically relevant components of leaf water status and, although still under development, the model illustrates the continued need for research in fundamental physical and mechanical (hydraulic) aspects of plant function.

Long-distance drought-signalling pathways were described at the whole-plant scale by Bill Davies (University of Lancaster, UK) in terms of interactions among ABA, ethylene and pH status modifying transpirational loss (Sobeih *et al.*, 2004). At the root interface, significant progress was reported by Christophe Maurel (CRNS/INRA Montpellier, France) and Francois Chaumont (Université Catholique de Louvain, Belgium) in elucidating the role, precise positioning and physiological significance of aquaporins, characterized in plants little more than a decade ago. The structural gating mechanism of action has been explained (Tornroth-Horsefield *et al.*, 2006), and these membrane-bound water channels are now functionally known to have a regulatory role in water uptake, with down-regulation of uptake under environmental stress. Several classes of aquaporins, known as isoforms, are known to exist in roots of *Arabidopsis thaliana* and links to other well-known signal pathways were confirmed under stress treatments involving reactive oxygen species (ROS) and pH regulation (Boursiac *et al.*, 2005).

A genomic approach was presented as part of a long-term study of changes in poplar trees (*Populus × euramericana*) grown under increased CO₂ concentration in a Free-Air CO₂ Enrichment (FACE) facility by Gail Taylor (University of Southampton, UK). Several changes in leaf morphology and physiology were observed which impacted on whole-plant water use. Leaf-level conductance was reduced, but whole-tree transpiration rates increased, as a result of increased leaf area and changes in stomatal numbers (Taylor *et al.*, 2003). Crossing of generations grown in treatment over a period of years allowed the use of quantitative trait loci (QTL) analyses to map the location of several candidate genes potentially important in water use changes under high CO₂ concentrations (including an *ERECTA* homologue; see previous section).

The canopy level

Global warming and climate-change effects on vegetation have stimulated the need to understand how environmentally induced transpirational regulation affects large-scale systems, whether agricultural or natural. This requirement has ushered in new techniques for quantification of canopy-wide measurements. Russell Monson (University of Colorado, Boulder, USA) described the use of national and international networks of flux towers, which provide on-going observations for eddy-covariance and model-data fusion analyses, allowing ecosystem evapotranspiration (ET) rates to be separated into component fluxes, for example soil and tree ET (Law *et al.*, 2002; Wilson *et al.*, 2003).

Despite Joseph Berry's (Carnegie Institution of Washington, USA) eloquent description of stomata as 'the most significant biological regulatory system on the planet', many complexities and uncertainties associated specifically with larger scale environments were highlighted as constraints to the incorporation of transpiration into vegetation models. One of these, rates of night-time respiration, was presented by

Maggie Caird (University of California, Davis, USA) as being up to 30% of daytime rates, with little or no knowledge of the cost to either the individual plant or the system as a whole. Furthermore, the case was made by Carl Bernacchi (Illinois State Water Survey, USA) that a lack of understanding of plant processes at this level could have large implications for regional climates undergoing climate change. The extent to which we understand these processes will affect how we predict such changes in the future, illustrating the need for increasingly sophisticated techniques of measurement. Remote sensing continues to progress in the measurement of field-scale canopy stomatal conductance. However, as highlighted by Hamlyn Jones (University of Dundee, UK), calibration methods remain limited in their ability to cope with the variation in environmental conditions encountered in the field; for example, using the energy balance equation, the difference between a wet and dry canopy can produce as much as 25°C difference in calculated temperatures.

The global level

The global aspects of transpiration and stomatal impacts were presented by Jenny McElwain (University College Dublin, Ireland). Stomatal numbers change with atmospheric CO₂ concentration, which provides the potential to use fossil stomatal numbers as proxy estimators of the CO₂ content of the atmosphere (McElwain, 2004). Going further, it was also suggested that, as far back as the Carboniferous period (some 300 million years ago), changes in stomatal numbers have been instrumental in driving the evolution of vessel architecture – a ‘pull’ vs ‘push’ hypothesis.

Current and future vegetation dynamics were presented by Ian Woodward (University of Sheffield, UK) using largely dynamic vegetation models, to aid understanding of the nature of global changes in vegetation structure as affected by long-term successional processes, perturbations of climate, such as El Niño events, and historical changes in atmospheric CO₂ concentrations. Model projections up to the year 2100 indicated that a warmer world will have significantly reduced tropical forest cover, even combined with increases in CO₂ that reduce transpiration and increase primary productivity. The stimulation of productivity by CO₂ is further predicted to increase the probability of fire, reducing mature forest cover and opening the forest to a savannah-like structure. These advances in model development are strongly reliant on advances at all smaller scales that link regional effects to global consequences.

The conference was brought to a close by Graham Farquhar (Australian National University, Canberra, Australia), who described a technique using isotopically heavy water to detect genetic and environmental effects on transpiration, with experiments showing that transpiration consists of two fluxes, leaf-to-air and air-to-leaf. Isotopically distinct material has been obtained by growth of maize (*Zea mays*) at different humidities, and thus different transpiration rates

(Gan *et al.*, 2003). Such material can be used to monitor the effects of climate change on a scale from single leaves to forests, and has great potential for aiding our understanding of how climate change affects global transpiration.

Summary

It is clear that much progress towards understanding plant transpiration has been achieved, not only at the cellular level but, through independent disciplines, on a full range of scales, both temporal and spatial. There is still a requirement to fully integrate findings – the coupling of each scale to the next will be an interdisciplinary challenge, but is necessary if we are to understand the entire transpiration system, from guard cell to globe.

Janice A. Lake^{1*} and Julie E. Gray²

¹Department of Animal and Plant Sciences, The University of Sheffield, Western Bank, Sheffield S10 2TN;

²Department of Molecular Biology and Biotechnology, The University of Sheffield, Western Bank, Sheffield S10 2TN, UK

(*Author for correspondence: tel +44 (0)114 2220088; fax +44 (0)114 2220002; email j.a.lake@sheffield.ac.uk)

References

- Bergmann D. 2006. Stomatal development: from neighbourly to global communication. *Current Opinion in Plant Biology* 9: 478–483.
- Bergmann DC, Lukowitz W, Somerville CR. 2004. Stomatal development and pattern controlled by a MAPKK kinase. *Science* 304: 1494–1497.
- Boursiac Y, Chen S, Luu DT, Sorieul M, Van Den Dries Maurel C. 2005. Early effects of salinity on water transport in Arabidopsis roots. Molecular and cellular features of aquaporin expression. *Plant Physiology* 139: 790–805.
- von Caemmerer S, Lawson T, Oxborough K, Baker NR, Andrews TJ, Raines CA. 2004. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. *Journal of Experimental Botany* 55: 1157–1166.
- Coursol S, Fan LM, Le Stunff H, Spiegel S, Gilroy S, Assmann SM. 2003. Sphingolipid signalling in Arabidopsis guard cells involves heterotrimeric G proteins. *Nature* 423: 651–654.
- Doi M, Wada M, Shimazaki K. 2006. The fern *Adiantum capillus-veneris* lacks stomatal responses to blue light. *Plant and Cell Physiology* 47: 748–755.
- Gan KS, Wong SC, Yong JWH, Farquhar GD. 2003. Evaluation of models of leaf water ¹⁸O enrichment using measurements of spatial patterns of vein xylem water, leaf water and dry matter in maize leaves. *Plant, Cell & Environment* 26: 1479–1495.
- Hetherington AM. 2001. Guard cell signalling. *Cell* 107: 711–714.
- Israelsson M, Siegel RS, Young J, Hashimoto M, Iba K, Schroeder JI. 2006. Guard cell ABA and CO₂ signaling network updates and Ca²⁺ sensor priming hypothesis. *Current Opinion in Plant Biology* 9: 654–663.
- Lai LB, Nadeau JA, Lucas J, Lee EK, Nakagawa T, Zhao L, Geisler M, Sack FD. 2005. The *Arabidopsis* R2R3 MYB proteins FOUR LIPS and MYB88 restrict divisions late in the stomatal cell lineage. *Plant Cell* 17: 2754–2767.
- Law BE, Falge E, Gu L, Baldocchi DD, Bakwin P, Berbigier P, Davis K, Dolman AJ, Falk M, Fuentes JD, Goldstein A, Granier A, Grelle A,

- Hollinger D, Janssens IA, Jarvis P, Jensen NO, Katul G, Mahli Y, Matteucci G *et al.* 2002. Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation. *Agricultural and Forest Meteorology* 113: 97–120.
- Li S, Assmann SM, Albert R. 2006. Predicting essential components of signal transduction networks: A dynamic model of guard cell abscisic acid signaling. *Public Library of Science Biology* 4: e312.
- Marten H, Konrad K, Dietrich P, Roelfsema MR, Hedrich R. 2007. Ca²⁺-dependent and -independent ABA activation of plasma membrane anion channels in guard cells of *Nicotiana tabacum*. *Plant Physiology* 143: 28–37.
- Masle J, Gilmore SR, Farquhar GD. 2005. The *ERECTA* gene regulates plant transpiration efficiency in *Arabidopsis*. *Nature* 436: 866–870.
- McElwain JC. 2004. Climate-independent paleoaltimetry using stomatal density in fossil leaves as a proxy for CO₂ partial pressure. *Geology* 32: 1017–1020.
- Ng CKY, Carr K, McAinsh MR, Powell B, Hetherington AM. 2001. Drought-induced guard cell signal transduction involves sphingosine-1-phosphate. *Nature* 410: 596–599.
- Ohashi-Ito K, Bergmann DC. 2006. *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development. *Plant Cell* 18: 2493–2505.
- Sobeih WY, Dodd IC, Bacon MA, Grierson D, Davies WJ. 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial root-zone drying. *Journal of Experimental Botany* 55: 2353–2363.
- Sutter JU, Campanoni P, Tyrrell M, Blatt MR. 2006. Selective mobility and sensitivity to SNAREs is exhibited by the *Arabidopsis* KAT1 K⁺ channel at the plasma membrane. *Plant Cell* 18: 935–954.
- Takemiya A, Kinoshita T, Asanuma M, Shimazaki K. 2006. Protein phosphatase 1 positively regulates stomatal opening in response to blue light in *Vicia faba*. *Proceedings of the National Academy of Sciences, USA* 103: 13549–13554.
- Taylor G, Tricker PJ, Zhang FZ, Alston VJ, Miglietta F, Kuzminsky E. 2003. Spatial and temporal effects of free-air CO₂ enrichment (POPFACE) on leaf growth, cell expansion, and cell production in a closed canopy of poplar. *Plant Physiology* 131: 177–185.
- Tornroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorhid E, Neutze R, Kjellbom P. 2006. Structural mechanism of plant aquaporin gating. *Nature* 439: 688–694.
- Wilson KB, Baldocchi D, Falge E, Aubinet M, Berbigier P, Bernhofer C, Dolman H, Field C, Goldstein A, Granier A, Hollinger D, Katul G, Law BE, Meyers T, Moncrieff J, Monson R, Tenhunen J, Valentini R, Verma S, Wofsy S. 2003. Diurnal centroid of ecosystem energy and carbon fluxes at FLUXNET sites. *Journal of Geophysical Research – Atmospheres* 108: 4664.
- Zwieniecki MA, Boyce CK, Holbrook NM. 2004. Functional design space of single-veined leaves: Role of tissue hydraulic properties in constraining leaf size and shape. *Annals of Botany* 94: 507–513.

Key words: ecosystem, genetic control, global consequences, stomatal function, transpiration.

Towards forest community and ecosystem genomics

Population genetics and genomics of forest trees: from gene function to evolutionary dynamics and conservation – a joint conference of IUFRO working

groups 2.04.01 (Population, ecological and conservation genetics) and 2.04.10 (Genomics) and COST action E-28 (Genosilva: European Forest Genomics network), Alcalá de Henares, Madrid, Spain, October 2006

These are exciting times for students of ecological and evolutionary genetics. New ‘-omics’ approaches increasingly allow biologists to understand adaptively important differences between individuals, populations and species at the organismal level, and simultaneously to unravel the functional and mechanistic underpinnings of their origins (Feder & Mitchell-Olds, 2003). At the same time, new methodological and conceptual developments permit studies of heritable traits with effects on entire communities and ecosystems (Whitham *et al.*, 2006). In some ways, the ecological and evolutionary genetics of forest trees and associated organisms (‘forest genetics’ from here onwards) comprise a microcosm within this field, with tremendous opportunities and prospects.

Forest communities provide livelihoods for millions of people around the world, they prevent local environmental deterioration, and they act as carbon sinks in the face of global climate change (Day *et al.*, 1991; United Nations Framework Convention on Climate Change (<http://unfccc.int/2860.php>), UN Millennium Development Goals (<http://www.un.org/millenniumgoals/>)). Not surprisingly therefore, forest genetics has long had an active scientific community. Whereas in the final decades of the 20th century, efforts were focused mainly on understanding spatial patterns of ‘neutral’ genetic marker variation or heritable growth-related characters in single tree species, forest genetics in the 21st century promises to go a significant step further. A recent conference, entitled ‘Population Genetics and Genomics of Forest Trees: from Gene Function to Evolutionary Dynamics and Conservation’ (<http://www.genfor2006.fgua.es/>), organized jointly by the International Union of Forest Research Organizations (IUFRO) and a European Union COST programme in Alcalá de Henares, Madrid, Spain, provided a glimpse of these developments.

‘... the search for adaptively important genes sometimes includes the detection of genes or genomic segments under divergent selection pressures strong enough to overcome the homogenizing effect of gene flow’

Genetics of adaptation and ecological differences

Two major trends are emerging in forest genetics research, visible also at the Madrid conference. One is the identification and study of adaptive genetic variation using a combination of approaches: quantitative trait locus (QTL) mapping, functional genomics, association mapping, population genomics work to identify 'outlier' loci subject to selection and molecular evolution studies of candidate genes (recently reviewed by González-Martínez *et al.*, (2006), the lead author being one of the organizers of this meeting). These approaches include a search for the locus-specific signature of directional selection during population divergence (Storz, 2005). Adaptive genetic variation in widespread trees is often arranged clinally in response to ecological (e.g. climatic) gradients. Efficient dispersal mechanisms and large effective population sizes imply that, at small to intermediate scales, gene flow and selection are important factors in determining patterns of differentiation. Thus, the search for adaptively important genes sometimes includes the detection of genes or genomic segments under divergent selection pressures strong enough to overcome the homogenizing effect of gene flow.

Although this combination of approaches has so far been applied only to a small number of tree species, traits and genes, some first results are already available. In pines, for instance, genes with DNA sequence signatures suggestive of selection were found to be related to biotic and abiotic stress tolerance and key metabolic pathways, including the pathway responsible for lignin formation (reviewed by González-Martínez *et al.*, 2006). Several association genetics projects are underway in temperate forest trees, with a focus on *Pinus* spp. and *Populus* spp. David Neale (University of California, CA, USA) and Outi Savolainen (University of Oulu, Finland) presented their current projects on variation in candidate genes for abiotic stress tolerance and disease resistance in pines at the 'Association mapping in forest trees' workshop and 'Quantitative genetics, QTL studies and adaptation' session. Since amounts of linkage disequilibrium (LD) in wind-pollinated outcrossing *Populus* and *Pinus* species are extremely low (Neale & Savolainen, 2004; Ingvarsson, 2005), most of these projects utilize a candidate gene-based approach rather than complete genomic scans for association.

Studies of admixed populations or 'hybrid zones' between divergent species or populations hold the potential of widening the region of a chromosome that is affected by LD, thus increasing the size of the chromosomal window that can be tested for genetic association by a given set of markers. Christian Lexer (Royal Botanic Gardens Kew, UK) presented results on this, which indicate that this admixture-based approach is suitable for dissecting the genetic basis of the barrier to gene flow between the ecologically divergent European species *Populus alba* (white poplar) and *P. tremula*

(European aspen; Lexer *et al.*, 2006). Similar approaches may also be useful for studying the genetic basis of ecological differences in other genera with closely related hybridizing species, such as ash (*Fraxinus* spp.) or oak (*Quercus* spp.).

Linking forest genetics and community ecology

A second trend emerging in forest genetics is the establishment of research programmes directed at the level of biological communities rather than single tree species. 'Community and ecosystem genetics' holds the potential of predicting the 'extended phenotype', that is, the downstream effects of major genes of forest trees on other trophic levels of associated forest communities (Whitham *et al.*, 2006). This approach has been remarkably successful in detecting correlations between genetic composition and multiple community and ecosystem components in North American *Populus* species (*P. angustifolia* and *P. fremontii*; reviewed in Whitham *et al.*, 2006). Although this approach has not yet been utilized at a comparable scale and depth in other tree species, several signs indicate a tendency of forest genetics to 'scale up' to the community and ecosystem level.

A 'Network of Excellence' funded recently by the European Commission (EVOLTREE; Evolution of Trees as Drivers of Terrestrial Biodiversity) and presented by its coordinator, Antoine Kremer (INRA, France), has the goal of studying genes of adaptive significance in the face of climate change in several European forest trees (*Pinus*, *Populus*, *Quercus*), phytophagous insects and mycorrhizal fungi, and to assess the impact of trees on the composition of forest communities. Another development with great potential impact is the establishment of spatial models that embrace patterns of species distributions and spatial genetic patterns within species. Studies of fine-scale spatial genetic structure in forest trees have recently experienced a boom, following the development of highly variable codominant markers in numerous tree species and the creation of novel statistical and interpretive tools. During the 'Simulation models of tree population genetics' workshop, Olivier Hardy (Université Libre de Bruxelles, Belgium) discussed how, by adopting theoretical concepts from population genetics, spatial models promise to predict patterns of species distributions from seed dispersal distances and habitat heterogeneity (Hardy & Sonke, 2004). Linking small-scale spatial structure analysis at the genetic and community levels may yield valuable insights into the factors that control diversity in ecological communities, a topic of particular relevance for the conservation and management of tropical forests (Condit *et al.*, 2002).

Conclusions and future prospects

A growing number of neutral marker-based conservation genetics studies are becoming available for tropical trees,

resulting in increased knowledge on the genetic consequences of different dispersal mechanisms and recruitment patterns (Hardy *et al.*, 2006; Jones & Hubbell, 2006). These developments, and the opportunities they bring for future research, were tangible in several lectures, for example, those by Frank Jones (Smithsonian Tropical Research Institute, Panama), Peter Smouse (Rutgers University, NJ, USA), and Olivier Hardy. An exciting area was examined by Peter Smouse, who discussed refined approaches of studying contemporary gene flow at the landscape level (Sork & Smouse, 2006) and how this is beginning to be applied to tropical species, including comparisons between populations with varying degrees of human-mediated disturbance. Although these signs all point in the right direction, much larger numbers of tropical species will have to be studied to account for the higher species richness found in many tropical compared with temperate forests. We expect that these developments will be made easier not only by practical improvements in genetic marker (e.g. microsatellite) isolation procedures, but also by the increased opportunity for cross-species transfer of these genetic tools as the network of species with available codominant markers grows.

In temperate species, 'scaling up' of forest genetics from the intra- to the interspecific level is expected to benefit from meta-genomic analyses of trees and associated forest organisms. For example, the first genome sequence of a forest tree is now available in the genus *Populus* (Tuskan *et al.*, 2006), and genome sequences for associated mycorrhizal and pathogenic fungi are underway. We expect that studies of hybridizing species groups will contribute to this 'scaling up' process as well. Hybridizing taxa such as oaks (*Quercus* spp.) or poplars (*Populus* spp.) exchange genes across semipermeable species barriers. To the extent that ecological divergence forms part of the barrier, such hybridizing systems will allow the genetic dissection of traits relevant to ecological differences and species interactions.

As pointed out by Whitham *et al.* (2006), taking genetics to the community and ecosystem level will require a collaborative vision that emphasizes integration of tools from multiple disciplines of biology. In the case of forest trees, this will require commitment to, and funding for, long-term experiments to accommodate the long generation spans of trees. Long-term projects, such as the establishment and maintenance of common gardens, provenance trials and crossing programmes, or the genetic monitoring of the regeneration of both natural and disturbed stands, would allow forest geneticists to move beyond producing molecular marker-based 'snapshots' of neutral genetic diversity. To close with an example from a different corner of the ecology and evolution community: long-term commitment has allowed biologists to unravel some of the mysteries of the adaptive radiation that gave rise to Darwin's finches, from studying phenotypic diversity to identifying the pathways and genes involved in phenotypic evolution (Abzhanov *et al.*, 2006). Trees may

have longer generation times than Darwin's finches, but their immense significance for human well-being should justify long-term investments.

Acknowledgements

Many thanks to Maria Teresa Cervera, Carmen Díaz-Sala, Santiago C. González-Martínez, Salustiano Iglesias, Álvaro Soto, Miguel Verdú and Dolores Abarca, for organizing a great meeting.

Christian Lexer*, Marcela Van Loo and Thelma Barbará

Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK (*Author for correspondence: tel +44 (0)20 8332 5367; fax +44 (0)20 8332 5310; email c.lexer@kew.org)

References

- Abzhanov A, Kuo WP, Hartmann C, Grant BR, Grant PR, Tabin CJ. 2006. The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature* 442: 563–567.
- Condit R, Pitman N, Leigh EG, Chave J, Terborgh J, Foster RB, Nunez P, Aguilar S, Valencia R, Villa G, Muller-Landau HC, Losos E, Hubbell SP. 2002. Beta-diversity in tropical forest trees. *Science* 295: 666–669.
- Day PR, Allard RW, Alvim PT, Barton JH, Buttel FH, Chang T-T, Evenson RE, Fitzhugh HA, Goodman MM, Hardon JJ, Marshall DR, Sastrapradja S, Smith C, Spence JA. 1991. *Managing global genetic resources: forest trees*. Washington, DC, USA: National Academy Press.
- Feder ME, Mitchell-Olds T. 2003. Evolutionary and ecological functional genomics. *Nature Reviews Genetics* 4: 649–665.
- González-Martínez SC, Krutovsky KV, Neale DB. 2006. Forest tree population genomics and adaptive evolution. *New Phytologist* 170: 227–238.
- Hardy OJ, Maggia L, Bandou E, Breyne P, Caron H, Chevallier MH, Doligez A, Dutech C, Kremer A, Latouche-Halle C, Troispoux V, Veron V, Degen B. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 neotropical tree species. *Molecular Ecology* 15: 559–571.
- Hardy OJ, Sonke B. 2004. Spatial pattern analysis of tree species distribution in a tropical rain forest of Cameroon: assessing the role of limited dispersal and niche differentiation. *Forest Ecology and Management* 197: 191–202.
- Ingvarsson PK. 2005. Nucleotide polymorphism and linkage disequilibrium within and among natural populations of European aspen (*Populus tremula* L., Salicaceae). *Genetics* 169: 945–953.
- Jones FA, Hubbell SP. 2006. Demographic spatial structure of the neotropical tree, *Jacaranda copaia*. *Molecular Ecology* 15: 3205–3217.
- Lexer C, Buerkle A, Joseph J, Heinze B, Fay MF. 2006. Admixture in European *Populus* hybrid zones makes feasible the mapping of loci that contribute to reproductive isolation and trait differences. *Heredity*. Available advanced-online.
- Neale DB, Savolainen O. 2004. Association genetics of complex traits in conifers. *Trends in Plant Science* 9: 325–330.
- Sork VL, Smouse PE. 2006. Genetic analysis of landscape connectivity in tree populations. *Landscape Ecology* 21: 821–836.
- Storz JF. 2005. Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology* 14: 671–688.

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U *et al.* 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313: 1596–1604.

Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, Leroy CJ, Lonsdorf EV, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks JC, Hart SC, Wimp GM,

Wooley SC. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* 7: 510–523.

Key words: adaptive evolution, community ecology, conservation, forest ecology and management, functional genomics, gene flow, population genetics, spatial genetic structure.



About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – our average submission to decision time is just 30 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £131 in Europe/\$244 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the US Office (newphytol@ornl.gov; tel +1 865 576 5261).