Commentary

Global biogeography of plant chemistry: filling in the blanks

It would perhaps come as a surprise to many nonbiological scientists (or even some biologists) to learn that despite our ability to characterize a number of environmental variables, such as climate, along regional or continental gradients, until recently we have had almost no basis for doing so for plant and soil chemistry. New work, including a paper by Han *et al.* in this issue (pp. 377–385), is beginning to fill in the blanks on this otherwise empty slate. It is well known that long-term climate records exist in a relatively well-distributed network across much, but not all, of the globe. Hence, we are able to quantify the difference in climate between, for example, central Saskatchewan, Canada and central Nebraska, USA but not the differences in plant or soil nutrient concentrations or contents between these two regions. Given the importance of nitrogen (N) and phosphorus (P) to plant function, to production of agricultural and unmanaged ecosystems and to global biogeochemical cycles, including the carbon (C) cycle, one could argue that knowledge of biogeography of their biochemistry is as useful as knowledge of many other kinds, yet it has been little emphasized. Why?

'… global heterogeneity in leaf N and P is likely substantial enough that we will require sweepingly comprehensive data sets before we will be able to reconcile differences that may arise owing to differences in intensity of sampling in different 'ecoregions' of the Earth.'

Why do we know so little about the biogeography of plant chemistry?

Several factors likely contribute to our lack of understanding of the biogeography of plant chemistry. First, whether one was interested in agricultural crop yield, ecological physiology or biogeochemistry, research in plant chemistry – here represented by the simple stoichiometry of C, N and P – has historically been process-oriented or site-based. (As a reminder, given slight variation in leaf C concentration, leaf N and P concentrations are excellent indicators of C : N and C : P ratios.) For example, the emphasis has traditionally been on understanding how biochemistry fundamentally regulates plant physiological function, and on potential consequences for interactions with competitors, consumers and decomposers. Spatially, we might have asked what regulates leaf N or P concentration (hereafter just leaf N or P, for brevity) at the microsite scale, such as in a forest gap or in a low or high spot in an agricultural field. I would guess that less than 1, 0.1 or even 0.01% of all publications that reported plant nutrient contents have been concerned with spatial patterns at regional to global scales. Second, plant chemistry is phenomenally heterogeneous both temporally and spatially, with many factors playing a role in generating these patterns.

Among these important factors are climate, geomorphology, vegetation type and site history (Reich & Oleksyn, 2004; Wright *et al.*, 2005). Temperature and moisture gradients can directly influence leaf chemistry and can indirectly influence soil biogeochemical processes and vegetative composition, each of which can influence the average foliar N or P. Geomorphology influences the kinds of mineral substrate from which soils develop and thus soil characteristics such as N cycling, P availability and cation exchange capacity, all of which influence plant composition and nutrient status. For a given type of mineral substrate, time since major geological disturbance (i.e. soil age) also influences soil nutrient supply and hence plant chemistry (Vitousek *et al.*, 1995; Richardson *et al.*, 2005). Finally, given the close coordination of leaf N and P with other leaf traits such as leaf life span and specific leaf area (Reich *et al.*, 1997; Wright *et al.*, 2004), communities or biomes dominated by certain kinds of species (e.g. deciduous or evergreen; those having a short leaf life span vs those having a long leaf life span; those having a high specific leaf area (SLA) vs those having a low SLA) will differ in leaf N and P. For example, even when growing on similar soils, evergreen trees always have lower N and P on average than deciduous ones. Spatial heterogeneity in this set of factors (climate, geomorphology, site history, vegetation type) at regional, continental and global scales is impressive, and so far has swamped our ability to develop predictive models of leaf N or P. However, as the work of Han *et al.* demonstrates via a comprehensive assessment of plant foliar N and P across all of China, we are beginning to characterize quantitatively and increase our understanding of these issues at local, regional or global scales.

China: one gap filled, several to go

There has been a recent increase or renewal of interest in the biogeography and the stoichiometry of ecological chemistry (e.g. Sterner & Elser, 2002; McGroddy *et al.*, 2004; Reich & Oleksyn, 2004). These studies have highlighted the general importance of stoichiometry to ecology across the range of biota and ecosystem types (Sterner & Elser, 2002), identified biogeographic patterns in terrestrial foliar stoichiometry across local, regional and global gradients (Vitousek *et al.*, 1995; McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Richardson *et al.*, 2005), and tested for global convergence in the relationships between foliar stoichiometry and other foliar metabolic and morphological characteristics (Reich *et al.*, 1997; Wright *et al.*, 2004). However, we likely know more about the processes involving links between ecosystem physiology and biogeochemistry than we do about its spatial patterns, especially at large scales.

Despite advances, even the most comprehensive studies published to date have had gaping 'holes' in their biogeographic coverage (Fig. 1). One area for which there have been few data reported in the peer-reviewed international literature is China. For example, in the Reich & Oleksyn (2004) study, 5086 records of 1287 species were used, and only 11 of the 5086 records were from China. The new report by Han *et al.* goes a long way towards filling this gap, and in so doing identifies some differences with past studies that illuminate the need for a more comprehensive global data base. The findings presented by Han *et al.* are consistent in some but not all respects with findings from earlier studies based on data from other regions. As seen in previous studies, Han *et al.* found that leaf N and P were significantly greater in herbs than in woody plants and in deciduous than in evergreen species. Han *et al.* also reported that leaf N and P increase with increasing mean annual temperature (MAT) and latitude, as also recently shown by McGroddy *et al.* (2004), Reich & Oleksyn (2004) and Kerkhoff *et al.* (2005).

Equally or more interesting are the ways in which the Chinese data differ from previously published data. Although Han and colleagues report a mean leaf N similar to that in two other recent broad studies (all $\approx$ 20.1–20.6 mg g^{-1}; Elser *et al.*, 2000; Reich & Oleksyn, 2004) that they use as benchmarks, they note that the mean leaf P in the Chinese data (1.46 mg g^{-1}) is significantly ($P < 0.05$) lower than in the Reich & Oleksyn (1.77) or Elser *et al.* (1.99) data sets. As a result, the mean leaf N : P ratio is also higher in the Chinese data (16.3) than in the two other data sets ($\approx$ 13). Han *et al.* logically interpret these differences as follows.

'Because leaf N : P mass ratio is a good indicator of the relative limitation of N vs P (N : P ratios < 14 often indicate N limitation and N : P ratios > 16 frequently signifying P limitation …), the higher N : P ratio of this study than in others … might imply that China's flora are relatively more limited by P than the world flora analysed by Reich & Oleksyn (2004)'.

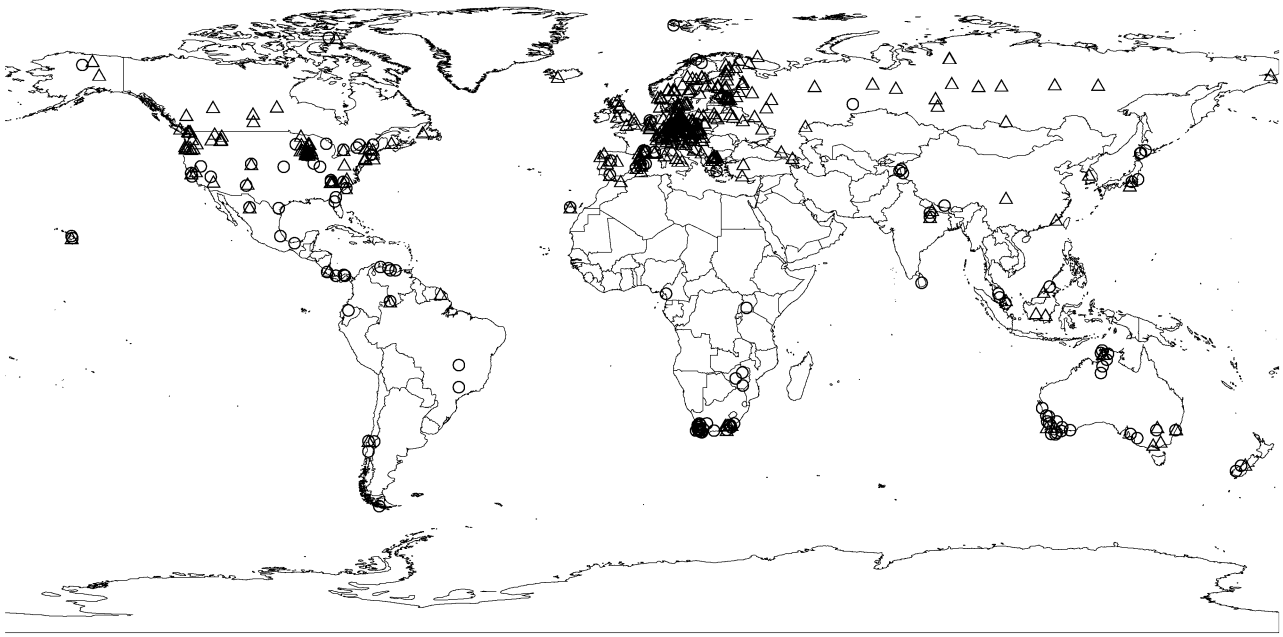

Fig. 1 Map showing sites of the Reich & Oleksyn (2004) (triangles) and Wright *et al*. (2004) (circles) global studies. Many sites are not visible owing to their proximity to other sites.

They go on to suggest that low soil P content may be the cause of low leaf P and high leaf N : P ratio in Chinese flora, given that leaf P is related, albeit loosely, to soil P content, and that data compilations suggest that soil P in China is on average lower than the global average. Their conclusion, although tentative, seems appropriate, given our current state of knowledge.

It is also of interest to compare these results to those of another almost entirely independent global-wide data survey, the Glopnet study of Wright *et al.* (2004), which Han *et al.* did not refer to. The Glopnet data have a lower mean leaf P (1.11 mg g^{-1}; 58 sites, $n = 752$) than even the Chinese data; a slightly lower leaf N (19.3 mg g^{-1}; 143 sites, $n = 2061$) than the Elser *et al.* (2000), Reich & Oleksyn (2004) or Han *et al.* data; and a higher N : P ratio (18.2; 58 sites, $n = 745$) than all three of these data sets. Although a formal analysis needs to be done, it appears that the Glopnet data set (Wright *et al.*, 2004) contains a greater fraction of data from Australia and other regions known to have predominantly infertile soils with low P contents than do the Reich & Oleksyn (2004) or Elser *et al.* (2000) data sets. If so, this is consistent with the explanation of Han *et al.* for lower leaf P and higher N : P ratio for the Chinese data than for the Reich & Oleksyn (2004) or Elser *et al.* (2000) data. However, these comparisons suggest that global heterogeneity in leaf N and P is likely substantial enough that we will require sweepingly comprehensive data sets before we will be able to reconcile differences that may arise owing to differences in intensity of sampling in different 'ecoregions' of the Earth.

The Chinese data of Han *et al.* also differ from other recent studies in the nature of the relationships of leaf N, leaf P and the leaf N : P ratio with latitude or MAT (McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Kerkhoff *et al.*, 2005). Although both leaf N and leaf P increase with latitude and MAT for the Chinese data as in the earlier publications, the correlations differ (slopes differ significantly: $P < 0.001$) when comparing Chinese data to the Reich & Oleksyn (2004) global data (Fig. 2). The relationship of leaf N with MAT has a steeper slope and better fit in the Chinese than the global data set, with the reverse true for the relationship of leaf P with MAT. Moreover, there is virtually no relationship of leaf N : P ratio with MAT in the Chinese data, whereas Reich & Oleksyn (2004) reported 31% of total variation in leaf N : P ratio could be associated with variation in MAT, and McGroddy *et al.* (2004) and Kerkhoff *et al.* (2005) also noted a positive relation between the two. Although a number of factors could lead to such differences, what those are is not clear at present.

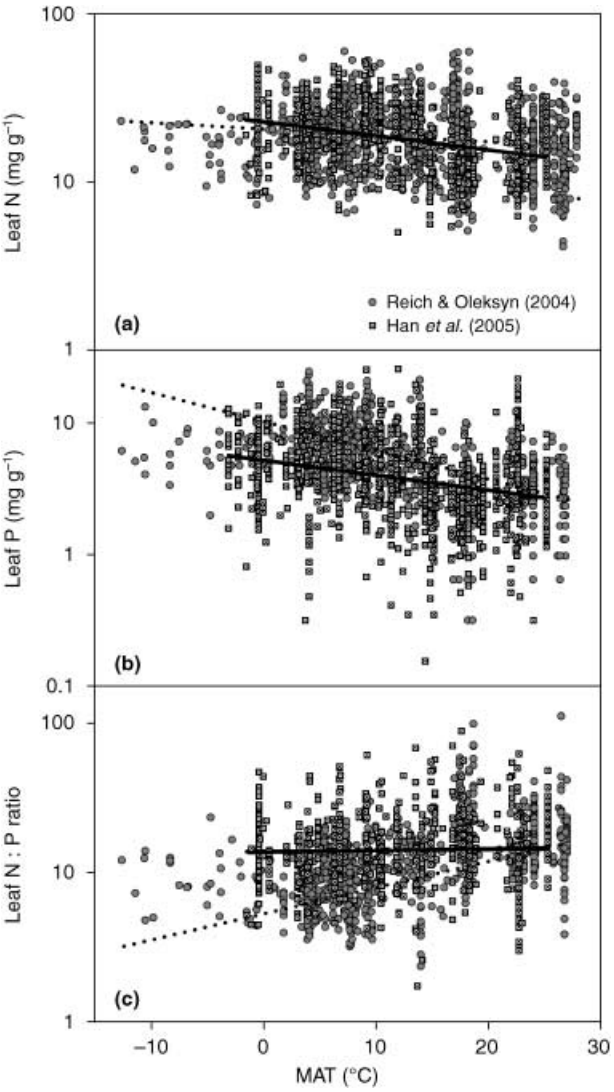

Fig. 2 Regression of leaf N (mg g^{-1}), leaf P (mg g^{-1}) and leaf N : P ratio in relation to mean annual temperature (MAT, °C) for Chinese (open symbols, solid lines; Han *et al*., 2005) and global (filled symbols, dotted lines; Reich & Oleksyn, 2004) data compilations. For consistency with the published record, for the Chinese data, the data were at the species level within sampling areas (as in Han *et al*., 2005, fig. 4) and for the global data set, the data were species averages (as in Reich & Oleksyn, 2004, fig. 1). However, to normalize the data and enable statistical comparison of the data sets, the Han *et al*. data were converted to logarithmic values and the relationships with MAT were considered to be linear. Slopes of the two data sets were significantly different ($P < 0.001$) in all three cases. Relations of (a) leaf N vs MAT for the Chinese data ($r^2 = 0.14$, $P < 0.001$, $n = 813$) and the global data ($r^2 = 0.03$, $P < 0.001$, $n = 1251$); (b) leaf P vs MAT for the Chinese data ($r^2 = 0.10$, $P < 0.001$, $n = 1177$) and the global data ($r^2 = 0.37$, $P < 0.001$, $n = 923$); and (c) leaf N : P ratio vs MAT for the Chinese data ($r^2 < 0.01$, $P = 0.53$, $n = 786$) and the global data ($r^2 = 0.31$, $P < 0.001$, $n = 894$).

What do we need to know in the future?

Knowledge of broad biogeographic patterns of leaf N and P not only is important for and contributes to understanding

of continental- to global-scale issues, but also to local and process-oriented questions. The latter may seem counterintuitive, but in fact, if we can make sense of how multiple global drivers collectively influence plant N and P, this will provide a foundation and context within which to view patterns and processes at local scales. Thus, in conclusion, we simply need more data about leaf and soil chemical attributes for as many ecosystem types in as many geographic regions as possible, especially when those attributes can be linked to quantitative information about vegetation type and history, geomorphology, soils, land use history, etc. I look forward to the day when an accurate global contour map of plant N or P can be made. You would not likely plan a picnic around it (although in an N- and P-rich site, the ants might be less likely to hone in on your greens), but it would provide an important information layer for both ecological science and global environmental management that could help us better predict responses of terrestrial ecosystems to disturbances such as elevated atmospheric CO_2, N deposition, pest outbreaks or alternative land management scenarios.

Peter B. Reich

Department of Forest Resources, University of Minnesota, Saint Paul, MN 55108, USA
(tel +1 612 624 4270; fax +1 612 625 5212; email preich@umn.edu)

References

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL, Siemann EH, Sterner RW. 2000. Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408**: 578–580.

Han W, Fang J, Guo D. 2005. Leaf N and P Stoichiometry across 753 Terrestrial Plant Species in China. *New Phytologist* **168**: 377–385.

Kerkhoff AJ, Enquist BJ, Elser JJ, Fagan WF. 2005. Plant allometry, stoichiometry, and the temperature-dependence of primary productivity 2005. *Global Ecology and Biogeography*. (In press.)

McGroddy ME, Daufresne T, Hedin LO. 2004. Scaling of C: N: P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios. *Ecology* **85**: 2390.

Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences, USA* **101**: 11001–11006.

Reich PB, Walters MB, Ellsworth DS. 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences, USA* **94**: 13730–13734.

Richardson SJ, Peltzer DA, Allen RB, McGlone MS. 2005. Resorption proficiency across a chronosequence: responses among communities and within species. *Ecology* **86**: 20–25.

Sterner RW, Elser JJ. 2002. *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*. Princeton, NJ, USA: Princeton University Press.

Vitousek P, Turner DR, Kitayama K. 1995. Foliar nutrients during long-term soil development in Hawaiian montane rain forest. *Ecology* **76**: 712–720.

Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Groom PK, Hikosaka K, Lee W, Lusk CH, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Warton DI, Westoby M. 2005. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* **14**: 411–421.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin FS, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Key words: climate, geomorphology, global biogeography, land-use history, leaf nitrogen content, leaf phosphorus content, plant chemistry, vegetation type.

Towards a multifunctional rhizosphere concept: back to the future?

The *Rhizosphere* 2004 Congress held in Munich, Germany, was organised to mark the centenary of the publication of a paper by Lorenz Hiltner in which he introduced the term 'rhizosphere' that was defined as the 'soil compartment influenced by the root' (Hiltner, 1904; Hartman, 2005). From extensive laboratory and field work on seed germination and on growth of legumes and nonlegumes under different soil management regimes, Hiltner not only proposed that plant root exudates support the development of dense bacterial communities in the rhizosphere but also confirmed that healthy roots are often colonised by nonpathogenic endophytic bacteria, which was termed the 'bacteriorhiza'. Hiltner's 'rhizosphere concept' incorporated the observed phenomena of induced soil supressiveness and rhizosphere-microflora-linked host resistence to plant pathogens that clearly laid the foundations for the later development of biological control theory. At the time, Hiltner envisioned the application of integrated management systems that included green manure, crop rotation and use of nitrogen-fixing bacterial inoculants for maintenance of plant productivity and of soil nitrogen (N) and phosphorus (P) fertility. These ideas, a century later, are regarded as central tenets in the development of low-input sustainable agricultural production. Reviews presented at the *Rhizosphere* 2004 Congress and published in this issue (Hinsinger *et al.*, pp. 293–303; Rengel & Marschner, pp. 305–312) provide timely updates on developments relating to rhizosphere development, functioning and management.

'A hundred years after publication of Hiltner's seminal paper on his 'rhizosphere concept', there are increasing calls for a return to more sustainable low-input agricultural production.'

Rhizosphere dimensions and dynamics

Hinsinger *et al.* focus on rhizosphere geometry, dynamics and functioning, which are driven by root-mediated physical, chemical and biological processes. Physical processes that distinguish the rhizosphere from bulk soil remain poorly understood, although their impacts on soil bulk density, porosity and soil structure have major implications for soil nutrient and water transport. Soil aggregate stability is of critical importance in this context, being clearly influenced by the presence of root- and microbe-derived exudates, mainly polysaccharide and glycoproteins. Mineral weathering and pedogenesis activity in the rhizosphere continues to receive attention and more recently has been the subject of interdisciplinary investigation in mycorrhizal roots systems in temperate and boreal forest ecosystems (Hoffland *et al.*, 2004).

In their review, Hinsinger *et al.* point out that the rhizosphere has no absolute geometry but represents a dynamic continuum with actual dimensions that are dependent on the process under consideration – for example, nutrient diffusion coefficients, pH, etc. Root architecture, which determines the degree of rhizosphere overlap, is a major factor controlled by plant genotype and environmental factors (see Rengel & Marschner). Factors influencing rhizosphere heterogeneity and spatio-temporal dynamics are discussed in detail. Positional redox potential (Eh), pH, water and nutrient gradients along growing roots (e.g. at apical, subapical and basal locations) have been well established, but rhizosphere heterogeneity resulting from specialised root development (e.g. cluster roots and mycorrhizas) were also highlighted. Classical and molecular tools for monitoring spatio-temporal rhizosphere dynamics are continually being developed and refined for application in rhizosphere research. Integration of microscale sampling and *in situ* physico-chemical analyses with molecular methods (e.g. FISH, GFP-reporters, *in situ* PCR) coupled to high-resolution noninvasive imaging and image analyses will allow integrative modelling of rhizosphere processes and dynamics under relevant conditions in the field.

Plant breeding and rhizosphere management

Our current understanding of rhizosphere processes contributing to plant productivity in unmanaged nutrient-limited soils could, in practice, be used in the selection of nutrient-efficient plant genotypes. Focusing on P and manganese (Mn) availability and management in limited soils, Rengel & Marschner provide a review of plant genotype-linked traits and activities that enhance nutrient availablity and uptake in the rhizosphere. In their own work, depleted rhizospheric Mn concentrations were detected in Mn-efficient as opposed to Mn-inefficient *Triticum aestivum* genotypes. Again, plant genotype-linked traits such as root morphology and rhizosphere acifidification, exudation of organic acids (e.g. citrate, malonate) and P-mobilising enzymes (phosphatases and phytase) were highlighted as being important for P or Mn uptake in limited soils. The potential importance of microbial-P-solubilising microbes in the rhizosphere was acknowledged but the authors argue the need for continued investigation of their roles under more realistic field conditions. A strong environmental case was made for development of plant breeding programmes to select nutrient-efficient crop genotypes for use in low-input agricultural production, although it was proposed that more detailed information is needed on the genetic basis of efficiency traits such as root morphology, exudation and rhizosphere microbial interactions.

The mycorrhizosphere

Hiltner was aware of the pioneering work of Frank (1885), which has been recently translated from the original German (Frank, 2005), and recognised the role of endomycorrhizas in his own studies (Hartmann, 2005). Yet, the importance of this near-ubiquitous mutualistic root–fungal symbiosis (Smith & Read, 1997) remains poorly appreciated in plant physiology and ecology (Trappe, 2005). Although the term 'mycorrhizosphere' was coined by Linderman (1988), the importance of mycorrhizas as a fungal derived rhizosphere compartment was first proposed over a decade earlier (Rambelli, 1973). Mycorrhizal fungi, in symbiosis with roots, effectively act as a bridge connecting the rhizosphere to 'bulk' soil. Through active growth of extraradical mycelium into the soil, the mycorrhizosphere greatly extends root–fungal interactions with and soil microbial communities (Jones *et al.*, 2004; Leake *et al.*, 2004; Whipps, 2004; Timonen & Marschner, 2005). Plant-derived activities in the rhizosphere (e.g. exudation of polysaccharides, glycoproteins, organic acids and nutrient-mobilising enzymes) are now known to be agumented by corresponding fungal activities in the mycorrhizosphere. Thus, it is imperative that there is more interdisciplinary effort on common questions relating to rhizosphere/mycorrhizosphere functioning and management.

Perspectives

The reviews by Hinsinger *et al.* and Rengel & Marschner published in this issue and those to appear in other plant

and soil science journals (http://www0.gsf.de/iboe/congress) provide a comprehensive view of the state-of-the-art in rhizosphere research. It is therefore only fitting that a hundred years after publication of Hiltner's seminal paper on his 'rhizosphere concept', there are increasing calls for a return to more sustainable low-input agricultural production. The integrated methods he proposed are already being applied in temperate and tropical agriculture but these can be still further optimised through a better understanding of rhizosphere/mycorrhizosphere processes that are so central to maintenance of soil fertility and productivity.

Robin Sen

Department of Environmental and Geographical Sciences,
Manchester Metropolitan University,
Manchester M1 5GD, UK
(tel +44 (0)161 2471600; fax +44 (0)161 2476318;
email r.sen@mmu.ac.uk)

References

Frank AB. 1885. Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. *Berichte der Deutschen Botanischen Gesellschaft* **3**: 128–145.

Frank B. 2005. On the nutritional dependence of certain trees on root symbiosis with belowground fungi (an English translation of A.B. Frank's classic paper of 1885). *Mycorrhiza* **15**: 267–275.

Hartmann A. 2005. Prof. Dr. Lorenz Hiltner, a pioneer in soil bacteriology and rhizosphere research. In: Hartmann A, Schmid M, Wenzel W, Hinsinger P, eds. *Rhizosphere 2004: perpectives and challenges – a tribute to Lorenz Hiltner*. Neuherberg, Germany: GSF-Forschungszentrum, 1–4.

Hiltner L. 1904. Über neurer erfahrungen und probleme auf dem gebiete der bodenbakteriologie unter besonderer berücksichtigung der gründüngung und brache. *Arbeiten der Deitschen Landwirtschaftlichen Gesellschaft* **98**: 59–78.

Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW. 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phytologist* **168**: 293–303.

Hoffland E, Kuyper T, Wallander H, Plassard C, Gorbushina A, Haselwandter K, Holmström S, Landeweert R, Lundström U, Rosling A, Sen R, Smits M, Van Hees P, Van Breemen N. 2004. The role of fungi in weathering. *Frontiers in Ecology and the Environment* **2**: 258–264.

Jones DL, Hodge A, Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* **163**: 459–480.

Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. *Canadian Journal of Botany* **82**: 1016–1045.

Linderman RG. 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathologist* **78**: 366–371.

Rambelli A. 1973. The rhizosphere of mycorrhizae. In: Marks GC, Kozlowski TT, eds. *Ectomycorrhizae, their ecology and physiology*. New York, USA: Academic Press, 299–349.

Rengel Z, Marschner P. 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. *New Phytologist* **168**: 305–312.

Smith SE, Read DJ. 1997. *Mycorrhizal Symbiosis*, 2nd edn. Cambridge, UK: Academic Press.

Timonen S, Marschner P. 2005. Mycorrhizosphere concept. In: Mukerji KG, Manoharachary C, Singh J, eds. *Microbial activity in the rhizosphere*. Heidelberg, Germany: Springer Verlag. (In press.)

Trappe JM. 2005. A.B. Frank and mycorrhizae: the challenge to evolutionary and ecologic theory. *Mycorrhiza* **15**: 277–281.

Whipps JM. 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany* **82**: 1198–1227.

Key words: low-input sustainable agriculture, mycorrhiza, plant productivity, plant–microbe interactions, rhizosphere, root architecture, soil management.

Measuring quality of service: phosphate 'à la carte' by arbuscular mycorrhizal fungi

Inorganic phosphate (P) is an essential nutrient for all living organisms, required for the synthesis of nucleic acids, phospholipids and cellular metabolites, and has an important role in the fine-tuning control of the activity of many signalling proteins. However, whereas concentrations required for proper cell function are in the millimolar range, most environmental concentrations are significantly lower. For plants, after nitrogen, P is the second most frequently limiting macronutrient for growth. In soil, P might be present in large amounts, but the preferred form for assimilation, orthophosphate (Pi), is usually very depleted owing to adsorption to soil particles or conversion into organic complexes, ranging from 1 to 10 μM (Marschner, 1995). The essential role of P in the metabolism has strained cells to develop specific mechanisms to obtain the P necessary for their growth. In order to concentrate P in the cytoplasm, a range of specific molecular transporters is set into action to adapt to different P scenarios. Plants are not an exception – for example, rice has 13 Pi transporters (Paszkowski *et al.*, 2002). However, uptake of P by plants seems to be by far much faster than rates of P release and diffusion in the soil, with the consequence that P-depleted areas form around the root. Among other less sophisticated ways to overcome this problem, such as release of phosphatases, organic acids or changes in root morphology, most terrestrial plants have chosen to use the 'Pi catering service' provided by arbuscular mycorrhizal fungi. A nice example of how arbuscular mycorrhizal (AM) fungi are able to feed their plant customers with Pi is provided in this issue (pp. 445–454) by Poulsen *et al.* These authors present molecular evidence of how the quality of the catering service can be checked – in other words, the link between the expression of specific symbiotic plant P transporters and the efficient Pi transfer to the plant.

'A complex and regulated Pi supply … how much will this symbiotic transport cost to the plant in terms of carbon?'

The symbiotic Pi uptake

Most plants have two Pi uptake pathways (Versaw *et al.*, 2002; Smith *et al.*, 2003). The so-called direct uptake, at the plant–soil interface, and the mycorrhizal or symbiotic uptake, at the plant–fungal interface. The direct pathway involves two types of Pi transporters: (i) a low-affinity transporter that is constitutively expressed; and (ii) a high-affinity transporter inducible under Pi-deficient conditions. Molecular experiments demonstrated that plants are able to suppress/reduce the direct high-affinity uptake pathway when colonized by AM fungi (Liu *et al.*, 1998; Burleigh & Harrison, 1999). Thus, Burleigh & Harrison (1999) showed very elegantly, using a split root system experiment, that a systemic signal, induced upon mycorrhization, is responsible for the shutting off of Pi transporters operating at the root epidermis or root hairs. In some cases, the plant is able to rely completely on the fungal delivery of Pi, as demonstrated earlier with radiotracer element experiments (Pearson & Jakobsen, 1993). This decision involves the active participation of fungal P transporters able to load Pi from the soil into their cytoplasm, the further translocation of phosphorous towards the plant, the release of Pi into the plant–fungal interface and the Pi uptake by the plant cells. Several of the molecular components of this complex Pi symbiotic uptake pathway have been elucidated in the last decade, including a fungal Pi transporter operating at the soil interface at low Pi concentrations (Harrison & Van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001) and several plant Pi transporters induced in cortical cells colonized by AM fungi and thus responsible for the transfer of Pi from apoplast to plant cytoplasm (Rausch *et al.*, 2001; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Nagy *et al.*, 2005). All that means a much more complex and regulated Pi supply – this restaurant is going to be expensive! and that is one of the most intriguing questions related to Pi transport: how much will this symbiotic transport cost to the plant in terms of carbon? Can we think in those terms at all?

One would assume that a higher capacity of exploring larger volumes of soil in search of P would report immediately into larger plant growth and higher concentrations of plant P. However, very often, attempts to correlate symbiotic P uptake with responses in plant growth and/or total plant P content led to very controversial results, showing that there is not a clear-cut phenotype between mycorrhizal and nonmycorrhizal plants. This is because not all plants eat the same, and neither do they dine at the same restaurants; a good example is illustrated in the article by Smith *et al.* (2004). They determined that tomato plants able to feed solely on symbiotic Pi showed no positive responses in terms of plant growth or total P content – a so-called nonresponsive plant.

Which fungus is actively delivering Pi to a specific plant? How to monitor functional mycorrhizal P uptake?

Poulsen *et al.* propose solutions to these questions using the symbiotic tomato Pi transporters, LePT3 and LePT4 (Fig. 1), as molecular markers. They show that they are effective markers to monitor functional mycorrhizal Pi uptake, even when used with an impaired mutant plant showing abnormal colonization ability. A set of experiments, performed comparing the Pi nutrition in the *rmc* tomato mutant and its wild-type tomato plant 76R when colonized by different AM fungi, allowed conclusions to be drawn about the expression pattern of several plant P transporters. Thus, although *rmc* does not differ from its wild-type genotype 76R in terms of growth or Pi absorption from the soil, it is, in contrast, unable to form symbiosis with the vast majority of AM fungi, with the exception of the *Glomus intraradices* isolate WFVAM 23. After an initial delayed phase of penetration, this fungus is able to form the usual AM fungal structures within the root cortex. The authors find that, when colonized with a compatible fungus, *rmc* obtains similar fungal Pi delivery to the wild type. This is tractable at the genetic level, showing that expression of LePT3 and LePT4 correlates with the presence of AM fungal structures within the root cortex and with an operative mycorrhizal Pi delivery route.

Interestingly, *rmc*, when inoculated with other fungi, shows a resistant phenotype, with aborted penetration attempts. These attempts did, however, not induce the expression of LePT3 or LePT4, showing that cortex colonisation is a prerequisite for LePT3 and LePT4 induction. Is it then a plant signal from AM-fungal-colonized cortical cells that induces expression of the symbiotic transporters? Or is it a fungal signal, produced once the AM fungus has reached the cortex? In any case, their results show the possibility of using these molecular markers to search for the mycorrhiza-induced signal that allows the accommodation of the mycosymbiont in the cortical cell.

One or many Pi transporters?

The obvious question is, why are so many transporters required at the symbiotic interface? Are there subdomains at the symbiotic interface? Are they all expressed at the very same time? A beautiful experiment by Harrison *et al.* (2002) showed that the low-affinity transporter MtPT4 from *Medicago truncatula* is located at the periarbuscular membrane and, more precisely, at the fine branches of mature arbuscules. Further, MtPT4 was not detected in young or senescent arbuscules, indicating that expression is coordinated with

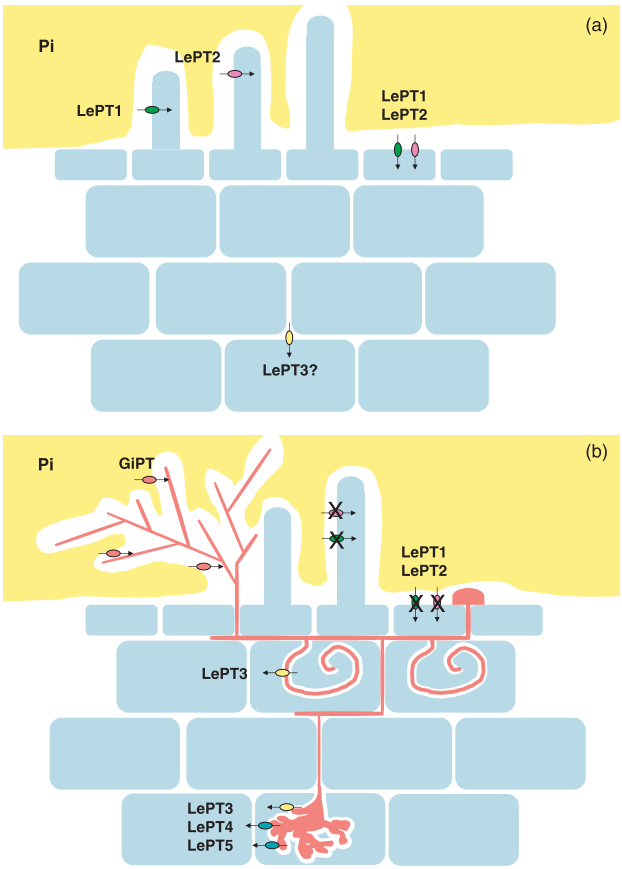

Fig. 1 Schematic overview of phosphate uptake in tomato. (a) Phosphate (P) uptake in a nonmycorrhizal root. P uptake follows the direct pathway through orthophosphate (Pi) transporters located in root hairs or epidermal cells, leaving a P-depleted area around the root. In tomato, transport is carried out by LePT1 and LePT2. Although at a very much lower level of expression, the mycorrhizal-induced LePT3 is also expressed. Localization of this transporter under nonmycorrhizal conditions is not known. (b) P uptake in a mycorrhizal root. P uptake takes place through the extraradical hyphae from the arbuscular mycorrhizal (AM) fungus spreading into the soil. Specific fungal Pi transporters (GiPT, in the case of *Glomus intraradices*) take up Pi from the soil solution beyond the P-depleted area created by the root. P is downloaded at symbiotic interfaces. Mycorrhiza-induced transporters, located at the perifungal membrane, are highly expressed in coils (LePT3) or arbuscules (LePT3, LePT4 and LePT5) taking Pi from the apoplastic space between plant and fungus. The direct Pi pathway is either down-regulated or suppressed.

arbuscule development and decay. In contrast, experiments from Karandashov *et al.* (2004) showed that the high-affinity transporter StPT3 from tomato is also expressed in cortical cells harbouring not only arbuscules but also other mycorrhizal structures, including coils. These contrasting but complementary results might explain the fact that different plants respond differently to colonization by AM. In this sense, if transporters such as StPT3 are absent in *Medicago*, then this plant will have a greater restricted window of opportunity to access fungal Pi than tomato, whose redundancy in symbiotic Pi transporters might account for a more plastic adaptation to different fungal Pi availability conditions. Therefore, yes, in some cases the fungal 'Pi catering service' can be too expensive. It is well known that several plants do not respond positively to mycorrhization and further still do not even show growth depression responses. In the case of *Medicago*, this could be directly correlated to the formation of more coils than arbuscules, with larger biomass that would demand a higher supply of carbon from the host plant (Smith *et al.*, 2004), and where in addition MtPT4 might not be expressed.

Future questions

Besides those raised above, many more questions regarding Pi transport remain unanswered. For instance, what about other fungal Pi transporters? Do some operate with low affinity, as predicted from kinetic studies? How is the Pi translocated throughout the fungal hyphae towards the plant? Is it in the form of polyphosphates, as reported? Which signals induce the further mobilization of Pi? What mechanisms are responsible for Pi efflux into the apoplast? Does the fungal alkaline phosphatase play an important role in this process, as suggested by enzymatic and gene expression analyses? How is this expensive route maintained? How can the cost in terms of assimilates transferred to the fungus be calculated? The novel approach by Poulsen *et al.*, in which molecular and physiological techniques are combined, provides new perspectives on the mechanisms of symbiotic Pi transport and will undoubtedly pave the way for elucidating this complex process further. The call now is to increase the interdisciplinary nature of this approach by integrating other disciplines to broaden our current understanding of AM symbiosis.

Natalia Requena

University of Karlsruhe, Institute of Applied Biosciences – Fungal–Plant Interactions Group, Hertzstrasse 16, D-76187 Karlsruhe, Germany
(tel +49 (0)721 6084626; fax +49 (0)721 6084509; email natalia.requena@bio.uni-karlsruhe.de)

References

Burleigh SH, Harrison MJ. 1999. The down-regulation of Mt4-like genes by phosphate fertilization occurs systemically and involves phosphate translocation to the shoots. *Plant Physiology* **119**: 241–248.

Harrison MJ, Dewbre GR, Liu J. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* **14**: 2413–2429.

Harrison MJ, van Buuren ML. 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* **378**: 626–629.

Karandashov V, Nagy R, Wegmüller S, Amrhein N, Bucher M. 2004. Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **101**: 6285–6290.

Liu H, Trieu AT, Blaylock LA, Harrison MJ. 1998. Cloning and characterization of two phosphate transporters from *Medicago*

truncatula roots: regulation in response to phosphate and colonization by arbuscular mycorrhizal (AM) fungi. *Molecular Plant–Microbe Interactions* **11**: 14–22.

Maldonado-Mendoza IE, Dewbre GR, Harrison MJ. 2001. A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. *Molecular Plant–Microbe Interactions* **14**: 1140–1148.

Marschner H. 1995. Nutrient availability in soils. In: Marschner H, ed. *Mineral Nutrition of Higher Plants.* London, UK: Academic Press, 483–507.

Nagy R, Karandashov V, Chague V, Kalinkevich K, Tamasloukht M, Xu Jakobsen I, Levy AA, Amrhein N, Bucher M. 2005. The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. *Plant Journal* **42**: 236–250.

Paszkowski U, Kroken S, Roux C, Briggs SP. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **99**: 13324–13229.

Pearson JN, Jakobsen I. 1993. The relative contribution of hyphae and roots to phosphorous uptake by arbuscular mycorrhizal plant, measured by dual labelling with 32P and 33P. *New Phytologist* **124**: 489–494.

Poulsen KH, Nagy R, Gao L-L, Smith SE, Bucher M, Smith FA, Jakobsen I. 2005. Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. *New Phytologist* **168**: 445–453.

Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, Bucher M. 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature* **414**: 462–466.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133**: 16–20.

Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* **162**: 511–524.

Versaw WK, Chiou T-J, Harrison MJ. 2002. Phosphate transporters of *Medicago truncatula* and arbuscular mycorrhizal fungi. *Plant Soil* **244**: 239–245.

Key words: LePT3, LePT4, *Medicago truncatula*, mycorrhiza, P transport, Pi transporters, tomato.

Letters

Why don't leaf-eating animals prevent the formation of vegetation? Relative vs absolute dietary requirements

Introduction

Hairston *et al.* (1960) asked why plant biomass accumulates in sufficient amounts to form vegetation, rather than being closely cropped by leaf-eating herbivores. They suggested a top-down hypothesis: predators/parasites prevent herbivore populations from building up to carrying capacity. Currently, however, plants are considered to be of too low a nutritional quality to be suitable as food for animals (e.g. Hartley & Jones, 1997; Polis, 1999). Most plants are ignored by most foliovores and that is why vegetation forms. Thus contemporary dynamic global vegetation models (DGVMs) typically ignore herbivory as a determinant of above-ground biomass (AGB) and mainly focus on net primary productivity (NPP) (e.g. Beerling & Woodward, 2001). However, NPP and AGB are not always correlated and herbivory is not always negligible. For example, temperate forests often have higher biomass than tropical forests (Midgley, 2001), although it remains to be seen whether differences in herbivory will explain this. In Africa, which uniquely still has most of its Pleistocene fauna, large herbivores such as elephants (Laws, 1970; Dublin *et al.*, 1990) can seriously reduce AGB below that expected from NPP. Generalist herbivores, such as goats (e.g. Hendriks *et al.*, 1992) overgraze many natural pastoral lands in Africa (e.g. Hoffman, 1997). This should not occur if plants are of too low a nutrient quality to be eaten.

Nevertheless, for many areas of Africa and elsewhere on the globe, abiotic or top-down factors are apparently more important in determining vegetation structure and AGB than are bottom-down factors such as herbivory. Plants appear to be ahead of foliovores in the evolutionary arms race and vegetation develops. Because forests date back to the Carboniferous, it appears that plants have always been ahead and vegetation has developed.

Besides nutrient quality, other factors also contribute to the reasons why plants are not totally consumed (e.g. Hartley & Jones, 1997; Polis, 1999). Secondary and other chemicals

New Phytologist

effectively lower food quality further, as do mechanical defences by increasing feeding time. The high patchiness of vegetation mosaics, high environmental variation (such as annual variation and interannual variation in climate), the vast numbers of plant species present, trophic structures and the impact of plant phenology/seasonality also limit herbivore reduction of AGB. Amongst nutrients, levels of protein (which largely corresponds to leaf nitrogen concentration, [N]) are seen as especially crucial (White, 1993; Polis, 1999).

Increasing quality of plant leaves as food through time

I argue that [N] in leaves in contemporary angiosperms is the highest it has ever been. Therefore leaf [N] can not presently be absolutely limiting because herbivory existed before angiosperms proliferated. For example, leaf-feeding arthropods existed in the late Carboniferous (Chaloner *et al.*, 1991) and suites of herbivorous dinosaurs, including gigantic sauroprods, are well known from the Jurassic (e.g. Bakker, 1986).

The prediction that [N] in contemporary angiosperm leaves is likely to be the highest ever is possible because it is well known that [N] levels correlate strongly and positively with hydraulic capacities of plant species; the photosynthetic assimilation rate A_{max} correlates strongly with [N] and with rates of stomatal conductance (e.g. Wright *et al.*, 2004). High rates of conductance require efficient hydraulic systems which are capable of delivering water at fast rates and of surviving cavitations during periods of stress. Thus the high levels of [N] in contemporary angiosperms, when compared with contemporary gymnosperms, cycads and ferns (e.g. Midgley *et al.*, 2002), have only been possible because the evolution of xylem vessels is largely an angiosperm invention. Therefore, compared with leaf nutrient status of present angiosperms, the dominant plants of previous geological periods such as the Jurassic, when cycads, conifers and seed ferns dominated (e.g. Willis & McElwain, 2002), were probably of a lower nutrient concentration. Gymnosperms are also well known for high levels of secondary chemicals and possession of sclerophyllous small leaves – these are attributes which further limit their food quality.

Not only did nonangiosperm plants have photosynthetic organs of inherently low rates of photosynthesis, but higher levels of CO_2 in previous times would have also acted to lower leaf [N] levels further per mouthful. For example, Beerling & Woodward (2001) argued that Palaeozoic plants must have had exceptionally low Rubisco (= leaf [N]) levels to explain the relative lack of fractionation of ^{13}C noted in leaves from these periods, given their low stomatal densities and high ambient CO_2 levels of that period. Recently, Korner (2004) summarised CO_2 enrichment studies by concluding that higher CO_2 levels tend to result in depleted leaf [N]. From the perspective of a foliovore, I suggest that high CO_2 levels in previous epochs would have resulted in a lowering of forage quality per mouthful or bite.

Angiosperms appeared to have increased in abundance geologically in parallel with declining CO_2 levels, whereas other groups such as gymnosperms have declined with declining CO_2 levels (e.g. Willis & McElwain, 2002). Whether this is an explanation or correlation, the point is that in previous times, with both high CO_2 levels and non-angiosperm plants, leaf quality must have been lower than it is at present, yet these periods supported the full range of foliovores. Present leaf quality is therefore not an absolute limit for it being considered as food for animal – it is a relative limit. Presumably, ancient nonmammalian foliovores would find contemporary angiosperms highly palatable.

A role for Red Queen?

I suggest the Red Queen Hypothesis (evolving to stay in the same relative place) explains in part why the world has vegetation despite the contemporary predominance of relatively nutrient-rich angiosperm leaves. This is because herbivore nutritional requirements will evolve in concert with food quality. Thus areas or epochs with food of lower relative quality will favour herbivores with relatively lower food quality requirements. As an example, Grubb (1992) noted the anomalous occurrence of extreme leaf spinescence in many Australian plants despite the low leaf [N] of these plants growing in this nutrient-poor continent. Again, low-nutrient plants should not be suitable food for herbivores and thus should not have experienced persistent significant vertebrate herbivory to have caused the evolution of antivertebrate herbivore defences such as spines. Grubb (1992) hypothesised that significant herbivory pressure was exerted by endemic Australian marsupials: their lower metabolical rates, when compared to eutherian mammals, would have allowed the former access to a relatively lower-quality diet. Marsupials also have ruminant-like fore-stomachs and an ability to recycle urinary nitrogen. The point is that herbivorous animals can deal with relatively low-quality food by the evolution of larger size, slower metabolisms/activity schedules and more efficient digestion and feeding behaviour.

The evolution of relatively higher-quality food in leaves as a consequence of the evolution of the angiosperms (nutritious 'seeds' are not an angiosperm invention) may have facilitated the spread of mammal foliovores with relatively higher metabolic rates. In part, the reason why we have vegetation then becomes the question of what caused the major evolutionary transitions between foliovore guilds. Possibly the evolution of angiosperms led to the competitive displacement of nonmammalian herbivores with lower metabolic rates.

Conclusion

I have argued that low food quality *per se* is not an absolute barrier to plants being considered as food. It is a relative barrier that depends on herbivore physiology, size, feeding habits and

activity schedule/behaviour. Even wood, possibly the lowest-quality plant product, is considered as food (i.e. energy to fund N fixation) by some organisms in some places (e.g. Martin, 1991). The reason vegetation develops is because the forage nutrient requirements of foliovores evolves, leaving most plant species as being relatively unpalatable to most animal species.

Acknowledgements

I thank Peter Grubb, Andrew Illius, William Bond, Ian Woodward, David Coomes, Norman Owen-Smith, Nicci Illing and referees for useful comments.

J. J. Midgley

Botany Department, University of Cape Town,
P. Bag Rondebosch, 7701, South Africa
(tel +27 21 6502447; fax +27 21 6504041;
email midgleyj@botzoo.uct.ac.za)

References

Bakker RT. 1986. *Dinosaur Heresies*. London, UK: Penguin.

Beerling DJ, Woodward FI. 2001. *Vegetation and the Terrestrial Carbon Cycle: Modelling the First 400 Million Years*. Cambridge, UK. Cambridge University Press.

Chaloner WG, Scott AC, Stephenson J. 1991. Fossil Evidence of Plant–Arthropod Interactions in the Palaeozoic and Mesozoic. In: Chaloner WG, Harper JL, Lawton JH, eds. *The evolutionary interaction of animals and plants*. London, UK: Royal Society, 1–9.

Dublin HT, Sinclair ARE, McGlade J. 1990. Elephants and fire as causes of multiple stable states in the Serengeti–Mara woodlands. *Journal of Animal Ecology* **59**: 1147–1164.

Grubb PJ. 1992. A positive distrust in simplicity – lessons from plant defences and from competition among plants and among animals. *Journal of Ecology* **80**: 585–610.

Hairston NG, Smith FE, Slobodkin IB. 1960. Community structure, population control and competition. *American Naturalist* **94**: 421–425.

Hartley SE, Jones CG. 1997. Plant chemistry and herbivory, or why the world is green. In: Crawley MJ, ed. *Plant Ecology*. London, UK: Blackwell Science, 284–324.

Hendriks H, Novellie PA, Bond WJ, Midgley JJ. 2002. Food-choice of goats in Richtersveld. *African Journal of Range and Forage Science* **19**: 1–11.

Hoffman MT. 1997. Human impacts on vegetation. In: Cowling RM, Richardson DM, Pierce SM, eds. *Vegetation of Southern Africa*. Cambridge, UK: Cambridge University Press, 507–534.

Korner C. 2004. Through enhanced tree dynamics carbon dioxide enrichment may cause tropical forests to lose carbon. *Phiosophical Transactions of the Royal Society London, B* **359**: 493–498.

Laws RM. 1970. Elephants as agents of habitat and landscape change in east Africa. *Oikos* **21**: 1–15.

Martin MM. 1991. The evolution of cellulose digestion in insects. In: Chaloner WG, Harper J, Lawton JH, eds. *The Evolutionary Interaction of Animals and Plants*. London, UK: Royal Society, 105–111.

Midgley JJ. 2001. Do mixed-species mixed-size indigenous forests following the self-thinning line? *Trends in Ecology and Evolution* **12**: 661–662.

Midgley JJ, Midgley GF, Bond WJ. 2002. Why were some dinosaurs so large? A food quality hypothesis. *Evolutionary Ecology Research* **4**: 1093–1095.

Polis GA. 1999. Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos* **86**: 3–15.

White TCR. 1993. *The Inadequate Environment. Nitrogen and the Abundance of Animals*. Berlin, Germany: Springer-Verlag.

Willis KJ, McElwain JC. 2002. *The Evolution of Plants*. Oxford, UK. Oxford University Press.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavendar-Bares J, Chapin T, Cornelissen JH, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Key words: above-ground biomass, angiosperms, herbivory, nitrogen content, vegetation structure.