Tansley review

Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change

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Contents

Summary

Biological stoichiometry theory considers the balance of multiple chemical elements in living systems, whereas metabolic scaling theory considers how size affects metabolic properties from cells to ecosystems. We review recent developments integrating biological stoichiometry and metabolic scaling theories in the context of plant ecology and global change. Although vascular plants exhibit wide variation in foliar carbon : nitrogen : phosphorus ratios, they exhibit a higher degree of 'stoichiometric homeostasis' than previously appreciated. Thus, terrestrial carbon : nitrogen : phosphorus stoichiometry will reflect the effects of adjustment to local growth conditions as well as species' replacements. Plant stoichiometry exhibits size scaling, as foliar nutrient concentration decreases with increasing plant size, especially for phosphorus. Thus, small plants have lower nitrogen : phosphorus ratios. Furthermore, foliar nutrient concentration is reflected in other tissues (root, reproductive, support), permitting the development of empirical models of production that scale from tissue to whole-plant levels. Plant stoichiometry exhibits large-scale macroecological patterns, including stronger latitudinal trends and environmental correlations for phosphorus concentration

(relative to nitrogen) and a positive correlation between nutrient concentrations and geographic range size. Given this emerging knowledge of how plant nutrients respond to environmental variables and are connected to size, the effects of global change factors (such as carbon dioxide, temperature, nitrogen deposition) can be better understood.

I. Introduction

Plant ecophysiologists and terrestrial ecosystem ecologists are confronted with the challenge of connecting simultaneous changes in multiple biogeochemical cycles (e.g. rising CO_2, enhanced nitrogen (N) deposition) with alterations in climate (temperature, rainfall) and land use to make useful predictions about the future of plant communities and their functional characteristics. Many of the pieces of knowledge needed for this effort are already in hand. For example, we know that climatic variables, such as water availability, have major impacts on key traits, such as plant size and stature (Niklas, 1994) and rates of plant production (Huxman et al., 2004). We know that variations in plant growth rate and physiological rates closely track variations in limiting nutrients (Lawlor, 1994; Van der Werf et al., 1994), and that multiple leaf characteristics show close inter-relationships of limiting nutrients with physiological and metabolic functions (Reich et al., 1997; Wright et al., 2004, 2005). Furthermore, we are beginning to gain a larger scale picture of how such traits are distributed in ecological communities and across broad geographic gradients (Wright et al., 2001; Westoby et al., 2002; Swenson & Enquist, 2007; Chave et al., 2009). However, we do not yet have generalizable theoretical and conceptual frameworks to explicitly interconnect key ecophysiological and ecological variables with environmental features relevant to global change. In this article, we attempt to show how recent developments in biological stoichiometry theory (BST) and metabolic scaling theory (MST) can contribute to these efforts.

BST involves the study of the balance of multiple chemical elements (especially carbon (C), nitrogen (N) and phosphorus (P)) in living systems (Elser et al., 2000b; Elser & Hamilton, 2007). An extension of the theory of ecological stoichiometry (Sterner & Elser, 2002), BST attempts to determine the underlying physiological, cellular and molecular underpinnings of the organismal processing of chemical elements, to understand their evolution and to connect both of these to ecosystem material flows. Although much of the work relevant to BST has focused on trophic interactions and heterotrophic biota (crustacean zooplankton, insects, bacteria), the research approach of BST also has broad relevance for photoautotrophic taxa, whose C : N : P ratios should also reflect their underlying biochemical allocations and life history strategies (Sterner & Elser, 2002). MST seeks to explain the observed patterns of allometric scaling in terms of the geometry of the hierarchical branching of vascular networks that distribute energy and materials in organisms, including plants (Niklas, 1994; West et al., 1997; Enquist et al., 1999; Enquist, 2002; Price et al., 2007). West et al. (1997) showed that, based on the geometric consequences of the underlying assumptions of MST, for a volume-filling network a measure of total metabolic rate (Y) should scale exponentially with body mass (M) with an exponent of $3/4$. Although evidence for the generality of this exponent is equivocal (Muller-Landau et al., 2006; Reich et al., 2006c; Enquist et al., 2007a, 2009), there is little dispute that plant size is a key component of a plant's life history strategy (Marba et al., 2007)

Connections between BST and MST have been forged in a number of recent publications (Gillooly et al., 2002, 2005; Kerkhoff et al., 2005; Niklas et al., 2005; Jeysasingh, 2007; Allen & Gillooly, 2009). Allen & Gillooly (2009) pointed out that, although MST and BST have generally emphasized different currencies (energy and elements, respectively), they are closely linked on three levels. First, at the subcellular level, the fundamental 'machines' of energy and material capture and transformation (e.g. chloroplasts, mitochondria and ribosomes) generally have characteristic elemental compositions. Second, at the level of the whole organism, the relationships among size, composition and function probably depend on the relative allocation to structural (e.g. bone, sclerenchyma) vs metabolically active (e.g. muscle, palisade layer) tissues, and their respective stoichiometric compositions. In plants, structural investment is likely to be of some considerable importance in understanding size-dependent variation in C : nutrient ratios, as biomechanical support involves the production of C-rich, low-nutrient, low-turnover woody materials. Finally, at the cellular, organismal and even the whole ecosystem level, material and energy perspectives are complementary (*sensu* Reiners, 1986) in that the uptake and transformation of materials and energy require both metabolic energy together with material substrates and the molecular machinery of life. By drawing explicit links between energetic and material currencies across multiple levels of organization, BST and MST may provide an integrative framework for understanding how plants and vegetation systems respond to global change.

In this article, we attempt to further integrate recent theoretical, observational and experimental work related to BST and MST in vascular plants. We first provide a brief review of the environmental, size and taxonomic influences

on plant C : N : P ratios and describe how stoichiometric traits can be incorporated into MST-based approaches to plant performance and productivity. Next, we present an overview of some new and recently published macroecological analyses of plant C : N : P stoichiometry at hemispheric scales and as a function of plant dispersal strategy. We end by describing how these relationships relate to important aspects of global change. The range of topics and related accumulation of data relevant to the interface of plant allometry, macroecology and stoichiometry is extremely large and increasing. Thus, we cannot hope to provide a comprehensive review. Instead, in completing this effort, we hope to highlight recent progress in connecting theories of plant size, growth and metabolism, and stoichiometry, and highlighting macroecological patterns of stoichiometric variation. In the process of linking these theories and documenting these macroecological patterns, we point towards some ways in which these connections might help in predicting plant and terrestrial ecosystem response to global change.

II. Variation in plant C : N : P ratios: how much and what are the sources?

Because the stoichiometry of autotroph biomass is associated with many physiological (e.g. in the context of the 'leaf economics spectrum,' Wright *et al.*, 2004) and ecosystem (Sterner & Elser, 2002) processes, perhaps the most fundamental question, at least initially, is: how much variation is there in the stoichiometric composition of plants? Elser *et al.* (2000a) provided an initial systematic macroecological overview of the elemental composition of the major elements (C : N : P) in plant tissues, focusing primarily on foliage, and compared these with observations of photoautotroph biomass in lakes. For both sets of data, Elser *et al.* (2000a) reported extensive variation in the C : N, C : P and N : P ratios. For example, in terrestrial plants, C : N ranged from *c.* 5 to > 100 and C : P ranged from < 250 to > 3500. Reflecting reduced allocation to low-nutrient structural materials, the range of variation in freshwater biomass C : nutrient stoichiometry was much less. Nonetheless, in both terrestrial plants and aquatic autotrophs, N : P similarly ranged from < 5 to > 65 and showed similar mean values (*c.* 28–30). Similar findings for terrestrial plants have also been reported by McGroddy *et al.* (2004), Reich & Oleksyn (2004) and Han *et al.* (2005). Interestingly, Kerkhoff *et al.* (2005) reported that the variation in N : P was not related to the total amount of standing biomass (g m^{-2}), indicating that vegetation ranging from grasslands, shrublands and forests does not differ systematically in N : P ratio.

Why are terrestrial plants characterized by such a wide variation in N and P concentrations as well as N : P ratio? Although C : nutrient ratios are clearly size depen-

dent, probably reflecting metabolic activity and/or investment in C-rich structural materials, it remains an open question as to how environmental, developmental, genetic and physiological factors interact to control variation in plant nutrient concentration. We have identified two potential contributors to the observed variation. First, plant nutrient concentration may simply reflect variation in the substrates in which the plants are growing (i.e. the 'you are what you eat' model or, for plants, 'you are what you root in'). Second, the observed variation may reflect genetically determined, physiologically necessary values, regardless of substrate. In other words, to what extent do plants exhibit stoichiometric homeostasis? Answering this question highlights the possible roles of both evolutionary and ecophysiological processes in modulating plant C : N : P ratios.

1. C : N : P homeostasis?

'Stoichiometric homeostasis' refers to the degree to which an organism maintains its C : N : P ratios around a given species- or stage-specific value (Sterner & Elser, 2002) despite variation in the relative availabilities of elements in its resource supplies. In early forms of stoichiometric theory, photoautotrophs (cyanobacteria, algae, plants) were considered to have very weak stoichiometric homeostasis, whereas metazoans, and perhaps bacteria, were considered to have strict homeostasis which allowed for no change in C : N : P ratios in biomass. The latter assumption was largely taken for purposes of analytical tractability in theoretical models (e.g. Sterner, 1990; Andersen, 1997), whereas the former is a direct corollary of Droop-type cell quota growth kinetics well known from algal physiology (Sterner & Elser, 2002). However, it is clear from various studies that neither of these assumptions is strictly true. That is, animals and bacteria do not have completely rigid C : N : P ratios, but instead exhibit variations, albeit muted, in their biomass C : N : P ratios in response to dietary and media variations, whereas many algae and plants cannot faithfully mirror the N : P ratios of the environmental supply across all ranges of variation. This led Sterner & Elser (2002) to propose a continuously variable regulation parameter (*H*) to quantify the degree of stoichiometric homeostasis exhibited by a particular organism, which can be readily quantified by growing an organism across a wide range of environmental or dietary elemental ratios (*x*), measuring the organism's resulting elemental composition (*y*) and then plotting the log-transformed values of each to find the slope (1/*H*) of the resulting relationship:

$$\log(y) = \log(c) + (1/H)\log(x) \qquad \text{Eqn 1}$$

Determining *H* for vascular plants is important, because it can tell us whether or not the observed variations in foliar

C : N : P ratios in ecological settings are simply a result of local physiological adjustment of extant species to local nutrient supplies, or whether they reflect species' turnover of taxa differing in some kind of taxon- and/or size-dependent C : N : P stoichiometric signature.

Classic data for the green alga *Scenedesmus* (Rhee, 1978) show a complete lack of stoichiometric homeostasis (*H* = 1) across the range of N : P ratios supplied (Sterner & Elser, 2002). Recognizing that bulk (total) soil N : P ratios may not be completely faithful indices of the N : P ratio of available nutrients, examples of similar analyses for vascular plants are shown in Fig. 1. In contrast with *Scenedesmus*, two species of grasses growing at N : P ratios ranging from < 0.2 to 75 (by mass) changed their N : P ratio from *c.* 3 to *c.* 25, yielding estimates of *H* of *c.* 3.5 (Ryser & Lambers, 1995). Data for the sedge *Carex curta* grown in the glasshouse also exhibited considerable stoichiometric plasticity in biomass N : P ratio, with relatively low *H* values of 2.5–2.9 under various conditions of light intensity and absolute nutrient supply (Güsewell, 2004). Güsewell (2004) summarized data for other plant studies, reporting *H* values for N : P ratios ranging from 1.7 to 4.6. Intriguingly, field analyses of 41 species of wetland plants and aquatic macrophytes suggested strong stoichiometric homeostasis with little observed variation in foliar N : P ratio within a species across a broad range of nutrient supply conditions (Demars & Edwards, 2007). For comparison, the data and examples summarized in Makino *et al.* (2003) for different bacterial species indicated strong (*H* = 5.2–6.0) or strict (*H* = infinity) homeostasis in C : P and N : P ratios. More analyses of these kind are needed for plants, so that a robust assessment of the strength of stoichiometric regulation on plant C : N : P ratios can be attempted. Laboratory or glasshouse studies are best suited for this because they remove the difficulties in assessing the N : P ratio of nutrients actually available to plants from natural soil. It is also important to assess whole-plant elemental composition rather than just focal tissues (e.g. leaves).

2. Ecological and evolutionary signals in the variation of C : N : P ratios

Despite potential influences of local environmental conditions, nutrient storage and tissue-specific differences in elemental composition, various studies have identified taxonomic or phylogenetic signals (*sensu* Blomberg *et al.*, 2003) in C : N : P ratios of field-collected plants (e.g. Thompson *et al.*, 1997; Broadley *et al.*, 2004; Kerkhoff *et al.*, 2006; Demars & Edwards, 2007; Townsend *et al.*, 2007). Recognizing that not all plants at a given site will experience precisely the same nutrient environment, another way to evaluate taxon-level contributions to plant stoichiometric variation is to examine the variation in C : N : P ratios among plants at a given local site. If plant stoichiometry is largely determined by highly variable uptake in response to local growth conditions, we would expect high homogeneity in plant C : N : P ratios among species in a given locality. Conversely, if plant stoichiometry is a reflection of functionally important ecological strategies associated with occupying different ecological niches that might permit coexistence, we would expect major differences in foliar nutrient concentration among plant species in a common site.

Several recent studies have provided data to test these ideas. Kraft *et al.* (2008) studied > 1089 species of tree within 25 ha of western Amazonian rainforest. They reported that plant N concentration varied by a factor of 10 across the species studied (P concentration was not measured). For comparison, this variation is similar to the 20-fold range observed across the entire range of all species
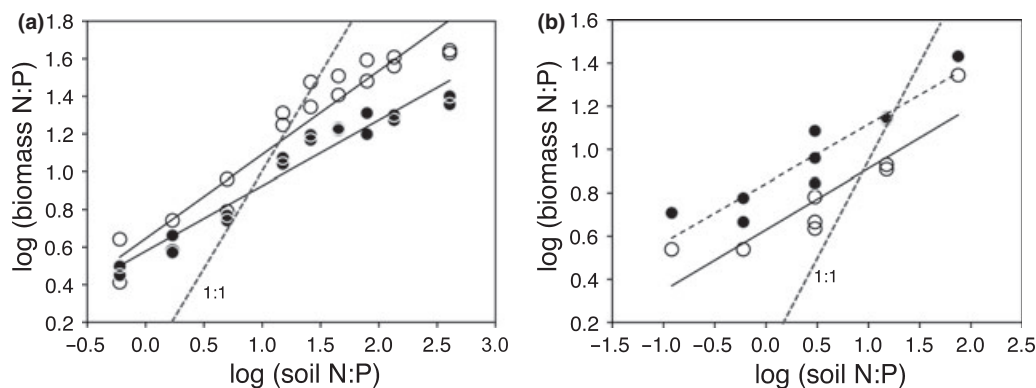

Fig. 1 Homeostasis in photoautotrophs. (a) Relatively strong stoichiometric homeostasis in the sedge *Carex curta* grown at two light intensities. Open circles, high light (*H* = 2.24); closed circles, low light (*H* = 2.88). (b) Stronger homeostasis in two species of grass. Open circles, fast-growing *Dactylis glomerata* (*H* = 3.54); closed circles, slow-growing *Brachypodium pinnatum* (*H* = 3.66). Note that the fast-growing species has a consistently lower N : P ratio than the slow-growing species, consistent with the 'growth rate hypothesis' (GRH) (see discussion of GRH in text). N : P values given as mass ratios. The 1 : 1 line is shown; data parallel to the 1 : 1 line indicate a complete lack of stoichiometric homeostasis (*H* = 1).

collected globally, reported by Elser *et al.* (2000a). Similar findings of large local variation in foliar nutrients were reported in studies of a smaller number of tree species in lowland forest in French Guiana (Hattenschwiler *et al.*, 2008) and in Costa Rica (Townsend *et al.*, 2007; also see review of Townsend *et al.*, 2008), summarized in Fig. 2. Indeed, at local rainforest sites considered by Townsend *et al.* (2008), the observed interspecific range of foliar N : P did not saturate as more species were added to the observation set, even for diversity levels as high as 150 species. A similar comprehensive analysis of environmental and phylogenetic contributors to plant N and P concentrations, and their covariance, has recently been completed for grassland plants in China (He *et al.*, 2009). Overall, these findings suggest that community-level processes, such as competition, that establish relative species' dominance will affect the coupled processing of C and nutrients in terrestrial ecosystems, and that the stoichiometry of plant biomass does not merely mirror local environmental conditions, regardless of which taxa are present.

These taxon-level signals can be readily understood because different species and phylogenetic groups are characterized by, among other things, differences in growth form, growth rate, stature and storage capacity, which impose a variety of structural- and size-related constraints on their construction and metabolism and thus on their C : N : P stoichiometry. Such linkages may not be surp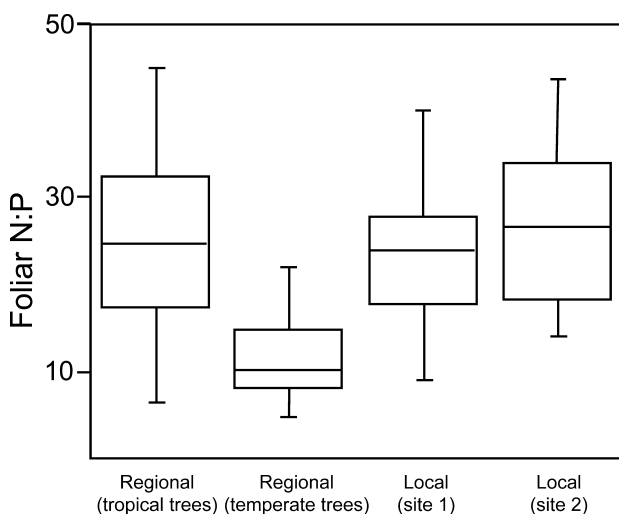rising to plant functional ecologists and may be increasingly useful to biogeochemists and ecosystem ecologists wishing to connect plant function to major environmental gradients and perturbations at larger scales.

III. The growth rate hypothesis in terrestrial plants and the scaling of whole-plant N : P stoichiometry and production

The growth rate hypothesis (GRH) was originally introduced to explain the variation in the C : N : P stoichiometry of crustacean zooplankton, and proposed that animals have low body C : P and N : P ratios because of increased allocation to P-rich ribosomal RNA in support of faster growth rates (Elser *et al.*, 2000b; Sterner & Elser, 2002). However, all living things invest in ribosomes to support the protein synthesis demands of growth, and thus the applicability of GRH to vascular plants has attracted recent interest. Indeed, in leaves, high nutrient concentrations (both N and P) tend to be associated with the 'live-fast/die-young' end of the leaf economics spectrum (Wright *et al.*, 2004). Such leaves tend to be short lived and structurally flimsy, with thin lamina (Nielsen *et al.*, 1996) and high specific leaf area (Reich *et al.*, 1998; Thomas & Winner, 2002), as well as high photosynthetic capacity and dark respiration rates (Reich *et al.*, 1992, 1997, 2008; Wright *et al.*, 2004, 2005). At the other end of the spectrum, low-nutrient leaves tend to be long lived and tough, with lower metabolic capacities. Together, these analyses establish a strong association between plant tissue nutrient concentration and tissue turnover time (Nielsen *et al.*, 1996; Wright *et al.*, 2004). This is important because plant investment in low-nutrient tissues that last longer may be coupled to ecological strategies related to space occupancy or canopy dominance (Poorter, 1994; Westoby *et al.*, 2002). Coupled with the lower rate of energetic return, such low-nutrient biomass is also likely to be less appealing to herbivores (Mattson, 1980; Coley *et al.*, 1985; Cebrian, 1999; Perez-Harguindeguy *et al.*, 2003), reflecting allocation to various chemical and structural defenses that may themselves differ in terms of stoichiometric investment (Craine *et al.*, 2003).

Multiple comprehensive reviews (Garten, 1976; Wright *et al.*, 2005; Kerkhoff & Enquist, 2006; Niklas, 2006; Reich *et al.*, 2010) have demonstrated that, although leaf N and P concentrations (N_L, P_L, respectively) are highly correlated across species, N tends to increase more slowly with P according to the function $N_L = aP_L^{\ b}$ with an exponent value $b < 1.0$ (Fig. 3). Although some studies have yielded estimates of b of $\sim^3/_4$ (e.g. Niklas *et al.*, 2005; Kerkhoff & Enquist, 2006; Niklas, 2006), the most comprehensive study to date (Reich *et al.*, 2010; a compilation of > 9500 observations of N_L and P_L across most taxonomic groups and biomes) suggests that the true value of b is $^2/_3$ and is independent of plant phylogenetic group or biome.

Fig. 2 Summary plot showing the variation in leaf-level N : P ratio at different spatial scales. Note that there exists considerable variation in foliar nutrients at small scales. The two local scales show that tree species within two tropical tree communities are characterized by similar variation that occurs at much larger geographic scales. Such high local variation suggests an important role of species-specific niche differentiation in driving the variation in plant C : N : P ratios, in addition to effects of physiological plasticity as a result of environmental conditions such as temperature, water availability and soil nutrient supplies. Redrawn from fig. 1(a) in Townsend *et al.* (2008) with permission.
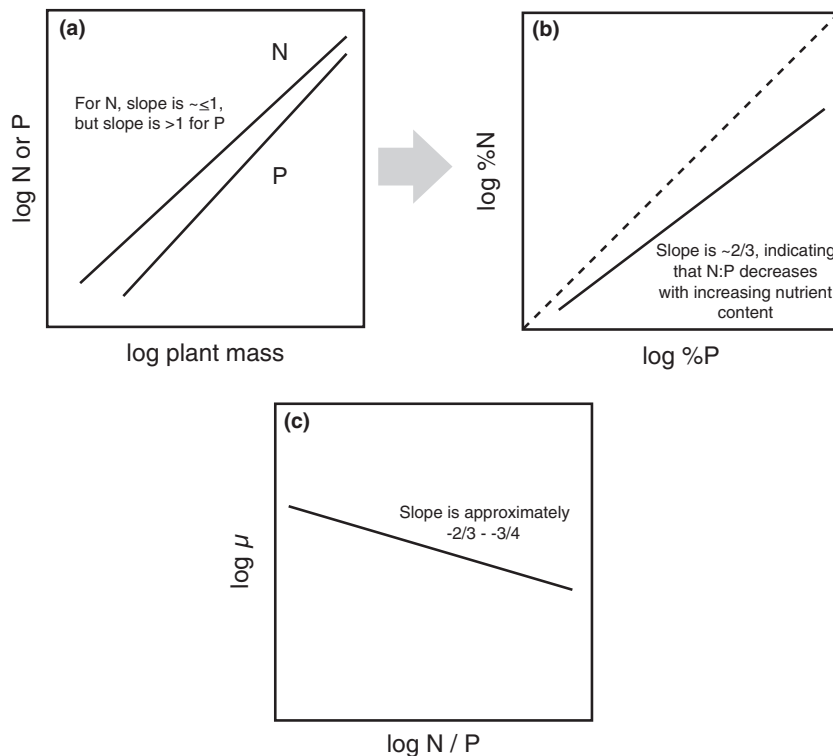
Fig. 3 A schematic view illustrating that the allometric scaling of N and P in leaves differs (a), such that the leaf N : P ratio decreases as the overall leaf nutrient concentration increases (b). Furthermore, the leaf growth rate (μ; Niklas *et al.*, 2005) or surrogates of the growth rate (such as specific leaf area; Reich *et al.*, 2010) are negatively correlated with the leaf N : P ratio (c), as predicted by the growth rate hypothesis. The figure is based on the studies of Niklas *et al.* (2005) and Reich *et al.* (2010).

Regardless of the specific value of the exponent ($^2/_3$ vs $^3/_4$), because the exponent is less than unity, N : P decreases systematically with increasing leaf nutrient concentration (as $N_L/P_L = aP_L^{b-1}$). This 'diminishing return' (*sensu* Niklas *et al.*, 2007) may reflect disproportionate investments in P-rich ribosomal RNA required to rapidly convert the products of metabolism and photosynthesis into organismal growth (consistent with GRH). This line of argument is supported by recent models of phytoplankton involving trade-offs between resource acquisition and biomass proliferation (Klausmeier *et al.*, 2004). Furthermore, the disproportionate increase in P_L with N_L in vascular plants is coincident with the scaling of key physiological parameters (specific leaf area, photosynthetic capacity) with growth rate (Reich *et al.*, 2010), providing support for GRH.

However, only a few experimental studies have directly assessed the GRH for particular plant species. Among those that have, the results are mixed. Ågren (2004) reported that P-limited birch (*Betula pendula*) seedlings displayed decreased N : P at high relative growth rates (consistent with GRH); however, N-limited plants did not show this pattern, probably reflecting P storage under N limitation. More recently, Matzek & Vitousek (2009) found that faster growth was correlated with increased nutrient concentrations and decreased protein : RNA and N : P ratios for fast- and slow-growing *Pinus contorta*. Further, in a glasshouse experiment, the increases in growth rate for seedlings of 14 species of *Pinus* grown at high vs low nutrient levels were accompanied by increased nutrient concentrations and decreased protein : RNA ratios, but no change in N : P. Finally, when they compared seedling growth rates across the 14 species under high nutrient conditions, they found no correlation with either N : P or protein : RNA ratios.

The results of both Ågren (2004) and Matzek & Vitousek (2009) suggest that the core prediction of GRH (negative correlation between N : P and growth rate) may not hold for plants when nutrients, especially P, are not limiting. The decoupling of leaf N : P from growth in the absence of nutrient limitation is intuitive if one considers the potential for so-called 'luxury' uptake and the nutrient storage capacity of plant vacuoles, as stored nutrients do not play an active role in metabolism and protein synthesis. Indeed, Matzek & Vitousek (2009) pointed out that the fraction of P in RNA has rarely been measured in plants and never exceeded 11% for their study, which is substantially lower than the values for the metazoans and unicells previously used to test GRH (Elser *et al.*, 2003). They concluded that, although plant protein : RNA ratio affects the speed and efficiency of growth, it does not, by itself, dictate leaf N : P stoichiometry. Thus, it appears that advances in understanding the interactions between N : P stoichiometry and growth require both further studies of plant allocation of P to RNA and the development of models that more explicitly account for the sizable and potentially quite

variable pool of stored nutrients, especially P. Along these lines, the model of Klausmeier *et al.* (2004) might be extended to terrestrial plants to assess such effects.

The coordination between N and P observed in leaves has recently been confirmed in other major plant organs (Kerkhoff *et al.*, 2006; Ågren, 2008; Reich *et al.*, 2008). Across a large number of species, Kerkhoff *et al.* (2006) found that, as in leaves, N and P concentrations are correlated in roots, stems and reproductive tissues (Fig. 4). Although parameters of the scaling relationships sometimes differed between woody and herbaceous species and among leaves, stems and roots, in all tissues, P increased disproportionately with N as seen for leaves (Niklas *et al.*, 2005; Reich *et al.*, 2010). Moreover, leaf nutrient concentrations were closely related to (and could be used to predict) the nutrient concentrations of stems, roots and reproductive structures. Thus, as demonstrated in the studies of stoichiometric homeostasis above, although plants display a substantial degree of developmental and environmental plasticity in nutrient concentration, they are still functionally integrated organisms whose stoichiometric composition probably reflects very general physiological constraints (such as required allocations to major nutrient-rich biomolecular apparatus, such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) and ribosomes, or to low-nutrient structural components, such as wood) and ecological strategies (such as root : shoot ratios and reproductive schedules) for a given species.

IV. Scaling from tissues to whole plants

The studies of Ågren (2004); Niklas *et al.* (2005); and Matzek & Vitousek (2009) just discussed involved either leaves or seedlings, whose pattern of biomass allocation is dominated by leaves. However, terrestrial plants vary enormously in size and, as they become large, the relative allocations to leaves, stems and roots change. Whole-plant nutrient concentration depends on the nutrient concentrations of different plant organs and the relative share of biomass allocated to these different organs. Thus, to understand the effects of plant size and stoichiometry on plant production, we need to understand the variation in the composition of plant organs, how the stoichiometry of plant organs affects production and how patterns of allocation vary with plant size. As described above, the primary metabolic machinery (e.g. chloroplasts, mitochondria, ribosomes) shares a common elemental composition across all plants, but the relationship between this metabolically active stoichiometry and the measured nutrient concentration of plant organs is complicated by the presence of 'inactive' nutrients in storage and structural tissues. As a first step towards linking whole-plant stoichiometry to production, and to provide an example of the type of synthesis we envision between scaling and stoichiometric approaches, we now develop a model of whole-plant nutrient concentration and how it scales across many orders of magnitude in plant size.

To move from organ-level stoichiometry to that of whole plants, we combine organ-level stoichiometric scaling with the allometric rules for biomass partitioning (Enquist & Niklas, 2002). First, we note that the total nutrient mass in a plant (nitrogen N_{plant}, phosphorus P_{plant}) is the sum of leaf, stem (S) and roots (R) (ignoring reproductive allocation). Here, we focus on N for simplicity. The individual pools are simply the product of the total mass of each component organ type and its nutrient concentration:
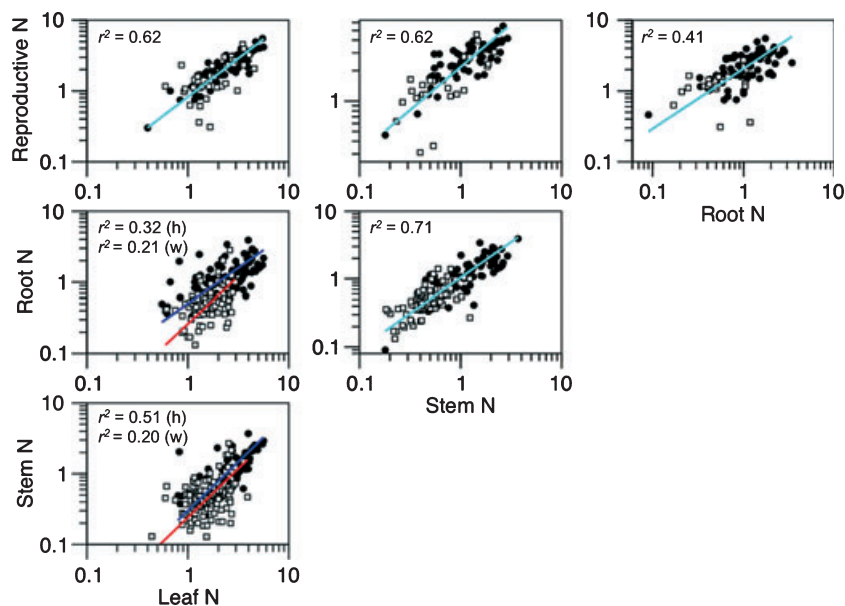
Fig. 4 Scatter-plot matrix showing the integration of whole-plant nutrient concentration via the scaling of N concentration (% dry mass) between organ types. Open squares, herbaceous taxa (h); closed circles, woody taxa (w). The separate lines are used for woody (red) and herbaceous (blue) taxa only when slopes or intercepts were significantly different (likelihood ratio tests, $P < 0.05$). A single cyan line is used otherwise. Lines indicate results of reduced major axis regression and all are significant (likelihood ratio tests, $P < 0.05$). Replotted from Kerkhoff *et al.* (2006).

$$N_{\text{plant}} = M_L N_L + M_S N_S + M_R N_R \qquad \text{Eqn 2}$$

Enquist & Niklas (2002) have shown that, for small to large plants (across nine orders of magnitude), leaf stem and root mass are related as:

$$M_L = \beta_1 M_S^{3/4} \qquad \text{Eqn 3a}$$

$$M_R = \beta_2 M_S, \qquad \text{Eqn 3b}$$

where we use the canonical $^3/_4$ value as it appears to best describe the overall scaling pattern across plants and, in principle, this value can be substituted with an empirically verified value for the taxon of interest. The values of β_1 and β_2 reflect allocation to leaf and root tissue, respectively. Eqn 3 provides a model for whole-plant mass as a function of stem mass:

$$M_{\text{plant}} = \beta_1 M_S^{3/4} + M_S + \beta_2 M_S \qquad \text{Eqn 4}$$

The scaling relationships relating N (and P) concentrations among organs can describe stem and root N (N_S, N_R) as a function of the more commonly measured leaf N (N_L):

$$N_S = \alpha_1 N_L^{b1} \qquad \text{Eqn 5a}$$

$$N_R = \alpha_2 N_L^{b2} \qquad \text{Eqn 5b}$$

Values for the scaling exponents vary among organs, nutrients and functional groups (e.g. woody vs herbaceous), but they typically fall between 3/2 and 4/3 (Kerkhoff *et al.*, 2006). It is important to note that these relationships may not apply within particular species or other restricted taxonomic groups that do not exhibit a wide range of organ N and P values. Nevertheless, they are useful for broadly comparative work of the kind described here.

Substituting into the equations for total plant nutrient concentration as a function of stem mass and leaf nutrient concentration, we can relate plant nutrient content to stem mass and leaf nutrient concentration:

$$N_{\text{plant}} = \beta_1 M_S^{3/4} N_L + M_S \alpha_1 N_L^{b1} + \beta_2 M_S \alpha_2 N_L^{b2} \qquad \text{Eqn 6}$$

Eqn 6 can be combined with Eqn 4 to graphically assess the relationship between whole-plant mass and whole-plant N for a given leaf N concentration, using empirical estimates for the other parameters. Based on data drawn from a recent compilation (Reich *et al.*, 2006c), combined with additional data that extend the range to larger plant size ($> 10^5$ g; Martin *et al.*, 1998), this simple empirical model appears to capture the overall pattern of variation (Fig. 5). However, consistent with expectations from stoichiometric

homeostasis, data drawn from glasshouse and growth chamber studies (Reich *et al.*, 2006c) are much more N rich than predicted, whereas larger field-grown trees are more N poor. Although the model predictions seem to be relatively insensitive to the small differences in scaling observed between woody and herbaceous taxa (Kerkhoff *et al.*, 2006), the fact that the empirical data exhibit a scaling exponent < 1 suggests that plants do, in fact, exhibit systematic reductions in tissue nutrient concentration with increasing size. Our elaborated theory for the scaling of whole-plant nutrient content shows that a common set of scaling 'principles' that guide biomass allocation and organ-level nutrient concentrations can be combined to quantitatively predict how size and nutrient content are related.

Although this particular model of whole-plant nutrient content has not yet been incorporated into models of plant production, several models have been developed that link stoichiometry to growth (e.g. Ågren, 2004; Kerkhoff *et al.*, 2005; Enquist *et al.*, 2007a; Allen & Gillooly, 2009). Although the particulars of model formulation vary, all of the models are based on the assumption that the relationship between plant stoichiometry and production is based on the relatively invariant elemental composition of metabolic and biosynthetic machinery. For example, Ågren's (2004) model of whole-plant carbon assimilation is based on the concentration of N in proteins, the concentration of
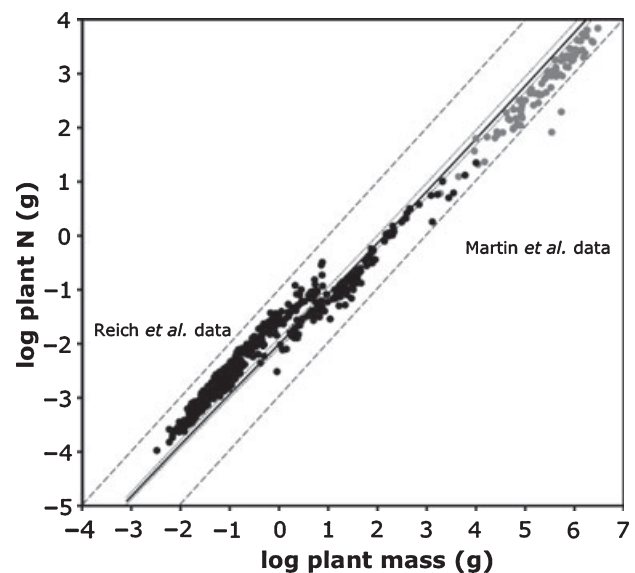
Fig. 5 Whole-plant N as a function of whole-plant mass. Data are drawn from Reich *et al.* (2006c) and Martin *et al.* (1998), separated into field-grown trees (grey) and tree seedlings and herbs grown in glasshouse and growth chamber studies (black). Broken lines bounding the data are isometric (slope = 1) reference lines. The heavy black line is the model (Eqns 4, 6) using the geometric mean leaf N value and scaling relationships from Kerkhoff *et al.* (2006). The dotted lines use N concentration and scaling relationships for herbaceous (upper) and woody (lower) species.

P in ribosomes, the rate at which proteins drive carbon assimilation and the rate at which ribosomes drive protein synthesis. Niklas (2006) assessed an interspecific version of Ågren's (2004) model and found that it adequately described the variation in growth rate across 131 species of herbaceous plant. However, the model is limited because it does not deal explicitly with how the density of metabolic machinery changes as plants grow increasingly large.

Applied to plants, MST provides a way forward to this problem by treating the physiology and dimensions of a leaf as parameters that are independent of plant size, then scaling up using the allometry of leaf mass (Eqn 3a). For example, Enquist *et al.* (2007b) built on the general framework of classical plant growth analysis (Poorter, 1989) to show how the variation in plant growth can be partitioned into a simple combination of leaf-level photosynthetic parameters (including leaf N) and the scaling of leaf mass. Further, they emphasized that the scaling of leaf mass is predicted to reflect the geometry of the plant's branching network (West *et al.*, 1997; Price *et al.*, 2007). As a result, MST provides a theoretical baseline to mechanistically link how the variation in tissue-level stoichiometry influences various physiological traits which then influence whole-plant growth.

Taking a slightly different approach, Kerkhoff *et al.* (2005) built on classical models of photosynthesis, respiration and growth (Penning de Vries, 1975; Amthor, 1984) to derive a plant growth model that integrates how variations in plant mass (M), N and P, as well as environmental temperature (T), influence plant growth. They modeled growth as the balance of assimilation (A) and maintenance respiration (R_M):

$$\frac{dM}{dt} = Y_G(N, P)[A_T(M, N, T) - R_M(M, N, T)], \quad \text{Eqn 7}$$

where both were functions of plant size, N content (reflecting the importance of N-rich mitochondria, chloroplasts and RUBISCO) and temperature. One advantage of this approach is that it separates the assimilation and biosynthesis components of growth. The latter is included in the growth yield (Y_G, which also entails growth respiration), which is treated as a function of both N and P content (reflecting the role of both mitochondria and ribosomes). Partitioning growth in this way provides a means of independently accounting for different components of the metabolic and biosynthetic machinery. At the same time, unlike Ågren's (2004) model and those of Klausmeier *et al.* (2004) and Allen & Gilloly (2009), the model does not effectively account for separate 'functional' and 'nonfunctional' (i.e. storage and structural) nutrient pools.

The combination of MST and BST reviewed and exemplified in this section suggests a common set of principles for modeling plant nutrient relations and productivity, and how they scale from leaves to plants and, ultimately, to ecosystems (see also Allen & Gilloly, 2009). First, models can capitalize on the fact that all plants share chloroplasts, mitochondria and ribosomes in common, and that these biomolecular 'machines' share a common stoichiometry and kinetics. That is, they can be treated as relatively constant, baseline components of any model. Second, models can also capitalize on the modular nature of terrestrial plants and the well-documented allometric aspects of plant biomass allocation to scale both stoichiometry and production from the leaf to the whole-plant level. Finally, models must account for the fact that, despite the common stoichiometry of their molecular machinery, terrestrial plants can harbor large and potentially highly variable nutrient pools in storage and as part of the structure of the plant body. All of the recent models reviewed here have incorporated these principles to some extent, and further unification of MST and BST provides the potential for developing a more integrated understanding of plant function based on the stoichiometry and kinetics of the metabolic machinery and the adaptive organization of the plant body.

V. Applications: large-scale patterns and processes associated with plant stoichiometry

Global variation in plant nutrients

A unique vantage point for assessing geographic variation in plant N and P comes from recent work by N.G. Swenson *et al.* (unpublished) using a large database on foliar N and P contents for woody plants in the Americas. Leaf nutrient data came from a number of published and unpublished sources (Wright *et al.*, 2004; Kerkhoff *et al.*, 2006; N.G. Swenson *et al.*, unpublished; BJ Enquist *et al.*, unpublished). In total, they were able to compile leaf N data for *c.* 6000 species and leaf P data for *c.* 4500 species, and woody plant distribution specimen data from several herbaria with collections that spanned the Americas. By merging the georeferenced database with the leaf stoichiometry database, it was possible to map, across the Americas, the mean species' trait value for each 1° grid cell. The mean leaf stoichiometry inside each grid cell was then correlated with the climate within that grid cell using a GIS-referenced database of global climate (Hijmans *et al.*, 2005).

While noting that the analysis was potentially limited by the fraction of extant species that were sampled within a specific area, several patterns emerged from this analysis (Fig. 6, Table 1). First, there was significant geographic variation in both foliar N and P. Second, the maps clearly showed a strong latitudinal gradient in P, but not so much in N. Warm tropical areas had lower values of foliar P content and colder high-latitude environments had higher values (Table 1). Third, and perhaps most importantly, variation in foliar P had a stronger relationship with the
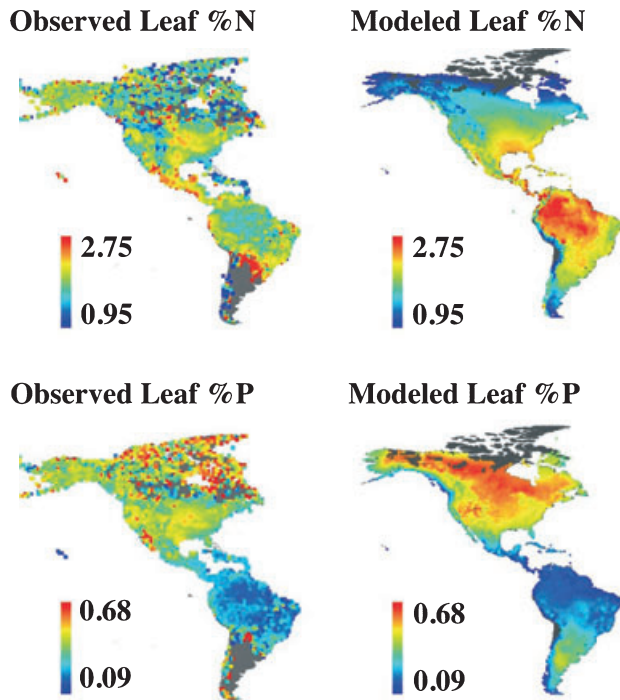
Observed Leaf %N   **Modeled Leaf %N**

Observed Leaf %P   **Modeled Leaf %P**

Fig. 6 Geographic variation in foliar nutrient concentrations in the Americas (N.G. Swenson *et al.*, unpublished). The left maps depict observed values for georeferenced samples of *c.* 4500 (% P) and *c.* 6000 (% N) woody plant species. The right maps depict the average foliar nutrient concentrations for all species within a given 1° grid cell based on recorded distributions of species obtained from *c.* 2.4 million georeferenced herbarium specimens.

climatic variables analyzed than did foliar N. Specifically, foliar P was lower in warmer and wetter climates, with the strongest correlation occurring with mean annual temperature. Foliar P values were also found to be lowest in climates that were largely aseasonal (i.e. tropical lowland rain forest). Variation in leaf N showed no detectable relationship with temperature, but higher values of foliar N tended to be mildly correlated with climates that experienced low annual precipitation that was strongly seasonal. In summary, this study shows that foliar P values tend to be more strongly associated with global trends in mean annual temperature and precipitation, whereas foliar N shows no correlation with temperature and weak correlations with precipitation.

Thus, at these geographic scales, patterns in plant nutrient concentration primarily involve variation in P rather than in N. This implies that large-scale vegetation shifts induced by climatic changes may more strongly impinge on P cycling, and the coupling of P to other elements, than on N cycling and N-intensive processes.

Species' geographic range size

Although the above studies have increased our understanding of geographic variation in plant stoichiometry, the existence and nature of stoichiometrically relevant *biogeographic* patterns remains unexamined. That is, to what extent are spatial variations in C : N : P stoichiometry, such as those observed across latitudes, associated with shifts in the species' composition of the vegetation? Considering the potential for ongoing global changes to shift species' geographic ranges, it is worth exploring what interspecific relationships might exist between stoichiometry and geographic range size.

Assuming that range size largely reflects species' biogeographic traits rather than the underlying environmental conditions (which is not unreasonable given that Kerkhoff *et al.* (2006) have already demonstrated that a substantial portion of interspecific variation in N and P reflects functional differences associated with evolutionary history), a correlation between geographic range size and tissue nutrient concentration may be expected under three different models. First, species with low foliar nutrient concentration (and hence low demand) might be expected to tolerate a wide diversity of habitats and, as a consequence, achieve large geographic ranges. Second, because of the tight relationship between nutrient concentration, photosynthetic rate and growth rate, species with greater nutrient concentration may be more prolific and, as a result, occupy larger geographic areas. Third, species with high foliar nutrient concentration (which is strongly correlated with nutritional investment in seeds and other reproductive organs, Kerkhoff *et al.*, 2006) might achieve and maintain widespread geographic distribution by virtue of their investment in reproduction and thus enhanced probabilities of dispersal.

To assess whether interspecific variation in plant N and P is related to interspecific variation in geographic range size, we merged two existing databases detailing foliar stoichiometry (detailed in Kerkhoff *et al.*, 2005) and latitudinal ranges (from herbarium specimens, see Weiser *et al.*,

Table 1 Pearson's correlation coefficients for mean leaf nutrient concentration in a grid cell and four climatic variables

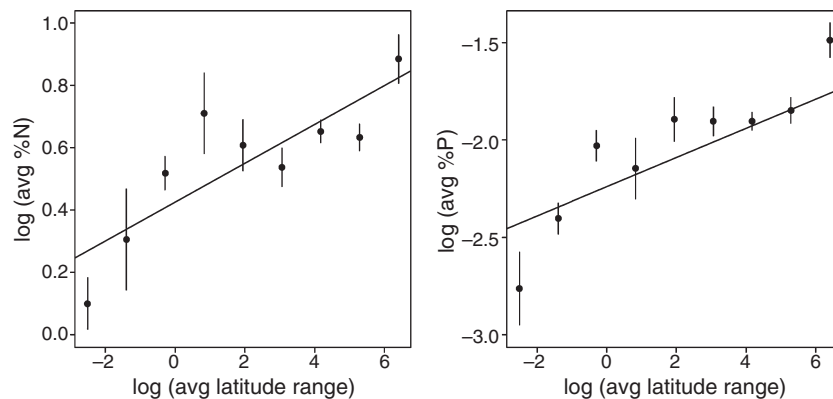| Trait	Mean annual temperature	Annual precipitation	Annual temperature range	Standard deviation of monthly precipitation	Absolute value of latitude
Foliar % N	0.14	−0.23*	0.05	0.44*	0.11
Foliar % P	−0.63*	−0.44*	0.52*	0.32*	0.68*

*$P < 0.05$.

Fig. 7 Association of the foliar nutrient concentration of a species and its geographic range size as determined by a phylogenetically controlled generalized least-squares analysis.

2007). Working with a dataset of 430 angiosperm species, we applied a phylogenetically controlled generalized least-squares analysis (Martins & Hansen, 1997) to evaluate associations between plant nutrient concentration and latitudinal range size. We uncovered a pattern in which plant species with broad biogeographic ranges tended to have nutrient-rich leaves (Fig. 7). The pattern persisted even after controlling for absolute geographic position (i.e. the different midpoints of species' geographic ranges) and for differences in sampling intensity across species. Leaves from plant species with the largest latitudinal ranges were, on average, *c.* 80% richer in N than leaves of the most narrowly distributed plants. Likewise, leaf P concentration for large-ranged species was nearly four times higher than in those with the most restricted ranges. We obtained comparable positive relationships between latitudinal range size and foliar N and P when we examined a particularly well-represented Angiosperm clade (the Cyperales) in isolation.

As foliar nutrient content scales inversely with plant size, these biogeographic patterns connected to leaf stoichiometry imply that small plants should also have large range sizes, given the size–stoichiometry patterns established elsewhere. Regardless, the positive relationships between nutrient concentration and geographic range size are almost certainly indirect and mediated by mutual correlations involving a third variable. As an operating hypothesis to be tested elsewhere, we suggest that the third variable may be investment of N and P to enhance reproduction and the viability of dispersed reproductive propagules. The recent findings of Morin & Chuine (2006) are suggestive of such a linkage. They found that plant species with large latitudinal extents tended to be characterized by a suite of plant life history traits, including those linked to enhanced dispersal ability. Because reproductive investment scales allometrically and differs between woody and herbaceous forms (Kerkhoff *et al.*, 2006), ecosystem-level impacts of changes in climate and biogeochemical cycling may hinge on species' differences in geographic range size.

VI. Global change and plants: a stoichiometric scaling perspective

A stoichiometric scaling perspective offers several insights into how global change may affect the coupling of chemical elements in terrestrial vegetation. Global change is anticipated to influence plant species' dominance and distribution, primary productivity and nutrient cycles worldwide (IPCC, 2001; Millenium Ecosystem Assessment, 2005). Shifts along three interrelated axes have considerable potential to differentially affect plant species in natural habitats, and these deserve revisiting in the light of a more complete understanding of stoichiometric scaling in plants.

Atmospheric CO_2

There are three potential scaling links between increased atmospheric CO_2 concentration ($p CO_2$) and plant stoichiometry. First, increases in $p CO_2$ are expected to stimulate plant photosynthesis and, perhaps, growth and overall production. As a result, there is the potential for increased sequestration of C in plant biomass globally as $p CO_2$ rises (IPCC, 2001). However, the duration of CO_2-enhanced plant growth in any one locality will probably also be influenced by available soil resources, particularly N (Körner, 2006; Reich *et al.*, 2006a,b). Second, increasing $p CO_2$ tends to increase plant root : shoot ratios (Luo *et al.*, 2006) and leaf area (e.g. Ferris *et al.*, 2001), which will influence whole-plant C : N : P ratios and, ultimately, photosynthetic capacity (Ainsworth & Long, 2005; Hyvonen *et al.*, 2007). Third, at the molecular level, RUBISCO, the key photosynthetic enzyme, operates more efficiently at higher atmospheric (and hence intracellular) CO_2 concentrations, especially in C_3 plants (Peterson *et al.*, 1999; Spreitzer & Salvucci, 2002; Tcherkez *et al.*, 2006). This increased efficiency relaxes the need for high expression levels of the RUBISCO gene to offset photosynthetic losses to photorespiration (e.g. Majeau & Coleman, 1996). Resources (e.g. N) that are not used to produce

RUBISCO can then be diverted to increase reproductive output (Ward & Kelly, 2004). Overall, these physiological linkages suggest that higher CO_2 should result in both higher C : N ratios in plant biomass and increases in plant size (Curtis & Wang, 1998; Taub & Wang, 2008; Taub *et al.*, 2008). Nonetheless, these effects may weaken if plant growth is strongly coupled to soil nutrient dynamics (Körner, 2006).

Global warming

Global warming will probably influence plant stoichiometry by changing which sizes of plants will be selected for. Warming influences plant species, plant communities and primary production via impacts on phenology, growing season length and, more importantly, growing season conditions (IPCC, 2001). However, these effects will be moderated by any increase in drought. For example, long-term warming with an increase in drought conditions in the Amazon may induce massive shifts in carbon from soils to standing biomass, increasing the dominance of relatively nutrient-poor, large-sized species (Raich *et al.*, 2006). However, allometric constraints on plant nutrient usage, in particular the scaling of nutrient concentration with plant size (Kerkhoff *et al.*, 2006; Ågren, 2008; Reich *et al.*, 2008; Figs 3–5) and size-related shifts in how plants partition resources among tissue types (Enquist & Niklas, 2002), suggest that such large-scale carbon shifts will also be accompanied by absolute shifts in nutrients from below ground to above ground.

Spatially variable increases in N and P supplementation

Most terrestrial ecosystems have been historically adapted to a natural limitation of these key nutrients (Vitousek & Howarth, 1991). The combustion of fossil fuels, use of N-based fertilizers, agricultural production of N-fixing legumes (Galloway *et al.*, 1994) and land clearing and conversion (Vitousek & Matson, 1993) have allowed a large-scale doubling of biologically available N input to ecosystems worldwide. Anthropogenic effects on circulating P in the biosphere appear to be even larger: the P cycle has been amplified fourfold by human action (Falkowski *et al.*, 2000).

In the short term, more available N and P can lead to increased productivity through a higher leaf area index (Hyvonen *et al.*, 2007) among other pathways, and biomass accumulation (Vitousek *et al.*, 1997; Elser *et al.*, 2007). In the long term, deposition-mediated increases in soil nutrients can shape community composition by differentially altering the growth rate and success of resident plant species (van Wijk *et al.*, 2004; Soudzilovskaia *et al.*, 2005; Kulmatiski *et al.*, 2007). Increases in species' dominance and

reductions in overall plant diversity have occurred in other ecosystems following N loading, including grasslands (Tilman, 1987, 1996; Bobbink *et al.*, 1988; Huenneke *et al.*, 1990) and heathlands (Aerts & Berendse, 1988). Most of these changes entail potential shifts in overall plant stature accompanying shorter term changes in plant nutrient concentration in response to altered soil fertility. Because major ecosystem-level features, such as above-ground net primary productivity, depend on the distribution of plant body sizes within a community (Kerkhoff & Enquist, 2006), changes in soil fertility that lead to differences in the stature of resident plants can scale up to affect ecosystem-level processes such as C sequestration. Changes in ecosystem nutrient cycling probably hinge upon the extent to which a community of plants responds to soil nutrient loading via plastic tissue stoichiometry or via species-level replacements as soil conditions change, highlighting the importance of characterizing the strength of whole-plant stoichiometric homeostasis (*H*).

VII. Synthesis and summary

Here we summarize some of the main 'take-home' points of our synthesis of stoichiometric and scaling perspectives in the context of plant responses to global change.

- Variation in plant C : N : P stoichiometry couples the carbon cycle to nutrient cycles. Because plant P and, to a lesser extent, N contents vary over large spatial scales in association with temperature and precipitation regimes that are expected to shift spatially under various global change scenarios, the details of this coupling of plant stoichiometry to elemental cycling deserves renewed attention. Recent advances in BST and MST can help in 'scaling up' cellular- and tissue-level variation in N and P to the whole-plant scale, which, in turn, can inform our view of large-scale patterns of C flux and nutrient use within and across ecosystems. Plant stoichiometry reflects both environmental and intrinsic (genetic) drivers because plants have considerable plasticity in nutrient storage, but also are constrained by the major element demands that result from investments in important metabolic molecules (enzymes and RNA) and structural materials (C-rich cellulose and lignins). Therefore, the observed variations in plant C : N : P stoichiometry probably reflect the combined effects of both plasticity in response to local growth conditions, but also species' replacements. Furthermore, major changes in supplies of CO_2 and limiting nutrients are likely to drive species' replacements in ways that might be better understood and predicted by knowing how plant size scales with C : N : P stoichiometry.
- Nearly as much variability in plant C : N : P ratios can be observed among species within a given site as observed globally among species. This suggests a major role for

ecological processes and evolved plant traits in determining biomass C : N : P ratios in terrestrial ecosystems, despite contributions from spatial variation in plant physiology. Because tropical forests play a major role in global C sequestration and cycling, a major challenge, also noted by Townsend *et al.* (2007), is to incorporate such species-level variation in plant C : N : P stoichiometry into regional and global C cycle models.

- In general, foliar nutrient concentration decreases with increasing plant size, but P concentration decreases more rapidly, yielding a $^2/_3$ slope for the N vs P scaling relationship. High-nutrient, fast-growing, small plants have lower N : P ratios. Plant stoichiometry shows considerable integration among tissues and organs, as well as strong size dependence because of major allocation shifts that accompany increased plant size (e.g. increasing allocation to structural vs metabolically active components).

- Because plant size and metabolic processes are strongly affected by environmental factors, perturbations associated with components of global change should have major stoichiometric consequences, which themselves represent potential feedbacks on global change. For example, increased CO_2 itself may result in higher foliar C : nutrient ratios both directly (via physiological effects on leaf allocations) and indirectly (if higher CO_2 results in increased plant growth and thus increased plant size, which would tend to lower overall plant nutrient concentration because of the scaling relationships reviewed here). Higher foliar C : nutrient ratios should allow increased C storage per unit nutrient in terrestrial ecosystems, a negative feedback on climate change. The net effects of such feedbacks remain critical unknowns, both regionally and at the global scale.

Acknowledgements

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