Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China

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Abstract
Understanding variation of plant nutrients is largely limited to nitrogen and to a lesser extent phosphorus. Here we analyse patterns of variation in 11 elements (nitrogen/phosphorus/potassium/calcium/magnesium/sulphur/silicon/iron/sodium/manganese/aluminium) in leaves of 1900 plant species across China. The concentrations of these elements show significant latitudinal and longitudinal trends, driven by significant influences of climate, soil and plant functional type. Precipitation explains more variation than temperature for all elements except phosphorus and aluminium, and the 11 elements differentiate in relation to climate, soil and functional type. Variability (assessed as the coefficient of variation) and environmental sensitivity (slope of responses to environmental gradients) are lowest for elements that are required in the highest concentrations, most abundant and most often limiting in nature (the Stability of Limiting Elements Hypothesis). Our findings can help initiate a more holistic approach to ecological plant nutrition and lay the groundwork for the eventual development of multiple element biogeochemical models.

Keywords

INTRODUCTION
Mineral nutrients sustain all plant life on our planet (Aerts & Chapin 2000; Epstein & Bloom 2004). It is necessary to maintain sufficient concentrations and relatively stable nutrient ratios in plant tissues (stoichiometric balance) for healthy growth (Marschner 1995). However, taxa may differ in the need for, and capability of obtaining and maintaining, specific ranges of concentrations and ratios of different nutrient elements in the plant body (stoichiometric homeostasis) (Sterner & Elser 2002). A better understanding of variation of all essential plant nutrients is critical to the development of a broad (rather than nitrogen-centric) perspective on the ecology of plant nutrition, as well as in long-range, holistic biogeochemical models. However, such understanding to date is largely limited to nitrogen (N) and to some extent phosphorus (P).

As all essential nutrient elements play a role in plant health and ecosystem function, a nuanced framework for understanding current biogeochemical cycles, including carbon (C) and N, and how they will change in the future, requires improved understanding of these elements and their interactions. Although globally N and P are considered of paramount importance to plant function, it is widely known that many other elements are also important in specific contexts or regions, either due to limitations or toxicity, or impacts on C/N/P cycling (Lynch & St. Clair 2004; Vitousek et al. 2010). For example, tissue calcium (Ca) and magnesium (Mg) deficiency and manganese (Mn) and aluminium (Al) toxicity are common in certain highly leached tropical soils (Lynch & St. Clair 2004); tissue Ca can act as a regulator of soil pH and cation exchange capacity (Reich et al. 2005); tissue molybdenum (Mo) and iron (Fe) can influence N fixation response to rising CO2 (Hugate et al. 2004; Van Groenigen et al. 2006; Barron et al. 2009), and micronutrients added to a tropical forest enhance leaf litter decomposition and leaf nitrogen content (Kaspari et al. 2008). Thus it is imperative that we begin to focus our attention in the direction of the full set of mineral elements (Lynch & St. Clair 2004; Ågren 2008; Townsend et al. 2011).

Despite the examples given above of the importance of elements beyond C and N, their broad patterns of tissue concentration and stoichiometry are very poorly documented compared with C and N (McGroddy et al. 2004; Reich 2005; Elser et al. 2007). Broadly speaking, it is not well understood how or why the biogeography (including both means and variation) of different plant minerals is created and maintained, nor whether patterns should differ for different elements (Lynch & St. Clair 2004; Marschner & Rengel 2007).

China spans large gradients of climate and vegetation, from tropical rainforest to cold alpine meadow or dry Gobi desert, covering nearly

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all the vegetation types in the world (Fang et al. 2002; Zhang 2007). It thus provides a good representation of much of global biome heterogeneity and a unique opportunity to examine how climate, soil and plant species interact in controlling leaf chemistry. The variation results from north-south and east-west gradients in climate, as well as variation in geomorphology and soil substrate materials, and resulting plant compositional variation (Hou 1983). Over a prolonged period, a large body of plant nutrient data has been accumulated in China. These data were obtained for leaves of nearly 2000 plant species across China (Fig. S1), coupled with information on location, climate and soil nutrients, involving concentrations of 11 mineral elements [N, P, potassium (K), Mg, Ca, sulphur (S), silicon (Si), Fe, sodium (Na), Mn and Al].

Herein, we first explore the biogeographic patterns of multiple elements in plants at the national scale; and whether and how these elements show similar or differential heterogeneity among plant functional groups and sensitivity to environmental factors (e.g. climate and soil nutrient availability). We then investigate variations in these elements and their possible differential responses to the environmental factors. We hypothesise that nutrients required in higher concentrations in leaves and considered most frequently limiting in nature should show smaller variations in their concentration and lower sensitivity along environmental gradients than the elements at the opposite end of the spectra (Stability of Limiting Elements Hypothesis). We examine the hypothesis by analysing the variation in the 11 plant minerals and their responses to the gradients of climate and soils.

MATERIAL AND METHODS

Concentrations of 11 plant leaf minerals

The concentrations of 11 leaf minerals (N, P, K, Ca, Mg, S, Si, Fe, Na, Mn and Al) in 1900 higher terrestrial plant species, belonging to 788 genera and 175 families, at 752 sites across China, were obtained from our field measurements and published literature (Fig. S1; see also Data S1 for details). In total, 4796 records on the whole, or about 2392 observations for each mineral on average, are involved in the dataset. The leaves for mineral analyses in this dataset were sampled during the growing season (June to September, mostly July and August). Site-related information, including the longitudes, latitudes, climate and soil mineral background values, were also documented in the dataset.

Climatic variables, soil data and functional types

Geographic patterns of leaf minerals may be related to climatic variables, including temperature, precipitation, length of growth season and climatic variability (Reich & Oleksyn 2004). In this study, five climatic variables were employed to analyse the climatic controls on leaf mineral spatial patterns: mean annual temperature (MAT, °C), mean annual precipitation (MAP, mm), growing season length (GSL, days), and average diurnal range of temperature (DRT, °C) and annual precipitation seasonality (coefficient of variation of monthly mean precipitations) (APS, %).

For sites where MAT/MAP and latitude/longitude were recorded, these values were used for the analyses. For the records lacking detailed geographic coordinates, we used the latitude/longitude of the geographical centre of the sample areas. For the sites where MAT/MAP were not recorded, estimates of MAT/MAP were extracted from a global climate dataset with a resolution of 0.0083 × 0.0083 (ca. 1 km × 1 km) obtained from http://www.worldclim.org/. GSL was defined as the number of days with diurnal mean temperature > 5 °C, and together with DRT and APS, was estimated with records of 740 climatic stations in China (during 1950–1999) using a Kriging extrapolation method.

Soil N, P and K data were obtained from the national soil survey and our field measurements; other soil minerals were from another national soil survey, except that soil S data cover only part of the country and were collected from several separate studies (for details, see Fig. S2 and the corresponding Supplementary references).

All species in the dataset were primarily classified into seed plants and ferns; seed plants were further divided into six groups according to their respective functional types. The four woody plant groups are deciduous broadleaves, deciduous conifers, evergreen broadleaves and evergreen conifers; the herbaceous groups are grasses (families of Cyperaceae and Gramineae) and forbs (all others).

Indices of physiological requirement and relative limitation

Both physiological (Ingestad 1997) and ecological (Sterner & Elser 2002) stoichiometry should increasingly constrain variability as elemental relative supply limitations grow and requirements increase. To explore such a trend, we used an index of physiological concentration requirement (following Marschner 1995; also see supplementary Appendix S1). We use this without implying that the absolute values hold for all taxa and all conditions, but instead proposing that the index is useful because it provides a relative measure of general physiological requirement. We also used a ranking of the elements (index of relative limitation) from those considered most (1) to least (8) frequently limiting in terrestrial plants (N > P > K > Ca > Mg > S > Fe > Mn). This relative limitation order differs from the rank order by requirement only in that P is moved ahead of K and Ca. We developed this ranking based on our synthesis of information from various sources (e.g. Marschner 1995; Jobbagy & Jackson 2001; White & Brown 2010; Townsend et al. 2011). Because this ranking is somewhat subjective, we developed two other rankings for comparison: (1) searching the number of fertiliser studies by individual elements as well as the relative amount of fertiliser consumption, and (2) using total soil contents of these elements as an indicator of soil supply potential (see supplementary Appendix S1 for details). Both the indices (of physiological requirement and of relative limitation) are limited to the eight elements known to have specific physiological requirements and whose limitations in soils are best understood. Results shown herein are similar if rank order of some elements is reversed, so the conclusions are not dependent on either choice of or the absolute accuracy of the rank order. The coefficient of variation (CV) was calculated for each element as the metric for the variation in chemical concentration.

Data analysis

All leaf mineral concentrations were log10-transformed before analyses to improve the data normality. The leaf mineral concentrations were averaged at the species or species-by-site (site-species) level in the same way as Han et al. (2005). As carbon concentration is relatively
stable, we use elemental concentrations as our index of stoichiometry relative to carbon.

Stepwise multiple regressions were applied to identify the most influential climatic variables among the five climate variables (MAP, MAT, GSL, APS and DRT). To explore the possible effects of soils on leaf minerals, Spearman’s rank correlations were performed between leaf minerals and the soil mineral background contents at the national scale; Student–Newman–Keuls (S–N–K) post hoc tests were then employed to compare the leaf mineral concentrations among plants growing in soils with different soil mineral levels for each single element. To demonstrate the relative effects of climate, soil and species composition (functional type), partial general linear model (GLM) analyses were applied. Partial GLM separates the variance explained by different factors into the independent effects of each individual factor and interactive effects between factors.

Considering that global scale modelling and understanding of vegetation function is confined to functional type groups (e.g. Woodward 1987), it is important to analyse the data at this level of classification and given the study goals it is also a useful attempt to assess the indirect effect of climate on plant nutrient status through climate impacts on the distribution of functional types. For such purposes, we analysed how leaf chemistry responds to climate and soil chemistry by functional type. Because the reduced major axis (RMA) regression slopes of the relationship between leaf chemical concentration and temperature, precipitation and soil chemistry can be used to indicate the response of leaf chemistry to variation in climate and soil chemistry (Sokal & Rohlf 1995), we calculated these slopes for all the 11 elements for five functional types (deciduous broadleaf, evergreen broadleaf, evergreen conifer, grass, and forb) but not for deciduous conifers and ferns due to their small sample sizes. Positive RMA slopes at the functional-type level indicate increases in chemical concentration with increasing temperature, precipitation or soil elemental content level, and vice versa. RMA slopes for the three variables (temperature, precipitation, and soil) were transformed to eliminate effects of the different unit of MAT, MAP and soil nutrient level (For full details, see supplementary Appendix S1).

All analyses were conducted with statistical software soss 13.0 (SPSS Inc., Chicago, IL, USA, 2004) and R 2.2.1 (R Development Core Team, 2005). For full details, supplementary Appendix S1.

RESULTS

Statistics and biogeographic patterns of leaf minerals

The mean concentrations of the 11 leaf minerals in China’s plants vary greatly – from 0.11 mg g⁻¹ (or 0.002 mol kg⁻¹) for Mn to 20.5 mg g⁻¹ (or 1.463 mol kg⁻¹) for N (Fig. 1 and Table S1), with ratios of N : P : K : Ca : Mg : S : Si : Fe : Na : Mn : Al = 100 : 6.8 : 50 : 43 : 11 : 7.7 : 19 : 1.4 : 7.0 : 0.54 : 2.2. These are generally within the normal range for healthy growth of plants (Marschner 1995; Epstein & Bloom 2004) [for the ratios, also see Knecht & Göransson (2004) and Watanabe et al. (2007)].

These leaf minerals show significant latitudinal trends (in the unit of kilometres, see supplementary Appendix S1 for full details) (P < 0.001; Fig. S3a): Nine minerals (N, P, K, Ca, Mg, S, Si, Fe, and Na) increase from south to north, whereas Mn and Al display an opposite trend. Similar to the latitudinal patterns, longitudinal (west-to-east) gradients also exhibit a decrease from west to east for all the minerals but Mn (P < 0.001 for all but except Al with P = 0.43) (Fig. S3b).

Climatic influence on leaf minerals

Temperature and precipitation are the two most critical climatic variables that shape vegetation distribution and structure (Woodward 1987; Brown & Lomolino 1998), but there are a number of ways they can be presented, including both the means and variability at annual and growing season scales (Reich & Oleksyn 2004). Considering the significant correlations among climatic variables (Table S2), we first quantify the role of MAT and MAP in shaping the biogeographic patterns of leaf minerals and then incorporate other climatic variables into the analysis.

We found that all leaf minerals were significantly correlated with both MAT and MAP (all P < 0.01; models 1 and 2 in Table S3; Fig. 2a, b). On average, MAP and MAT explained 10% and 6% of total variation in the 11 elements, respectively. When both climatic variables were entered into a stepwise multiple regression (SMR), with each of the leaf minerals as the dependent variable, MAP played a significant role for all elements except P and Al, whereas MAT had no significant influence on Mg, Si, Fe and Mn (i.e. MAP was removed from SMR; P > 0.10), or explained much less of the variance than...
MAP for N, K, Ca, S and Na (model 3 in Table S3). Even after adding other climatic variables (APS, DRT and GSL) into the predictor lists of the SMR models, MAP explained more of the variance than other climate variables for most of the leaf minerals (Ca, Mg, S, Si, Fe, Na and Mn) (Table S4).

Edaphic influence on leaf minerals

Terrestrial plants take up most of their nutrient minerals directly from soils. Soil chemical attributes (e.g. pH and mineral nutrient availability) are critical to plant growth and thus affect leaf mineral patterns (Foulds 1993; Vitousek & Farrington 1997; Partel 2002; Lynch & St. Clair 2004). We documented soil mineral and pH values to address their relationships with leaf mineral concentrations. As often observed elsewhere, our data demonstrated general positive correlations between plant leaf and soil mineral contents for most minerals (Table 1; Fig. S2).

The A or O horizon soil pH value was significantly and positively correlated with nine of the 11 leaf minerals (N, P, K, Ca, Mg, S, Si, Fe and Na) and negatively correlated with Mn and Al (all $P < 0.005$) (Fig. 2c).

Variation in leaf minerals among functional types

Species composition greatly affects the leaf mineral geography (Hou 1982; Reich & Oleksyn 2004) and biogeochemical cycling (Cornwell et al. 2008). To demonstrate this effect, we divided the seed plants (the largest part of the dataset) into six functional types: deciduous broadleaves, deciduous conifers, evergreen broadleaves, evergreen conifers, grasses and forbs. ANOVA results showed that there were significant differences in leaf minerals among different plant functional types (Table S5). In general, forb leaves were richest in most of the minerals; whereas grass foliage had low mineral concentrations, except for the highest Si concentration. The

Figure 2 Trends in plant leaf chemistry along the climatic and soil pH gradients in China. (a) Mean annual precipitation (MAP); (b) mean annual temperature (MAT). (c) Top soil pH. All $P < 0.001$ for MAP (model 1) except Al ($P = 0.01$) and for MAT (model 2) except Na ($P = 0.006$) in Table S3 and for soil pH except Si ($P = 0.004$).
The 11 elements differ in their sensitivities to the gradients in climate and soil (Figs. 2 and 4). Na, Mn, Si and Al respond to climate (MAP/MAT) with steeper RMA slopes than the other elements, with N (followed by P and K) showing the shallowest responses to MAP and MAT (Fig. 4). The sensitivity of concentrations (the absolute value of the regression slopes) to MAP and MAT were positively correlated with the index of relative limitation (Spearman’s ρ = 0.88, P < 0.005 for both MAP and MAT). There was also a positive correlation (Spearman’s ρ = 0.83, P = 0.010) between the RMA slopes of mineral concentrations vs. soil pH and the relative limitation rank.

The above results, together with an allometric relation between the mean concentrations of the minerals and their physiological requirements (Fig. 3c), suggest that nutrients required in a high concentration in leaves and considered most frequently limiting in environment should show a small variation in their concentration and lower sensitivity to the environmental factors (Stability of Limiting Elements Hypothesis), which is further discussed below.

**DISCUSSION**

**Biogeographic patterns and the environmental control**

Leaf minerals showed large variations among China’s terrestrial plants (Fig. 1; Table S1) and exhibited significant latitudinal and longitudinal trends for each element (Fig. S3). If we translate latitudinal and longitudinal patterns to the mean leaf elemental concentration and its coefficient of variation (CV). (a) Elemental requirement vs. CV, \( \log_{10}(y) = 2.067 - 0.407 \times x \) \((r^2 = 0.77, P < 0.005)\); (b) leaf elemental concentration vs. CV, \( \log_{10}(y) = 2.212 - 0.423 \times x \) \((r^2 = 0.70, P < 0.005)\); (c) elemental requirement vs. leaf elemental concentration, \( y = 0.182 + 0.859 \times x \) \((r^2 = 0.96, P < 0.005)\). 95% confidence bands for all the fitting curves are also shown.

**Table 1** Spearman’s rank correlations (ρ) between leaf minerals and soil nutrients. Leaf minerals (mg g⁻¹) are \( \log_{10} \)-transformed before analysis. Soil total N is density based (kg m⁻²), and the other soil nutrients are content based (mg g⁻¹). The 11 elements differ in their sensitivities to the gradients in climate and soil (Figs. 2 and 4). Na, Mn, Si and Al respond to climate (MAP/MAT) with steeper RMA slopes than the other elements, with N (followed by P and K) showing the shallowest responses to MAP and MAT (Fig. 4). The sensitivity of concentrations (the absolute value of the regression slopes) to MAP and MAT were positively correlated with the index of relative limitation (Spearman’s ρ = 0.88, P < 0.005 for both MAP and MAT). There was also a positive correlation (Spearman’s ρ = 0.83, P = 0.010) between the RMA slopes of mineral concentrations vs. soil pH and the relative limitation rank.

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<table>
<thead>
<tr>
<th>Element</th>
<th>ρ</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.064</td>
<td>1910</td>
<td>0.005</td>
</tr>
<tr>
<td>P</td>
<td>0.350</td>
<td>2510</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>K</td>
<td>0.272</td>
<td>2009</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ca</td>
<td>0.233</td>
<td>2052</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mg</td>
<td>0.347</td>
<td>932</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S</td>
<td>0.199</td>
<td>528</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Si</td>
<td>0.077</td>
<td>800</td>
<td>0.030</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.036</td>
<td>1864</td>
<td>0.119</td>
</tr>
<tr>
<td>Na</td>
<td>0.281</td>
<td>1320</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mn</td>
<td>0.433</td>
<td>1584</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Al</td>
<td>0.179</td>
<td>1086</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
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Deciduous angiosperms (with short leaf life-span) had generally mineral-rich (except for Mn and Al) leaves in contrast with the evergreen ones; while conifers were lower in most of the leaf minerals, compared with the broadleaved counterparts (see Table S5 for details).
longitudinal degrees into a spatial distance basis, the rates of the change of these leaf minerals displayed a surprising close consistency in both directions ($r^2 = 0.94$, $P < 0.001$; Fig. 5). The similar rates of the change of leaf mineral concentrations along the two gradients suggest similar underlying biophysical (environmental) and biological controls that shape the biogeographic patterns of the plant minerals.

Climatically, the north-to-south and west-to-east gradients in China both reflect shifts from cold, dry to warmer, moister conditions, although the thermal gradient is steeper in the former and the moisture gradient more pronounced in the latter (Fig. S4). Additionally, both the latitudinal and longitudinal gradients in plant mineral concentrations are associated with, and likely reflect, pervasive geographic patterns in the structure and function of terrestrial ecosystems (such as functional type, biodiversity, soil development, vegetation primary production and ecological traits of plants) (Brown & Lomolino 1998; Hedin 2004; Reich & Oleksyn 2004), which themselves reflect responses to climate gradations.

Climate, soil nutrient contents and species composition all influence plant mineral biogeography in complex ways (Hou 1982; Reich & Oleksyn 2004). General linear models (GLM) involving climate, soil and plant functional types (indicate that these three factors together account for a substantial part of the biogeographic variation in the concentrations of these leaf minerals (full models in Table S6): 37% for leaf N, and more than 20% for the other minerals except Fe and S (both less than 16%). In addition, the explanatory power of these three factors for different minerals varied greatly. Plant functional-type variation accounted for the largest explained fraction of the variances for leaf N, P, K, Ca, Mg, Si, Fe, Mn and Al, while climate explained the most for S and Na (Table S6).

However, significant collinearities between these factors could potentially obscure their true roles. Partial GLM regressions (Heikkinen et al. 2005) can separate the variance explained by multiple factors into independent effects of all individual factors and their interactive effects with the remaining factors (Legendre & Legendre 1998). Performing the partial GLM indicated that the independent effects of functional type were much larger than those of climate and soil nutrient contents, for leaf N, P, K, Ca, Mg, Si and Mn (Table S6). By contrast, the independent effects of climate on S, Na and Al were the largest.

Figure 4 Reduced Major Axis (RMA) regression slopes for leaf chemistry against MAT/MAP/soil chemistry for five functional types. RMA slopes for the three variables (MAT, MAP and Soil) were transformed to eliminate effects of the different units (see the Methods). Deciduous conifers and ferns are not shown because of their small sample sizes. The segmental lengths of the bars represent directly the slopes of the regression lines between leaf chemistry and temperature, precipitation and soil chemistry. Bars rather than points are shown here because the bars provide a clearer visual picture of the changes in the slopes across the different functional types and environmental conditions. Both leaf chemistry and MAP are in log-scale.
These results suggest that the geography of plant leaf minerals was largely controlled by plant functional types, favouring the species composition hypothesis (Reich & Oleksyn 2004). This functional-type effect can also be illustrated by the generally consistent (parallel) relationships between the deciduous percentage and the leaf mineral contents (except for Mn and Al) in the woody plants (deciduous vs. evergreen broadleaf) along the latitudinal gradient (Fig. S5). Species that tend to have high mineral concentrations, such as deciduous (relative to evergreen) plants and herbaceous (relative to woody) plants (Table S5), are more proportionately distributed in northern than southern regions, and in dry west inland than humid coastal areas (Hou 1983; Gaston 2000).

In previous studies, the effects on leaf traits of environmental factors and plant functional types and their interactions have rarely been analysed (but see Reich et al. 2007). In the current study, the interactive effects of climate and functional type accounted for 7.5% of the variation in leaf N, and those of climate, functional type and soil accounted for another 9.3% (Table S6; Fig. S6). The interaction of functional type, climate and soil accounted for significant portions of the variation in leaf P and Mn (8.6% and 10.5%, respectively). Note that the interactive effects on certain minerals will be negative when the relationship between two factors is mainly suppressive rather than additive (e.g. the interactive effects of climate and functional type on leaf Al) (Chevan & Sutherland 1991; Heikkinen et al. 2005).

Variation and sensitivity in relation to physiological requirements and relative limitation

We posit that both physiology and ecological stoichiometry should result in variability being increasingly constrained as elemental relative supply limitations grow and requirements increase (Ingestad 1997; Sterner & Elser 2002). However, heretofore it was unknown whether variability per se would differ among elements in any relation to their ecology. Equally unknown was whether different mineral elements vary in relation to environmental gradients in idiosyncratic or patterned fashion. We hypothesised here that elements with high physiological requirements, high average concentrations and most frequently limiting in nature would be more stable and less sensitive to environmental gradients (the Stability of Limiting Elements Hypothesis).

The stability of limiting elements can be explained as follows. For elements with high requirement and that are frequently limiting, extreme low values should be limited by stoichiometric requirements (i.e. growth would be suboptimal with concentrations below a certain threshold). Extreme high values are also less likely because higher supply would often lead to higher growth rate and thus elemental dilution (Marschner 1995).

The mean concentrations of the minerals were well correlated with an index of their physiological requirements (Fig. 3e) (log–log scaling, $r^2 = 0.96$). The RMA slope (0.87) of this log–log relationship is < 1 (although for this difference, $P = 0.138$, indicating only 86% likelihood that this is not by chance). A log–log slope < 1 is consistent with the idea that the extent to which the minimum requirement is exceeded would be less in elements required at the greatest concentrations. Additionally, P is the only element outside of the 95% confidence intervals of the relationship, and also has a mean concentration less than the index of physiological requirement, consistent with the idea that it is often found at limiting levels.

The patterns of CV of leaf minerals are consistent with the Stability of Limiting Elements Hypothesis. N, P and K (the three most frequently limiting nutrients) had the lowest CV values (41, 67 and 77, respectively), while Al, Na and Mn (the most often toxic elements) the highest (188, 266 and 479, respectively) (Table S1). In addition, the CV of these mineral elements (which was negatively correlated to both the index of physiological requirement and the mean concentration; see Fig. 3a,b) showed significantly positive correlation with the rank order index of relative limitation, suggesting that the more frequently limiting in nature, the more stable the concentration of an element should be. It is possible that geomorphological sources of variation could vary widely among elements (Hou 1982), leading to variation in their CV which would be unrelated to their requirements or rank order of limitation. However, the strength of the observed relationships (Fig. 3) suggests that such geomorphological ‘noise’ is low compared with the ‘signal’ from physiological requirement and stoichiometry. For example, there is no significant correlation between the index of relative limitation and the mean contents of the soil elements or their CVs (Spearman’s $\rho = -0.095$, $P = 0.82$ and $\rho = -0.50$, $P = 0.21$, respectively).

Results from the regression analyses were also consistent with the prediction by the Stability of Limiting Elements Hypothesis. According to the hypothesis, the environmental sensitivity (assessed as the RMA regression slope) should be lowest for elements that are required in the highest concentrations, most abundant, and most often limiting in nature. Leaf N and P display shallower slopes against the climate and soil gradients, compared with leaf Mn and Na (Figs. 2 and 4); and there exist positive correlations between the index of relative limitation and the absolute value of the slopes against MAP/MAT or soil gradients. These positive correlations imply that mineral elements considered more frequently limiting tend...
to vary less across the climate (e.g. precipitation) or soil (e.g. pH) gradients.

Implications of mineral variation across functional types

The RMA analyses indicated some similarity of overall functional type responses to MAP, MAT and soil nutrient concentration, but also considerable variation (Fig. 4). The analysis by functional type indicates that grasses and forbs respond in a similar fashion to MAP, MAT and soil nutrients. However, the tree functional types differ in all of these aspects, with evergreen broadleaf trees the most divergent. Therefore, if gradient analyses are any indication, future changes in climate will exert idiosyncratic effects on plant functional types, as well as modifying nutrient biogeography by altering spatial patterns of composition. Recent experimental imposition of warming, and reduced precipitation, on Mediterranean vegetation (Peñuelas et al. 2008; Sardans et al. 2009) led to species-specific differences in the response of a range of plant macro- and micro-nutrients. Those species that showed the greatest change in nutrients were also most affected in terms of growth.

CONCLUSION

Our study is, to our knowledge, the first to comprehensively document the foliar chemistry of multiple mineral elements and quantify the potential controls and variability at a large scale. Of the three major factors considered to influence the biogeochemical distribution, functional type shows the greatest direct influence for most leaf minerals. However, climate and soil are both directly and indirectly influential, as they contribute substantially to shaping the distribution of vegetation (species composition) (Brown & Lomolino 1998; Chapin et al. 2002) (Fig S7). In addition, we found that variation in elemental concentrations was more constrained (more stable and less sensitive to the environment) for nutrients with highest requirements, generally present in the highest concentrations and considered the generally most limiting to plant growth (the Stability of Limiting Elements Hypothesis).

Our findings broaden the knowledge of the biogeochemical cycling of elements through plants and the fundamental constraints on plant stoichiometry across wide gradients of environmental factors. They also provide a beginning of synthetic data compilation and analyses that will eventually make it possible to better parameterise complex multi-element biogeochemical models, that should be developed in the future (Hedin 2004; Wright et al. 2004; Lambers et al. 2008). As nutrients with highest requirements and most limiting in nature also are globally concentrated in shallower soil horizons (Jobbagy & Jackson 2001), it appears that physiological requirements in conjunction with biogeochemical availability influence elemental distribution spatially in soils and biogeographically across climate gradients, as well as constraining general levels of variability. Other signatures of relative requirements and availability of elements likely remain to be discovered at local to global scales.

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REFERENCES


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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Full description of the Material and Methods.
**Data S1** Dataset on concentrations of 11 leaf minerals in terrestrial plants of China and associated information.
**Figure S1** Distribution of the samples for 11 leaf minerals in plants of China.
**Figure S2** Relationships between minerals in China’s soils and plant leaves.
**Figure S3** Geographic patterns of plant leaf minerals in China.
**Figure S4** Geographic patterns of temperature and precipitation in the mainland and two large islands of China.
**Figure S5** Latitudinal trends in leaf minerals of deciduous vs. evergreen broadleaves.
**Figure S6** Variation partitioning of environmental factors in accounting for the variations in leaf nitrogen and phosphorus concentrations.
**Figure S7** The schematic diagram showing the climatic controls on the leaf mineral patterns.
**Table S1** Leaf mineral concentrations in 1900 plant species in China.
**Table S2** Correlations of five climatic variables, using climate data in all sites in this study.
**Table S3** Linear regressions of leaf minerals on MAT and MAP.
**Table S4** Model summary for the stepwise multiple regression of leaf minerals on five climatic variables.
**Table S5** Leaf minerals with different functional types of seed plants.
**Table S6** Summary of the (partial) general linear models for the effects of climate, plant functional type and soil nutrient contents on leaf minerals.

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