Coordinated pattern of multi-element variability in leaves and roots across Chinese forest biomes

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ABSTRACT

Aim The elemental composition of plants is of fundamental importance for plant physiology and biogeochemical cycling. Knowledge about how the pattern of multi-element variability is coordinated between above- and below-ground organs remains limited. Here, we quantify multi-element variability in the leaves and roots of terrestrial plants, in addition to trying to understand its taxonomic and environmental regulation at large scales.

Location China.

Methods Sixteen elements in the leaves and fine roots of 792 plant species across nine forests located along the north–south transect of eastern China were measured. General linear mixed models were used to partition taxonomic and environmental variation. Canonical discriminant analyses were conducted to identify elements with the highest discriminatory power for different plant orders.

Results Elemental composition differed significantly between the leaves and roots, with the roots containing higher concentrations of trace metal elements (aluminium, iron, sodium, zinc, copper, lead, nickel and cobalt). A coordinated pattern of multi-element variability and similar taxonomic regulation was observed between the leaves and roots of terrestrial plants. That is, elements with higher internal concentrations were less variable, with most of the variability being attributed to taxonomic effects rather than the environment.

Main conclusions Taxonomic and environmental regulation differed for different elements. Compared with microelements, macroelements exhibited a narrow range of internal concentrations, less environmental control and stronger taxonomic conservatism. The coordinated pattern of multi-element variability and similar taxonomic effects in the leaves and roots implies that above- and below-ground ecological processes are tightly linked.

Keywords Conservatism, element variability, environmental regulation, fine root, macronutrient, stoichiometry, taxonomy.

INTRODUCTION

Terrestrial plants are critical for biogeochemical cycling (Epstein, 1972). Plant growth requires approximately 30 of the 92 known elements on earth (Marschner, 2012). According to the quantity of the element required by plants, these elements are divided into macroelements (carbon, C; nitrogen, N; potassium, K; calcium, Ca; magnesium, Mg; sulphur, S; phosphorus, P; all involved in this study) and microelements (aluminium, Al; manganese, Mn; iron, Fe; sodium, Na; zinc, Zn; copper, Cu; nickel, Ni; cobalt, Co; lead, Pb; all involved in this study). The assimilation and utilization of multiple elements are interdependent and influence and regulate fundamental processes of plant physiology and the functioning of plant communities
(Chapin, 1980; Marschner, 2012; Melvin et al., 2013). It is critical for plants to keep a balance of elements between their basic metabolic requirements and the availability of elements in the environment (Sterner & Elser, 2002). Therefore, comprehensive knowledge about plant element composition, variation and the potential regulators is important for understanding plant physiology and biogeochemical cycling.

Elemental variability, the degree of variation in elemental concentrations, is often quantified by the coefficient of variation (CV), and is distinct for different elements. Carbon concentration in the leaves tends to be relatively stable, whereas N and P concentrations are much more variable with larger CV values (He et al., 2006, 2008; Ågren, 2008; Chen et al., 2011). Karimi & Folt (2006) found that the variability increased from macronutrients to microelements in invertebrates. Within the last 5 years, a similar pattern has been reported for terrestrial plant leaves, with macronutrients being less variable than micronutrients (Han et al., 2011).

The reason why macronutrients are less variable than micronutrients has yet to be elucidated. However, variability in plant elements is probably the result of environmental and evolutionary regulation (Chapin, 1980; Wright et al., 2004; Stock & Verboom, 2012; Zhang et al., 2012). Environmental regulation of plant element variation involves the interactive effects of climate [e.g. mean annual temperature (MAT) and mean annual precipitation (MAP)] and soil conditions (Güsewell, 2004; Reich & Oleksyn, 2004; Han et al., 2005, 2011; He et al., 2008; Chen et al., 2011; Sardans et al., 2011). Han et al. (2011) proposed that macronutrients (N, P, K, Ca, Mg, S) show lower variability and environmental sensitivity than microelements due to the higher physiological requirements of plants for them and the frequent limitation in nature. Evolutionary regulation of plant element variation is associated with taxonomic and phylogenetic effects (Thompson et al., 1997; Broadley et al., 2001, 2004, 2007; Watanabe et al., 2007; Hao et al., 2014). Evolutionary conservatism for leaf N, P, K, Ca and Mg has been detected (Stock & Verboom, 2012; Sardans et al., 2014); thus, plant lineages, families and species may retain a characteristic elemental composition as a result of control by biological processes (Sterner & Elser, 2002; Yu et al., 2010; White et al., 2012). Furthermore, macronutrients tend to be more tightly regulated by organisms than microelements due to their basic requirement for growth (Sterner & Elser, 2002; Karimi & Folt, 2006; Ladanai et al., 2010). Given the decreased environmental sensitivity and increased biological control of macronutrients compared with microelements, we hypothesized that elements with higher internal concentrations are less variable, with most of the variability being attributed to taxonomic effects rather than the environment.

Previous studies have almost exclusively focused on photosynthetic tissues, with only some considering the elemental composition and variation of plant roots (Gordon & Jackson, 2000; Yuan et al., 2011; Kong et al., 2014). Fine roots are responsible for plant water uptake and the assimilation of inorganic nutrients, and are considered as the below-ground analogue of leaves. Several studies have demonstrated covariation between leaf and root traits, including positive correlations between leaf and root N and P (Newman & Hart, 2006; Ågren, 2008; Liu et al., 2010). Given this tight linkage of nutrient assimilation between above- and below-ground organs, we hypothesize that there is a coordinated pattern of multi-element variability and similar taxonomic regulation between leaves and roots. That is, elements with higher internal concentrations should be less variable and exhibit stronger taxonomic regulation than those with lower concentrations.

To test these hypotheses, we conducted a comparative study on 16 elements in the leaves and fine roots of 792 plant species from nine forest ecosystems, ranging from cold temperate forests to temperate, subtropical and tropical forests in China. First, we used CV to quantify the variability of each element, and then explore the pattern of multi-element variability. Second, we analysed variation in leaf and root elements to identify differences in taxonomic regulation between macronutrients and micronutrients. Our findings are expected to reveal differences in the variability and taxonomic regulation of different macronutrient and microelement concentrations.

MATERIALS AND METHODS

Study sites

This study was conducted in nine forests located across the north–south transect of eastern China (NSTEC; 108.9° E, 18.7° N to 123.0° E, 51.8° N; Fig. 1). MAT and MAP ranged from −3.7 to 23.2 °C, and 473.0 to 2265.8 mm, respectively (Appendix S1 in the Supporting Information). There is major

Figure 1 Location of the nine sampling sites in the forests of eastern China.
latitudinal variation in soil type, changing from brown soils with high organic matter to tropical red soils with low organic matter. Vegetation types ranged from cold temperate needleleaf forest to temperate broadleaf deciduous forest, subtropical broadleaf evergreen forest and tropical seasonal rain forests along the transect line.

**Plant and soil sampling**

Sample collection was conducted during July and August 2013. We obtained permission from the National Natural Reserves of China, and then established four experimental plots (30 m × 40 m) in representative plant communities of each forest. In each plot, the leaves and roots of dominant and common plant species were collected according to a unified protocol. For herbaceous species, a certain number of whole plants, which provided enough leaf and root samples for measurement, were excavated and transported to the laboratory for post-processing. For woody species, fully expanded and sun-exposed leaves were collected from five individuals of each species. To sample the roots of trees and shrubs, we first loosened the soil within a distance of 2 m from the target tree. Then the root branches were followed to the tree stem to confirm plant species. Subsequently, sections were cut from the main lateral roots. At each plot, soil samples were randomly collected from 30–50 points in 0–30 cm layers using a soil sampler (diameter 6 cm).

Leaf and root samples were cleaned carefully to remove soil and other material. The leaves and fine roots (diameter < 2 mm) were randomly selected and oven-dried at 60 °C in the laboratory. Soil samples were air-dried in a ventilation room after being sieved (2-mm mesh), with visible roots and organic debris being separated by hand. All samples were ground to fine powder using an agate mortar grinder (RM200, Retsch, Haan, Germany) for elemental analysis.

**Elemental analysis**

All plant samples were analysed for K, Ca, Mg, S, P, Al, Mn, Fe, Na, Zn, Cu, Pb, Ni and Co with an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 5300 DV, Perkin Elmer, Waltham, MA, USA). Plant samples were acidified with trace metal grade 68% HNO₃, overnight, and soil samples were acidified with HNO₃ and HF. Then samples were digested using a microwave digestion system (Mars X press Microwave Digestion system, CEM, Matthews, NC, USA) before elemental analysis. C and N from the leaf, root and soil samples were analyzed using an elemental analyser (Vario MAX CN Elemental Analyzer, Elementar, Hanau, Germany).

**Statistical analysis**

In total, we analysed 3030 leaf samples from 792 species belonging to 73 families from 33 orders and 1800 root samples from 465 species belonging to 55 families from 28 orders. In general, at least three species from each family were analysed. We excluded plant families that contained many species but for which samples from fewer than three species were collected. Species names were checked against The Plant List (http://www.theplantlist.org/). Angiosperm order and family assignments were based on the Angiosperm Phylogeny Group III (APG III) classification (Angiosperm Phylogeny Group, 2009). The software PHYLOMATIC v.3 (http://phylodiversity.net/phylomatic/) (Webb et al., 2008) was used to build a family-level resolved phylogenetic tree according to the megatree R20120829 (Appendix S2).

The elemental concentrations of leaves and roots were averaged at the species level. Variation in the concentration of each element was quantified by the CV. We used the CV to evaluate the multi-element variability in the leaves and roots. Elements with higher CV values were considered to be more variable, and vice versa.

To quantify the relative regulation of environmental and taxonomic effects, we used general linear mixed models (GLM) to partition the variation components for each element. The variance component of the total variance for each element was partitioned into taxonomic, environmental (site) and residual components by using residual maximum likelihood (REML) procedures. The taxonomic effect was defined as a hierarchically nested structure ’(order/family/species)’, which is described in detail by Broadley et al., (2004, 2007) and Watanabe et al., (2007). This method enabled us to investigate the degree of variability at each phylogenetic level, i.e. species and family levels. The overall random term within the GLM model was [(order/ family/ species) site], and no fixed factors were defined. Variation in the concentration of each element caused by environmental variables (including climate and soil element concentrations) was assigned to the ‘site’ component of the model (Broadley et al., 2001, 2004, 2007; Watanabe et al., 2007).

To identify how the environment affects elemental concentrations in the leaves and roots, we separately analysed the effect of latitude, climate (MAP and MAT) and soil. We applied linear regression and polynomial regression to identify the effect of latitude and soil element concentrations (C, N, K, Ca, Mg, P, Al, Mn, Fe, Na, Zn, Cu, Pb, Ni and Co), using each of the 16 elements in the leaves and roots as dependent variables. Stepwise multiple regression was used to identify the effect of MAP and MAT on leaf and root element concentrations, because of the remarkable collinearity between MAP and MAT.

Canonical discriminant analyses (CDA) were conducted to compare differences in multi-element composition among plant orders, and to identify elements with the highest discriminatory power (Karimi & Folt, 2006). All elemental concentrations were log₁₀-transformed before analysis to homogenize variance and normalize distributions. All analyses were conducted with R 3.1.0 (R Core Team, 2014).

**RESULTS**

**Elemental composition of leaves and roots**

The mean concentrations of the 16 elements in leaves and roots varied by seven orders of magnitude from the most to the least...
abundant element (Fig. 2). Concentrations in leaves and roots ranged from 457,439 mg kg$^{-1}$ and 461,190 mg kg$^{-1}$ for C to 0.18 mg kg$^{-1}$ and 0.87 mg kg$^{-1}$ for Co, respectively. The concentrations of the 16 elements differed significantly between leaves and roots ($P < 0.001$; Fig. 2). The concentrations of macroelements (N, K, Ca, Mg, S, and P, except for C) were noticeably higher in leaves than in roots ($P < 0.001$; Fig. 2a). In contrast, the concentrations of trace metal elements (Al, Fe, Na, Zn, Cu, Pb, Ni and Co, except for Mn) were significantly higher in roots than in leaves ($P < 0.001$; Fig. 2b, c).

**Pattern of elemental variability in leaves and roots**

The variability of the 16 elements differed greatly. The CV increased from 7.7% for leaf C to 112% for leaf Mn, whereas the values ranged from 7.0% for root C to 87.4% for root Mn. The CV varied 14- and 12-fold for leaf and root elements, respectively. The CVs of the leaf elements tended to be relatively higher than those of the root elements. Importantly, the CVs of the 16 elements were significantly negatively correlated with elemental concentrations in leaves ($R^2 = 0.32$, $P = 0.023$; Fig. 3a) and roots ($R^2 = 0.50$, $P < 0.001$; Fig. 3b). Of note, elemental variability significantly declined with increasing elemental concentration, in both leaves and the roots.

**Taxonomic regulation on elemental concentrations in leaves and roots**

The REML analysis showed that taxonomic effect was a considerable source of variation in elemental concentrations in leaves and roots. The variation explained by taxonomy (incorporating effects of order, family and species) accounted for an average of 48.9% and 42.6% of the total variation of elemental concentrations in leaves and roots, respectively (Appendix S3). The average taxonomic variation of macroelements (C, N, K, Ca, Mg, S and P) in leaves and roots was 63.7% and 61.4%, respectively, compared with 37.6% and 28.0% for microelements (Al, Mn, Fe, Na, Zn, Cu, Pb, Ni and Co). The average variation at the family level and above was 27.9% (39.5% for macroelements and 18.9% for microelements) and 21.6% (18.9% for macroelements and 9.6% for microelements) in leaves and roots, respectively (Appendix S3).

Of note, the effect of taxonomy increased significantly with elemental concentration in both leaves and roots (Fig. 4). The variation explained by taxonomy was positively correlated with elemental concentrations in leaves $R^2 = 0.55$, $P = 0.001$ at family level and above; Fig. 4a). Similar positive correlations were observed in roots $R^2 = 0.52$, $P = 0.001$ at family level and above; Fig. 4b). Compared with microelements, macroelements were largely regulated at the family level and above.

**DISCUSSION**

**Difference in elemental composition between above- and below-ground organs**

The above- and below-ground organs of forest plants exhibited different elemental compositions (Fig. 2). Plant leaves contained more macronutrients whereas roots contained more trace metals (Al, Fe, Na, Zn, Cu, Pb, Ni and Co, except for Mn). Higher concentrations of trace metals in the roots are largely determined by absorption and transportation of elements within plants. Nutrient elements and trace metals are transported to different plant tissues after absorption by the roots. There is also considerable variation in the mobility of different elements. For example, elements related to photosynthesis (e.g. N, P, K) are more mobile; consequently, more of these elements are transported to the leaves. In contrast, some trace metals are strongly bound in root cells (in order of strongest binding: Fe, Pb, Cu, Co, Zn, Ni; Kabata-Pendias, 2011), leading to their accumulation in the below-ground parts. Moreover, trace metals tend to accumulate and be immobilized by roots when the metal supply exceeds a certain threshold concentration. In this situation, roots may act as a barrier to element uptake and transport to protect the plant from metal toxicity (Baker, 1981; Kabata-Pendias, 2011). Therefore, the elemental composition of the above- and below-ground organs is regulated by their different functioning and physiological processes.
Coordinated pattern of elemental variability in above- and below-ground organs

Our results indicated that elemental variability declined with increasing elemental concentration. In general, macroelements were less variable than microelements. The pattern of multi-element variability was similar to the results obtained in previous studies on plant leaves across China (Han et al., 2011; Zhang et al., 2012) and in karst areas of China (Hao et al., 2014). However, the range of CV in this study (35–120%, except for C) was noticeably smaller than that (40–500%) reported by Han et al. (2011), who also analysed meadow and desert ecosystems. In contrast, White et al. (2012) reported in a fertilizer experiment that elemental variability differed among 21 herbage species, Zn being the most stable element and Na the most variable. In addition, research on Picea abies and Pinus sylvestris tree species in Sweden detected no clear difference in variability between macro- and micronutrients in needles (Ladanai et al., 2010). This discrepancy indicates that effects of sample size, species and geographical scale must be considered when investigating elemental variability.

We detected similar elemental variability between the above- and below-ground organs, supporting our initial hypothesis. This coordinated pattern is due to the covariation of multi-elements in the leaves and roots. Covariation in above- and below-ground functional and morphological traits has been widely observed (Reich et al., 2003; Craine et al., 2005; Newman & Hart, 2006; Niklas, 2006; Li et al., 2010; Liu et al., 2010; Geng et al., 2014; Yang et al., 2014). Newman & Hart (2006) reported covariation in macronutrients (N, P, Ca and Mg) between

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Figure 3 Relationship between elemental concentration and the coefficient of variation (CV).
above- and below-ground resource-acquiring tissues. In this study, we detected a noticeable positive relationship between concentrations of elements in leaves and roots (except for Na, Pb and Co; Appendix S4). The tight linkage in nutrient acquisition contributed to forming the coordinated pattern of elemental variability in the above- and below-ground organs.

The hypothesis of stability of limiting elements provides one possible explanation for why macronutrients are less variable than micronutrients (Han et al., 2011). This hypothesis states that elements that are required in higher concentrations during plant growth are less variable and show lower sensitivity to environmental variation (Han et al., 2011). Consequently, the physiological requirements of plants are closely related to elemental variability.

**Similar regulation of the environment and evolution on elemental variation in above- and below-ground organs**

Environmental factors are powerful regulators of the concentration of elements in terrestrial plants. In this study, leaf and root elements showed significant latitudinal tendencies (Appendix S5), with environmental factors explaining a large percentage of elemental variation (Appendix S3). In general, macroelements showed less environmental control than microelements, except for leaf P, leaf N, root P and root Ca (Appendix S6). In particular, MAP had relatively stronger effect on leaf N, K, Fe and Na and on root Pb and Ni ($R^2 > 15\%$; Appendix S7). In contrast, MAT had a stronger effect on leaf P and root P and Al ($R^2 > 15\%$; Appendix S7). Our result was consistent with previous studies showing that latitudinal variation in leaf element concentrations is associated with MAP and MAT (Reich & Oleksyn, 2004; Han et al., 2011; Zhang et al., 2012).

Soil element concentrations (total content) in nine of our study forests differed noticeably (Appendix S8). However, the total elemental content of soil did not appear to be a sufficient indicator for the soil-to-plant transfer of elements. Plant elements were positively correlated with soil element concentrations for seven elements in leaves and six in roots, other plant elements were negatively correlated or had no significant relationships with soil element concentrations (Appendix S9). Several published studies also detected weak coupling between soil and plant element concentrations (Ladani et al., 2010; Roivainen et al., 2012). Soil properties (such as soil temperature, moisture, pH and Eh) and environmental factors that affected the biological availability of elements (Chapin, 1980; Kabata-Pendias, 2011; Roivainen et al., 2012), obscured to some extent the relationship between soil and plant element concentrations.

**Figure 4** Relationship between elemental concentration and taxonomic regulation (taxonomic variation %) in leaves (a) and roots (b). Columns with different colours/shadings represent taxonomic regulation in hierarchically nested structures: order/family/species (dark blue/dark grey columns), order/family (light blue/light grey columns), and order (dotted columns). Insets for each panel show linear regressions between elemental concentration and variation at the order/family/species level (dotted lines), order/family level (short-dashed lines), order level (long-dashed lines) and at the family level and above (solid lines).
Taxonomic regulation increased with increasing elemental concentration in both leaves and roots (Fig. 4). This result implied that macroelements are more conserved and are under stronger evolutionary control than microelements. However, elemental concentration differed significantly among different plant growth forms (Appendix S10). To eliminate the potential variation caused by different plant growth forms, all data were divided into herbs, shrubs and trees and then variation component analysis was completed for each plant growth form separately. The results indicated that taxonomic regulation of elemental concentration was robust for different plant growth forms. The variation assigned to the family level and above was positively correlated with elemental concentration in leaves ($R^2 = 0.52$, 0.60 and 0.30 for herb, shrub and tree, respectively) and roots ($R^2 = 0.19$, 0.46 and 0.13 for herb, shrub and tree, respectively).

Of note, leaf N and P and root P were under the dual control of environmental and taxonomic effects (Appendices S3 & S6). Despite taxonomy explaining a great amount of variation, leaf N, P and root P were strongly influenced by the environment. Numerous studies have demonstrated that growth and reproduction of terrestrial plants are often limited by N and P supplies (Vitousek & Howarth, 1991; Elser et al., 2007). The leaf P of China’s flora (1.51 and 1.46 mg g$^{-1}$ in this study and Han et al., 2005, respectively) was significantly lower than the global averages of 1.77 and 1.99 mg g$^{-1}$ reported by Reich & Oleksyn (2004) and Elser et al. (2000), respectively. The deficiency of environmental supply might partially dilute the taxonomic characteristics of N and P in terrestrial plants.

CDA analyses were also conducted to further support our hypothesis. These analyses showed that different plant orders have a unique multi-element composition in the leaves and roots (Appendix S11). Based on the correlations with the canonical axis (a greater absolute size of the canonical correlations indicates a greater discriminatory power of the elements), leaf K, Ca and C and root K, C and Ca were the most effective elements for distinguishing different plant orders (Appendix S12). Our results were similar to those of a previous study showing that the most effective elements in the discriminant analyses are elements for which considerable variation in elemental concentrations is assigned to the family level and above (White et al., 2012). In general, the CDA results indicate that macroelements in the leaves and roots are more effective than microelements at distinguishing phylogenetic and taxonomic differences in plants. This result supported our hypothesis that macroelements are under stricter taxonomic regulation than microelements in both above- and below-ground organs. Macroelements are constituents of organic compounds, such as proteins and nucleic acids. In contrast, most microelements are predominantly constituents of enzymes (Marschner, 2012). Consequently, compared with microelements, macroelements are foundation elements of plant structural organization and basic metabolism. Thus, the relatively strong stability and taxonomic conservatism of macroelements should be the evolutionary outcome associated with the development of specific physiological nutrient requirements of different plant lineages (Broadley et al., 2001, 2004, 2007; Stock & Verboom, 2012).

CONCLUSIONS
Here, we demonstrated that variation in elemental concentrations in leaves and roots is associated with environmental factors and taxonomic effects. Environmental and taxonomic regulation differed for different elements. Compared with microelements, macroelements exhibited a narrow range of internal concentration, less environmental control and stronger taxonomic conservatism. A coordinated pattern of elemental variability and similar taxonomic regulation was observed for both above- and below-ground plant organs. Our findings advance our knowledge about plant elements beyond the framework of C, N and P, and the limitation on photosynthetic tissues. Further analyses of elemental composition, with well-resolved phylogenetic structures, are required to explore the evolutionary patterns and mechanisms that drive elemental variation in plants.

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### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web-site.

- **Appendix S1** Description of the vegetation and soil parameters in the study sites.
- **Appendix S2** Phylogenetic tree for the plant orders analyzed in the study.
- **Appendix S3** Variance components analysis of the 16 element concentrations in the leaves and roots.
- **Appendix S4** Pearson correlations between leaf and root elements.
- **Appendix S5** Biogeographical patterns of the leaf (a) and root (b) elements.
- **Appendix S6** Variations in leaf and root element concentrations explained by the environment (site).
- **Appendix S7** Model summary for the stepwise multiple regression of leaf and root element concentrations for mean annual temperature and mean annual precipitation.
- **Appendix S8** Total soil element concentrations of nine sample sites.
- **Appendix S9** Relationship between soil element concentrations and leaf (a) and root (b) element concentrations.
- **Appendix S10** Leaf (a–c) and root element (d–f) concentrations for different plant growth forms.
- **Appendix S11** Canonical discriminant analyses of multielement composition in the leaves (a) and roots (b) of diverse plant orders.
- **Appendix S12** Correlation coefficients between the elements and canonical axes.

### BIOSKETCHES

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