

Rapid report

Evolutionary control of leaf element composition in plants

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Summary

Key words: element accumulation, leaf, neutron-activation analysis, phylogeny, principal component analysis (PCA).

- Leaf nitrogen (N) and phosphorus (P) concentrations are correlated in plants. Higher-level phylogenetic effects can influence leaf N and P. By contrast, little is known about the phylogenetic variation in the leaf accumulation of most other elements in plant tissues, including elements with quantitatively lesser roles in metabolism than N, and elements that are nonessential for plant growth.
- Here the leaf composition of 42 elements is reported from a statistically unstructured data set comprising over 2000 leaf samples, representing 670 species and 138 families of terrestrial plants.
- Over 25% of the total variation in leaf element composition could be assigned to the family level and above for 21 of these elements. The remaining variation corresponded to differences between species within families, to differences between sites which were likely to be caused by soil and climatic factors, and to variation caused by sampling techniques.
- While the majority of variation in leaf mineral composition is undoubtedly associated with nonevolutionary factors, identifying higher-level phylogenetic variation in leaf elemental composition increases our understanding of terrestrial nutrient cycles and the transfer of toxic elements from soils to living organisms. Identifying mechanisms by which different plant families control their leaf elemental concentration remains a challenge.

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Introduction

Plants require at least 17 mineral elements to complete their life cycles, most of which are acquired primarily from the soil solution (Marschner, 1995). Highly specific mechanisms enable plants to take up, transport and store essential elements, although plants also accumulate nonessential elements, often in smaller quantities. The abundance of different elements in leaves tends to decrease as a function of atomic mass (Markert, 1987); all essential elements except Mo have an atomic number ≤ 30 . Since the accumulation of elements in leaves underpins the productivity and diversity of communities and ecosystems (Grime, 2001), integrating leaf-element data across species can increase our understanding of ecosystem, or even global scale processes (Grime *et al.*, 1997; Reich, 2005).

Variation in leaf N among species reflects physiological scaling relationships linking leaf N, leaf mass and leaf respiration rates (Reich *et al.*, 2006). Leaf P is stoichiometrically related to leaf N and general allocation constraints are likely to control N and P partitioning (Sterner & Elser, 2002; Ågren, 2004; Han *et al.*, 2005; Wright *et al.*, 2005; Kerkhoff *et al.*, 2006). For example, among 2548 species, 75% of the variation in leaf N and leaf P associates with a single-axis in a multidimensional trait space comprising just four other leaf traits: dry matter per unit leaf area, leaf longevity, photosynthesis and respiration rate (Wright *et al.*, 2004, 2005). Thus, both leaf N and P correlate highly across organs, species, and functional classifications. There also is evidence that higher-level phylogenetic scales (i.e. those above the species level) influence leaf N and leaf P (Kerkhoff *et al.*, 2006).

Leaf N and leaf P correlate less well with the concentration of other leaf elements (Grime *et al.*, 1997; Osaki *et al.*, 2003a,b,c; Broadley *et al.*, 2004; Wright *et al.*, 2005). However, higher-level phylogenetic effects also appear to influence the leaf composition of several other elements, including those with quantitatively lesser roles than N in metabolism and elements that are nonessential for plant growth. For example, concentrations of both leaf Ca and Mg are lower (Thompson *et al.*, 1997; Broadley *et al.*, 2003, 2004) and leaf Si concentration is higher (Hodson *et al.*, 2005) in many commelinid monocotyledon species than in species from other families of angiosperms. Higher-level phylogenetic variation in leaf Ca among species has been attributed to morphological and chemical differences in the leaf and cell wall Ca-binding properties of different groups of angiosperms (White & Broadley, 2003). In addition, leaf Mg concentrations tend to be higher among species from the order Caryophyllales, while extreme leaf metal (e.g. Al, Ni and Zn) accumulation patterns occur more frequently among species in certain families (Broadley *et al.*, 2001, 2004, 2007; Jansen *et al.*, 2002a,b, 2004). However, the hypothesis that phylogenetic effects above the species level are associated with variation in the leaf composition of multiple elements has not yet been tested on a single data set because of a lack of suitable data.

Materials and Methods

We analysed 2228 leaf samples from 670 species of terrestrial plants, representing 138 families. Raw data for all samples are presented in a database in the Supplementary material, Table S1. Angiosperm and gymnosperm family assignments were based on the Angiosperm Phylogeny Website (<http://www.mobot.org/MOBOT/Research/APweb/welcome.html>). Genera of ferns and their allies follow the classification of the Australian National Herbarium (<http://www.anbg.gov.au/fern/taxa/classification.html>). Species names were checked against the International Plant Names Index (<http://www.ipni.org/index.html>). All species were assigned to 30 'key clades', which represent monophyletic groups at relatively high taxonomic levels following recent phylogenetic insights (Pryer *et al.*, 2001; Soltis *et al.*, 2005), including Bryophytes, Lycopodiophytina, Polypodiophytina, Equisetophytina, Ginkgoales, Pinales, Cycadales, Austrobaileyales, Alismatales, Dioscoreales + Pandanales, Liliales, Asparagales, Commelinids, Magnoliids, Ranunculales, Sabiaceae, Proteales, Berberidopsidales, Caryophyllales, Santalales, Saxifragales, Vitales, Crossosomatales, Myrtales, Eurosids I, Eurosids II, Cornales, Ericales, Euasterids I, and Euasterids II (Fig. 1).

Samples were collected using statistically unstructured sampling techniques, from 1980 to 1984, at 26 sites in Japan (Aomori, Gifu, Gunma, Hokkaido, Hyogo, Ibaraki, Ishikawa, Kagoshima, Kanagawa, Kochi, Kyoto, Miyagi, Nagano,

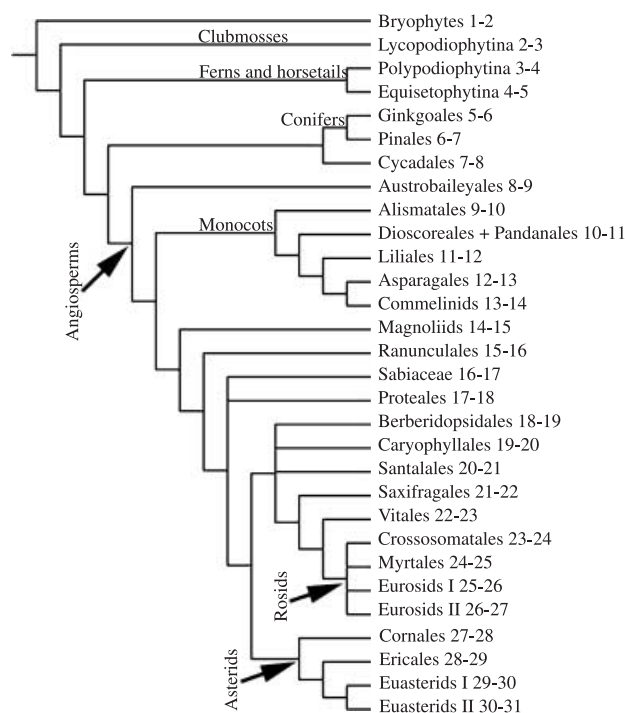


Fig. 1 Hypothetical tree of the 30 key clades represented in our study. The tree is based on recent phylogenetic insights. Numbers following clade names refer to the scaling of the y-axis in Fig. 4.

Nagasaki, Nara, Niigata, Okayama, Okinawa, Osaka, Saitama, Shiga, Tochigi, Tokushima, Tokyo, Tokyo-Ogasawara, Tottori) and at a further three sites in Indonesia, New Zealand and Sweden. The underlying soils, topography and climate (from cool-temperate to subtropic) of these sites varied substantially (data not available). Newly emerged and perennial leaves were collected randomly, rinsed in deionized water, and dried at 60°C. Leaf veins were removed, and the remaining sample was pulverized (Cyclotec 1093 Sample Mill; Foss Tecator, Höganäs, Sweden). Subsequently, 100 mg of sample was analysed by neutron-activation analysis (Koyama & Matsushita, 1980). Samples were packed in double polyethylene film bags with a neutron flux monitor. The bags were irradiated (Kyoto University Nuclear Reactor, Kyoto, Japan). For short-lived nuclides, samples were irradiated in a pneumatic transfer tube (Pn-3, thermal neutron flux; $\Phi_{th} = 2.3 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$) for 60 s. After sufficient cooling, gamma-ray spectra were measured for 200 s using a diode detector system of 90cc-Ge(Li) coupled to a 4096-channel pulse-height analyser. For longer-lived nuclides, samples were irradiated in a pneumatic transfer tube (Pn-2, thermal neutron flux; $\Phi_{th} = 2.8 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$) for 1 h. For intermediate- and long-lived nuclides, samples were cooled (164 h and 1 h, respectively), and gamma-ray spectra measured (1 h). Forty-four elements could be analysed using this method: Ag, Al, As, Au, Ba, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Dy, Eu, Fe, Gd, Hf, Hg, I, K, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, Ti, U, V, Yb and Zn. Note, despite splitting samples to analyse elements with different half-lives, it was still not possible to obtain data for all elements for all samples because of variation in half-lives among groups and overlap of Compton peaks. Raw leaf concentration data for 42 elements were \log_e -transformed (data available in a database, which is published as supplementary information on the *New Phytologist* website). Both I and Ti were excluded from the analysis because of sample sizes of $n \leq 10$.

A variance components model suited to analysing unstructured data was used to allocate variation in leaf mineral composition to a phylogenetic component defined as '(key clade/family/species)', using residual maximum likelihood (REML) procedures (Broadley *et al.*, 2001, 2003, 2004, 2007; White *et al.*, 2004; Hodson *et al.*, 2005). The overall random term within the variance components model was (site + (key clade/family/species)) and no fixed factors were defined. Thus, variation in leaf mineral composition caused by soil chemistry and/or climate was assigned to the 'site' component of the model. Variation in leaf mineral composition resulting from sampling (e.g. leaf age, sampling height, etc.) was assigned to the residual term. Therefore, the following questions can be addressed: how big is the phylogenetic effect, compared with the site + residual effects, how much of this phylogenetic variation is at the species level and how much at the family level, etc.? Such techniques have previously been used to extract evolutionary information from other unstructured data-sets

including data from the literature (Broadley *et al.*, 2001, 2003, 2004, 2007; White *et al.*, 2004; Hodson *et al.*, 2005).

Subsequently, species, family and key clade mean leaf mineral composition values were estimated using each term as a fixed factor, retaining site as random factor. Wald tests were used to identify significant differences in leaf element concentration between families and key clades. Correlation and principal components analyses (PCA) on species, family and key clade means were performed using REML-adjusted data. All statistical analyses were performed using GENSTAT (Release 8.1.0.152; VSN International, Oxford, UK).

Results and Discussion

Leaf element concentrations varied by seven orders of magnitude from the most (K) to the least abundant element (Au); these decreased as a function of atomic number (Figs 2 and 3; Table 1). Among the nine lanthanides, elements with an even atomic number were more abundant than those with the adjacent odd number, which conforms to the Oddo–Harkins rule. The frequency distribution of \log_e concentration of most essential leaf elements was (approximately) normally distributed. However, the distributions of some elements (e.g. Ca and Cl) were negatively skewed (Fig. 3), while the lanthanides and some heavy metal elements were positively skewed (Fig. 3), reflecting increased accumulation among fern (Polypodiophytina), horsetail (Equisetophytina) and clubmoss (Lycopodiophytina) species (Fig. 4). For 21 elements, > 25% of variation (including variation between sites) in leaf element concentration occurred at the family level or above and the leaf concentration of 36 elements differed significantly between plant families (Table 1). Phylogenetic variation in leaf element concentrations at the family level or key clade decreased in the order, V (64.4%) > Al > Dy > Ca > Ta > K >

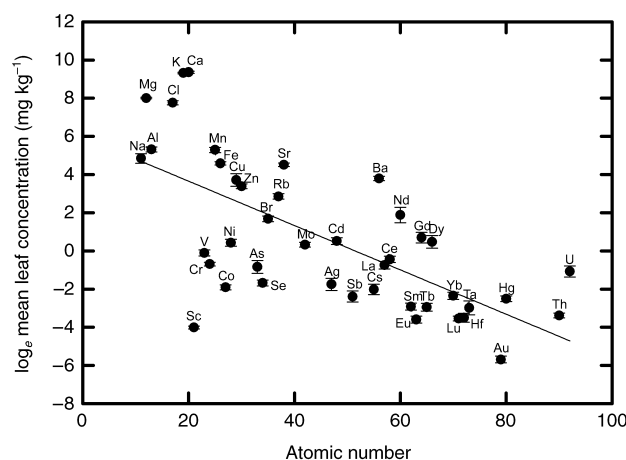


Fig. 2 Leaf concentration of 42 mineral elements as a function of their atomic number. Measurements are based on 670 species of terrestrial plants, sampled from 29 sites in four countries. Values are estimated means. The random effect within the variance components model was site and the fixed effect was species.

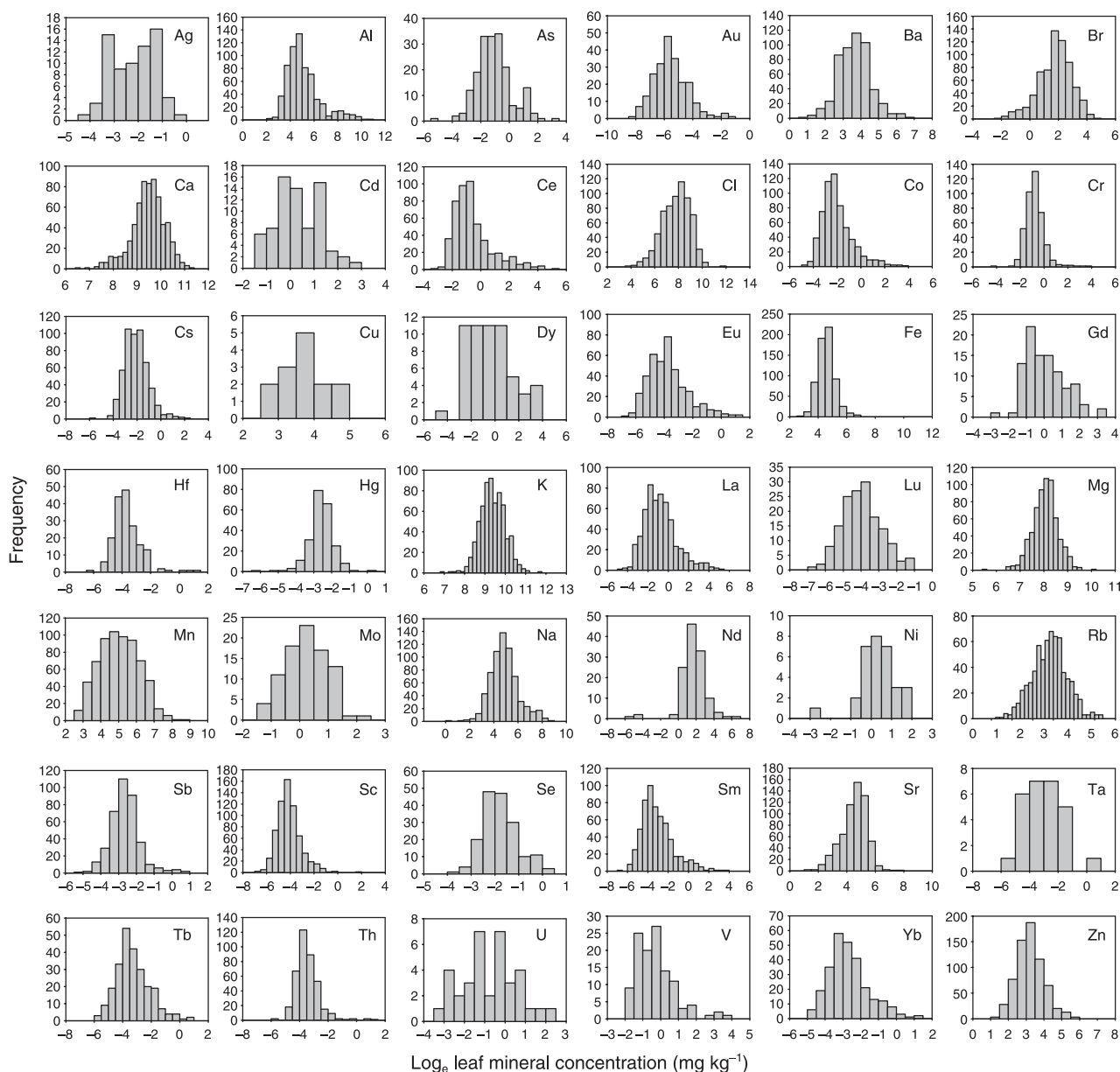


Fig. 3 Frequency distribution of leaf mineral concentration. Measurements are based on 670 species of terrestrial plants, sampled from 29 sites in four countries. Values are estimated means. The random effect within the variance components model was site and the fixed effect was species.

$U > La > Ce > Sm > Zn > Yb > Cl > Tb > Ni > Mg > Ba > Mn > Br > Gd > Eu > Sr > Sc > Lu > Rb > Co > Nd > As > Hg > Fe > Mo > Th > Cu > Na > Cr > Sb > Cs > Cd > Hf > Au > Se > Ag$ (4.1%). Notably, plant family or key clade was associated with 49.5% of the variation in leaf Al, 46.5% of the variation in leaf Ca, 36% of the variation in leaf K and 26.3% of the variation in leaf Mg across all sites.

A valid criticism of this analysis is that phylogeny will correlate with soil preference and so phylogeny and environment cannot be considered to be independent factors in determining the character state of a species. This is because 'phylogeny' represents the sum of all past selection pressures (i.e. soil

chemistry, climate, etc.). So, while it is reasonable to assume that there is an equal chance of over- or under-estimating any 'true' phylogenetic effect using the approaches outlined in this study, a (site + (key clade/family/species)) variance components model can be considered to be quantifying the relative contribution of past vs present-day selection pressures. Notably, family level variation in shoot Ca composition is not correlated with present-day soil pH preference in a UK herbaceous flora (Thompson *et al.*, 1997; Broadley *et al.*, 2004). Furthermore, detailed phylogenetic sampling within the orders Ericales (Jansen *et al.*, 2004), Gentianales (Jansen *et al.*, 2002a) and Myrtales (Jansen *et al.*, 2002b) shows that higher-level

Table 1 Variance components analysis of leaf composition of 42 mineral elements

Variation in element composition (% in total)	Variance component					Percentage variation at and above family level (including site)	Percentage variation at and above family level (adjusted for site)
	Site	Key clade	Key clade/family	Key clade/family/species	Residual		
Ag	55.6	2.4	1.7	9.9	30.4	4.1	9.3
Al	4.3	11.6	38	17.4	28.7	49.5	51.8
As	40.5	7.6	9.1	8.8	33.9	16.8	28.2
Au	26.4	0.8	3.5	10.2	59	4.4	5.9
Ba	19.2	5.6	20.3	9.8	45.1	26	32.1
Br	14.9	0	25.8	19.5	39.8	25.8	30.3
Ca	3.6	21.8	24.7	12.5	37.3	46.5	48.3
Cd	28.6	0	5.9	38.1	27.4	5.9	8.3
Ce	9.9	18.7	14	18.3	39.1	32.7	36.3
Cl	6.6	3.8	25.8	27.4	36.4	29.6	31.7
Co	4	0.7	19.2	30.1	46	19.9	20.7
Cr	8.8	1.5	7.5	13.2	69.1	8.9	9.8
Cs	56.9	2.7	4.4	5.7	30.3	7	16.4
Cu	70.4	10.6	0	11.9	7	10.6	36
Dy	8.3	14.1	34.2	0	43.5	48.2	52.6
Eu	11.6	11.8	13.7	12.9	50.1	25.4	28.8
Fe	10.4	0.9	13.2	7	68.5	14.1	15.7
Gd	18.2	16.9	8.7	10.2	45.9	25.6	31.3
Hf	36.2	2.4	3.4	28.4	29.6	5.8	9.1
Hg	31.5	9.4	5	11.6	42.5	14.3	21
K	4.4	8.7	27.3	11.7	47.8	36	37.7
La	10.8	20.8	13.4	12.9	42.1	34.2	38.3
Lu	9	9	13.3	10.6	58.2	22.3	24.4
Mg	3.8	5.5	20.8	18.4	51.6	26.3	27.3
Mn	10.6	7.1	18.9	25.2	38.2	26	29.1
Mo	10.2	2.4	9.3	8.8	69.3	11.7	13.1
Na	45.3	0.5	9.1	11.8	33.4	9.6	17.6
Nd	41.9	8.8	11.2	9.7	28.4	20	34.4
Ni	0	27.3	0	30.8	41.9	27.3	27.3
Rb	31.6	15	6.9	6.5	39.9	21.9	32
Sb	61.9	3.3	3.3	5.3	26	6.7	17.5
Sc	7.9	9.5	14.1	9.5	59	23.6	25.6
Se	39	1	3.3	20.3	36.4	4.3	7
Sm	11	17.6	15.1	14.2	42.1	32.7	36.7
Sr	9.9	4.5	19.5	10.5	55.6	24	26.7
Ta	41.7	25.4	13.2	0	19.6	38.7	66.4
Tb	13.8	11.7	17.5	11.5	45.5	29.2	33.9
Th	13.8	4.9	6.7	9.4	65.2	11.6	13.4
U	25.4	0	35.4	0	39.2	35.4	47.4
V	8.7	56.2	8.2	6.3	20.6	64.4	70.5
Yb	13.9	10.3	19.7	5.5	50.6	30	34.8
Zn	13.1	11.8	18.5	23.4	33.3	30.2	34.8

Site and phylogenetic variance components estimates for leaf element concentrations are based on a residual maximum likelihood (REML) analysis of 2228 leaf samples from 670 species of terrestrial plants, from 138 plant families and 30 key clades, sampled from 29 sites in four countries. The random effect within the variance components model was (site + (key clade/family/species)); no fixed effect was specified.

taxonomic/phylogenetic variation in Al accumulation is independent of present-day soil preferences. Therefore, higher-level phylogenetic effects observed in this study are not likely to be artefacts of present day soil preferences. It should be stressed, however, that for many of the elements studied here, variation in leaf mineral composition due to site is greater than variation in leaf mineral composition due to phylogeny.

Most leaf element composition patterns identified in this

study have not been reported previously. However, data for several elements are consistent with previous literature and experimental studies, for example, for leaf Al, Ca, K, Mg and Si, substantial phylogenetic variation has been observed above the species level (Thompson *et al.*, 1997; Jansen *et al.*, 2002a,b, 2004; Broadley *et al.*, 2003, 2004; Hodson *et al.*, 2005). In this study, phylogenetic variation in leaf Se above the species level was small, as observed previously (White *et al.*, 2004).

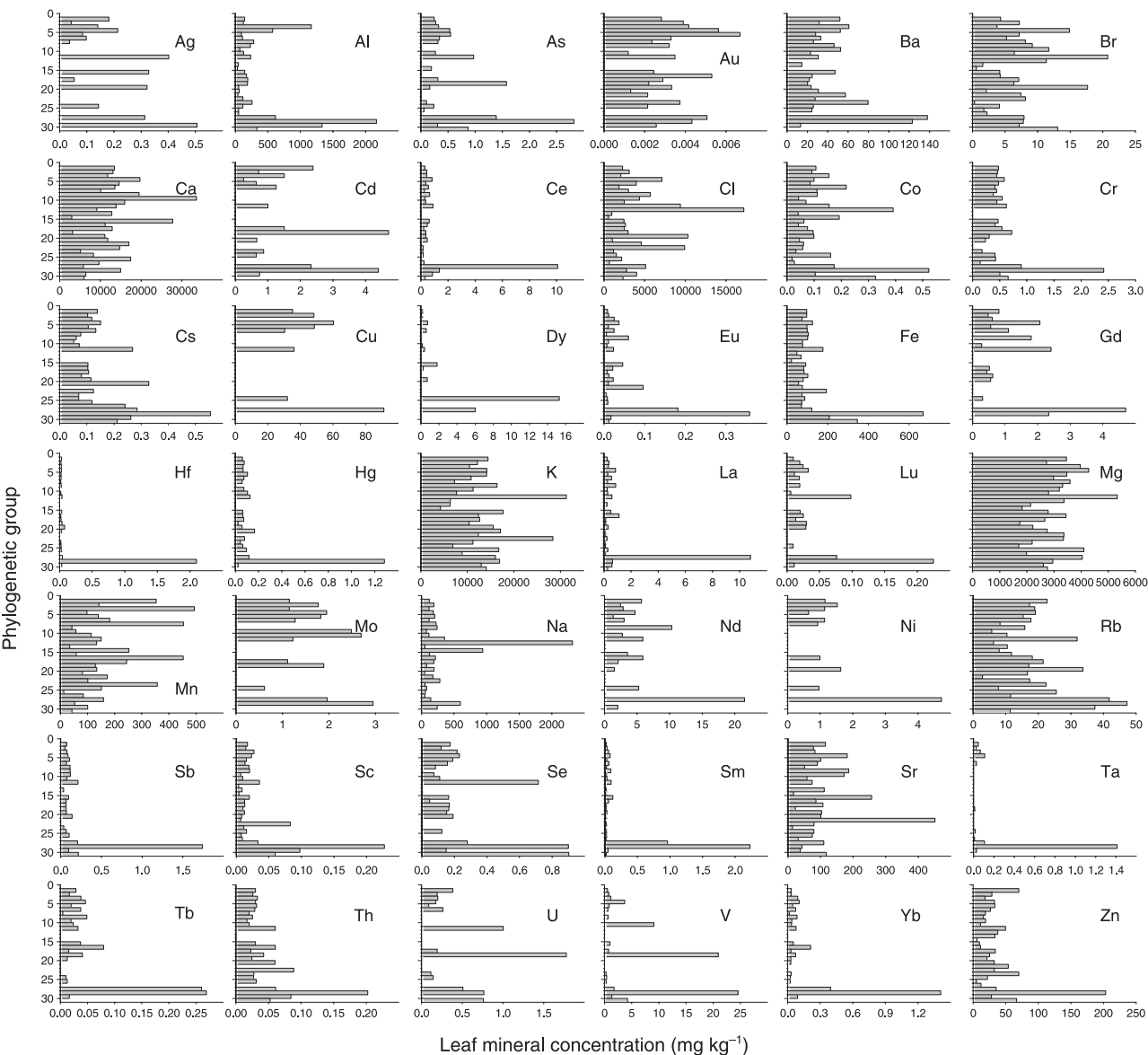


Fig. 4 Leaf mineral concentrations of 30 key clades of plant groups. Data are sampled from 29 sites in four countries; values are estimated means; the random effect within the variance components model was site; the fixed effect was key clade. The y-axis includes 30 monophyletic key clades based on recent phylogenetic insights. A key for the y-axis bins and a hypothetical phylogeny of the key clades is provided in Fig. 1.

Table 2 Correlation coefficients for leaf composition of Group I and II mineral elements in 138 plant families

Group I				Group II			
Na	0.171*			Mg	0.357****		
Rb	0.604****	0.064 ns		Sr	0.665****	0.322***	
Cs	0.237**	0.001 ns	0.52****	Ba	0.146 ns	0.280**	0.307***
	K	Na	Rb		Ca	Mg	Sr

Coefficients are derived from pair-wise regression of family means estimated using site as a random effect and family as a fixed effect in a variance components model.
*, **, ***, ****Significance of correlation at $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, based on 1134 df for the analysis of variance of the regression, corrected for the mean of the observations (SigmaPlot 9.01; Systat Software, Inc., Richmond, CA, USA).

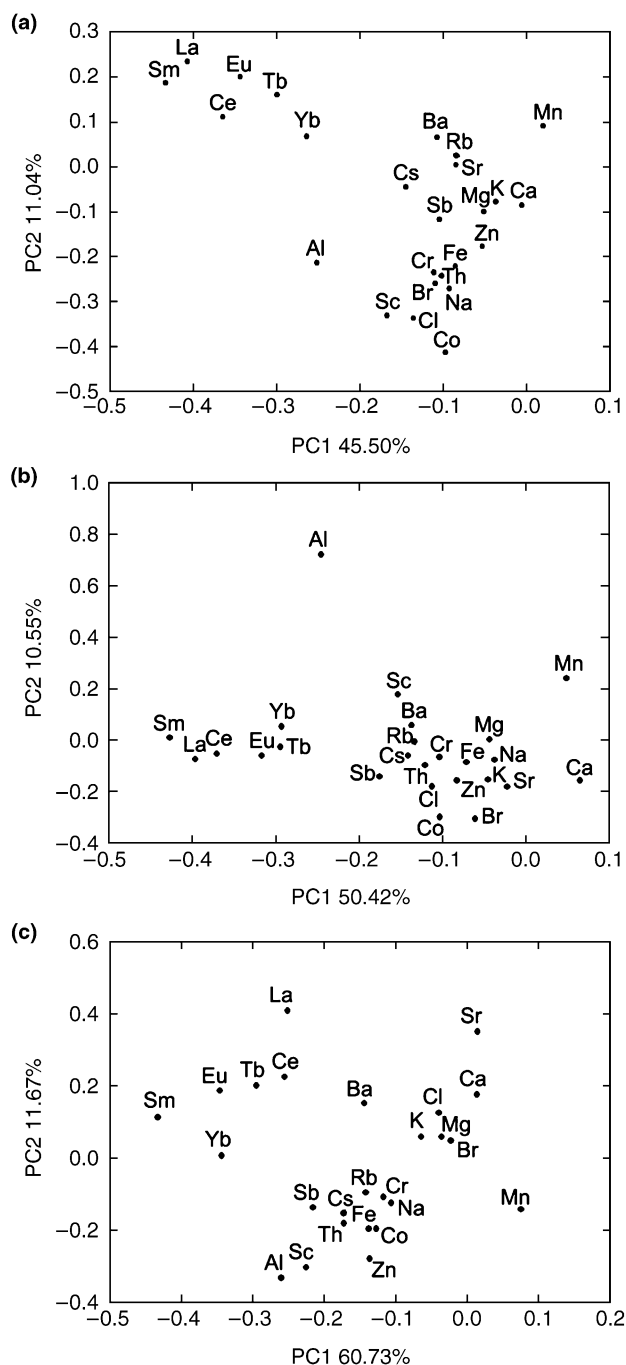


Fig. 5 Principal component analysis (PCA) of estimated leaf composition of 24 mineral elements. A variance–covariance matrix, minerals selected if $n > 500$. (a) Data from up to 670 species; (b) data from 138 families; (c) data from 30 key clades.

Principal component analysis was used to analyse data for 24 elements with > 500 sample observations (Fig. 5). We found that PC1 accounted for 45.5% and PC2 accounted for 11.0% of the variation at the species level. At the family (PC1 = 50.4%, PC2 = 10.6%) and key clade group (PC1 = 60.7%, PC2 = 11.7%) level, variation-capture increased between the

first two PCs. The lanthanides (i.e. elements of atomic numbers 57–70, represented in this analysis by Ce, Eu, La, Sm, Tb and Yb) formed a distinct group compared with the other 17 elements. Leaf-Mn and -Al (except at key clade group level) was an outliers (Fig. 5).

Multivariate correlations in the leaf concentration of elements among species have been identified previously. For example, among 43 herbaceous species, variation in 67 mature plant traits associated with variation in leaf-N, P, Ca, K and Mg. A metabolically active ‘nucleic acid-protein’ set of leaf elements, comprising leaf-N, P, Cu, Fe and S, forms associations distinct from structural and enzymatic sets of leaf elements, including leaf Ca, K and Mg (Garten, 1976, 1978). Thus, we show here for the first time that higher-level phylogenetic effects influence the leaf composition of a wide range of elements, including elements with quantitatively lesser roles than N in metabolism, and elements that are nonessential for plant growth. Most of the leaf elements measured in this study are likely to localize quantitatively to structural (cell-wall) or osmotic (vacuolar) fractions of leaf tissues.

Finally, pairwise correlations between 24 leaf-elements at the species, family, and key clade levels were conducted. These analyses revealed significant associations between numerous pairs of leaf-elements. For example, correlations in Group I and II leaf-element composition are highly significant for several pairs of elements at the family level (Table 2). Thus, in addition to describing a previously unreported source of variation in essential mineral accumulation patterns, this study provides direct evidence that phylogeny accounts for some of the variation in the plant accumulation patterns of nonessential minerals including Cs and Sr, isotopes of which can be radiologically harmful to humans and other biota (Beresford *et al.*, 2004).

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Supplementary Material

The following supplementary material is available for this article online:

Table S1 Raw data for 2228 leaf samples from 670 species of terrestrial plants, representing 138 families. Samples were collected from 1980 to 1984 at 26 sites in Japan and at a further three sites in Indonesia, New Zealand, and Sweden. Forty four elements were analysed by neutron-activation analysis. I and Ti were excluded from the analysis because of sample sizes of $n \leq 10$. Despite splitting samples to analyse elements with different half-lives, it was still not possible to obtain data for all elements for all samples because of variation in half-lives among groups and overlap of Compton peaks.

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