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Phylogenetic variation in the shoot mineral concentration of angiosperms

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Abstract

The calcium (Ca) concentration of plant shoot tissues varies systematically between angiosperm orders. The phylogenetic variation in the shoot concentration of other mineral nutrients has not yet been described at an ordinal level. The aims of this study were (1) to quantify the shoot mineral concentration of different angiosperm orders, (2) to partition the phylogenetic variation in shoot mineral concentration between and within orders, (3) to determine if the shoot concentration of different minerals are correlated across angiosperm species, and (4) to compare experimental data with published ecological survey data on 81 species sampled from their natural habitats. Species, selected pro rata from different angiosperm orders, were grown in a hydroponic system under a constant external nutrient regime. Shoots of 117 species were sampled during vegetative growth. Significant variation in shoot carbon (C), calcium (Ca), potassium (K), and magnesium (Mg) concentration occurred between angiosperm orders. There was no evidence for systematic differences in shoot phosphorus (P) or organic-nitrogen (N) concentration between orders. At a species level, there were strong positive correlations between shoot Ca and Mg concentration, between shoot P and organic-N concentration, and between shoot K concentration and shoot fresh weight:dry weight ratio. Shoot C and cation concentration correlated negatively at a species level. Species within the Poales and the Caryophyllales had distinct shoot mineralogies in both the designed experiment and in the ecological survey.

Key words: Calcium (Ca), cation, carbon (C), comparative analysis of independent contrasts (CAIC), content, nitrogen (N), potassium (K), phosphorus (P), magnesium (Mg).

Introduction

Plants require at least 17 mineral elements to complete their life-cycles (Marschner, 1995). Some minerals, such as carbon (C), nitrogen (N) and potassium (K), are required in large amounts, whilst other minerals, such as copper (Cu), manganese (Mn) and zinc (Zn), are required in trace amounts. Plants can also accumulate non-essential minerals such as cadmium (Cd) and lead (Pb) when these minerals are present in soils. The shoot concentration of essential minerals must be maintained within a certain range since mineral-deficiency limits growth and mineral-excesses can be toxic. Thus, a general proportionality between different essential minerals occurs in plant shoots (Epstein, 1972). However, species also differ systematically in their shoot mineral concentrations. For example, fast-growing species characteristic of nutrient-rich, disturbed habitats tend to have greater shoot phosphorus (P) and organic-N concentrations than slow-growing species characteristic of infertile habitats (Thompson *et al*., 1997; Grime *et al*., 1997; Grime, 2001). Systematic differences between species in the shoot concentration of other minerals have also been reported from plants grown under comparable conditions (Broadley *et al*., 2001, 2003) and when sampled from their natural habitats (Thompson *et al*., 1997). For example, commelinoid monocot species tend to have lower shoot calcium (Ca) concentrations than other angiosperm species (Broadley *et al*., 2003).

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Essential plant elements, with the exception of C, are primarily acquired by plant roots from the soil solution. Shoot mineral concentration is therefore largely determined by rates of root uptake and sequestration within root vacuoles, and by rates of transport of minerals in the xylem and in the phloem. Under constant environmental conditions, species differences in shoot mineral concentration may, therefore, reflect differences in the rates or selectivity of mineral ion uptake and transport to (and from) the shoot. However, in an ecological context, species differences in shoot mineral concentration may be due to specific root features, for example, interactions with mycorrhiza or *Rhizobium*, or may be due to an environmental factor constraining species distribution, for example, soil mineral composition (Wright and Westoby, 2003).

To date, there has been no attempt to quantify the phylogenetic variation in the shoot mineral concentration of angiosperms. However, in an ecological survey, Thompson *et al*. (1997) reported that a significant amount of the variation in the shoot concentration of Ca and Mg occurred at or above the level of the plant family. This implies that systematic differences in shoot mineral concentration occur between species from different clades. Similarly, Kinzel (1982) and others have postulated that different angiosperm families are characterized by different shoot mineralogies (reviewed in Broadley *et al*., 2003). Despite these systematic differences between clades, Garten (1976) observed correlations in the shoot Ca and Mg concentration and in the shoot N and P concentration of mineral elements across vascular, non-vascular, and aquatic plant species. However, it is not known if these relationships occur across all clades.

Broadley *et al*. (2001, 2003) have developed a method to assess the phylogenetic variation in the shoot mineral concentration of angiosperms. This method samples species pro rata from angiosperm orders, i.e. in proportion to the number of species within each order, which can subsequently be assayed under identical experimental conditions. Thus, trait means can be estimated for species-rich orders and phylogenetic variation can be partitioned between and within these orders. Pro rata sampling allows phylogenetic variation to be estimated and partitioned systematically between and within orders. Although this approach does not provide complete coverage of angiosperm species, it provides information on regions of the phylogeny that are amenable to further comparative study and to hypothesis testing (Broadley *et al*., 2001). A small proportion of trait variation within orders indicates that evolutionary processes during early angiosperm diversification (possibly during the late Cretaceous Period) may influence the trait. A large proportion of trait variation within orders indicates that more recent evolutionary processes (for example, during the more recent late Neogene/Quaternary Periods) may influence the trait. Partitioning the phylogenetic variation in shoot mineral concentration can inform agricultural strategies to optimize mineral delivery to humans and livestock, and improve our understanding of the structure and function of plant communities (Thompson *et al*., 1997) and of the cycling of minerals in ecosystems (Broadley *et al*., 2001).

This paper quantifies the dominant mineral constituents of shoots of different angiosperm orders, using a pro rata sampling technique, and it describes the partitioning of phylogenetic variation in shoot mineral concentration between and within orders. This paper also explores correlations between plant traits and compares the results with a published ecological survey of leaf mineral concentration.

Materials and methods

The aims of this study were (1) to quantify the shoot mineral concentration of different angiosperm orders, (2) to partition the phylogenetic variation in shoot mineral concentration between and within orders, (3) to determine if the shoot concentration of different minerals are correlated across angiosperm species, and (4) to compare experimental data with published ecological survey data on species sampled from their natural habitats. Shoot mineral concentration data for 117 angiosperm species from a phylogenetically-balanced experiment were compared with shoot mineral concentration data from an ecological survey of 81 herbaceous angiosperm species growing in Central England (Thompson *et al*., 1997). For all analyses and comparisons, a recent angiosperm phylogeny (APG, 1998; Soltis *et al*., 1999) was used as a phylogenetic framework.

Experimental conditions

The experimental conditions are outlined in detail elsewhere (Broadley *et al*., 2003). Briefly, 144 species of (mainly herbaceous) angiosperms were selected using pro rata sampling, i.e. species were sampled in proportion to the number of species in each order. Seeds of each species were germinated and seedlings transplanted to rockwool blocks. Once established, rockwool blocks were transferred to a nutrient film technique (NFT) hydroponic system in a glasshouse. The nutrient solution contained 2 mM $Ca(NO_3)_2$, 2 mM NH_4NO_3, 0.75 mM $MgSO_4$, 0.5 mM KOH, 0.25 mM KH_2PO_4, 0.1 mM FeNaEDTA, 30 μM H_3BO_3, 25 μM $CaCl_2$, 10 μM $MnSO_4$, 3 μM $CuSO_4$, 1 μM $ZnSO_4$, and 0.5 μM Na_2MoO_4. The nutrient solution was adjusted daily to pH 6, using H_2SO_4, and solutions were replaced completely twice each week. Plant shoots from 117 species were harvested during vegetative growth. The duration of growth of each species is reported in Broadley *et al*. (2003).

Analyses of plant shoot tissues

Shoots were separated into leaves and stems where possible and the fresh weight (f. wt) of each was recorded. Samples were dried in paper bags for 72 h in a fan-assisted oven set to 80 °C. The dry weight (d. wt) of leaves and stems was measured and dry shoot tissue was subsequently milled to a powder using a ball-mill. Total Ca, K, Mg, sodium (Na), organic-N, and P concentrations were determined on dry leaf or whole-shoot material using the micro Kjeldahl method, with *c*. 0.1 g subsamples of dried plant material digested for 1 h, following the addition of 1 ml of H_2O_2 and 2 ml of a H_2SO_4/Se catalyst (Bradstreet, 1965). Inductively-coupled plasma emission spectrometry (JY24, Jobin-Yvon ISA, France) was used to determine final mineral concentrations in digested material. When sufficient shoot material was available, total-C and total-N concentration were quantified directly on 0.5–1 g of dried and milled plant

material using a C:N analyser (CN2000, LECO, Stockport, UK). All concentrations were calculated on d. wt basis.

Partitioning the phylogenetic variation in shoot traits

The experimental variation was removed using a residual maximum likelihood (REML) analysis (Broadley *et al*., 1999, 2001, 2003). Variation in shoot traits was assigned between and within informal plant division (n=3; eudicot, commelinoid monocot, non-commelinoid monocot), and between and within order plus one unassigned family (n=25), using further REML analyses and hierarchical, nested analyses of variance (ANOVA). All statistical analyses were performed using GenStat (Fifth Edition, Release 4.2, VSN International, Oxford, UK).

Determining which shoot traits are correlated

Possible evolutionary associations between shoot mineral concentration, shoot f. and d. wt, and shoot and leaf f. wt:d. wt ratio were explored using phylogenetically independent contrasts (Harvey and Pagel, 1991). Phylogenetically independent contrasts (PICs) of traits were calculated using comparative analyses of independent contrasts (CAIC) computer software (Purvis and Rambaut, 1995). Equal branch lengths within the phylogeny were assumed. The use of CAIC to study associations between shoot mineral concentration traits is described elsewhere (Broadley *et al*., 2001).

The PICs were treated in two ways. In the first approach, separate null hypotheses of independent evolution were tested for each pair-wise combination of PICs obtained for shoot Ca, K, Mg, Na, organic-N, and P concentration and leaf f. wt:d. wt ratio. Response PICs were regressed onto explanatory PICs for each possible pair-wise combination. Explanatory PICs were always positive and response PICs were calculated using the same algebraic comparison of nodes. Since the CAIC procedure considers the true phylogeny to bifurcate and thus splits daughter taxa of multiple nodes into two monophyletic groups according to the explanatory trait value (Purvis and Rambaut, 1995), each trait, therefore, defined a slightly different phylogeny and a slightly different set of PICs. Thus, regressions were performed twice for each pair of traits, each trait representing the explanatory (phylogeny-defining) variable in turn. Fitted regression lines were constrained to pass through the origin. Fitted slopes that differed significantly from zero indicated that traits might not have evolved independently (Harvey and Pagel, 1991).

In the second approach, PICs for all traits were analysed simultaneously using principal components analysis (PCA), as described in Broadley *et al*. (2001). A PCA was used to summarize the joint variation between the PICs for all traits simultaneously by fitting linear principal components (PCs) to the PIC correlation matrix representing all traits. The first PC accounted for the greatest proportion of the total variation in the PIC correlation matrix representing all traits. Subsequent PCs, orthogonal to all preceding PCs, accounted for the greatest proportion of the remaining variation in the PIC correlation matrix representing all traits. Loadings were calculated as the contribution of the PIC for each trait to each PC. Subsequently, the PIC for each trait could be described as a vector from the origin to the point specified by the loadings of each PC. The angle (θ) between any pair of vectors (PICs of two different traits) can be calculated from the vector dot product (Smyrl, 1980),

$$\cos\theta = \frac{\boldsymbol{ab}}{|\boldsymbol{a}||\boldsymbol{b}|} \quad (1)$$

where $\boldsymbol{a}$, $\boldsymbol{b}$ are the two vectors and |*| denotes the magnitude of the vector. For three dimensions, x, y, and z, this result becomes,

$$\cos\theta = \frac{x_1x_2 + y_1y_2 + z_1z_2}{\sqrt{(x_1^2 + y_1^2 + z_1^2)}\sqrt{x_2^2 + y_2^2 + z_2^2}} \quad (2)$$

where x_1, y_1, z_1 are the loadings for the first vector $\boldsymbol{a}$ and x_2, y_2, z_2 are the loadings for the second vector $\boldsymbol{b}$. Correlation coefficients, calculated as cosines of these angles, indicated the direction and strength of associations between PICs for two traits. Since 14 traits (shoot mineral concentration, shoot f. wt and d. wt, and shoot and leaf f. wt:d. wt ratios) were measured in 62 species, and seven traits (shoot Ca, K, Mg, Na, organic-N, and P concentration, and leaf f. wt:d. wt ratio) were measured in 115 species, separate PCAs were performed on two PIC correlation matrices; a 14-trait matrix and a seven-trait matrix. Within each of the two PIC correlation matrices, the mean correlation coefficient was calculated for pairs of vectors with each trait considered in turn as the explanatory (phylogeny-defining) variable. A 95% confidence interval was similarly calculated using the mean and standard deviations of the angles between each pair of vectors from the 14-trait or seven-trait PIC correlation matrices.

An ecological survey of leaf mineral concentration in a regional herbaceous flora

Data were obtained from a published ecological survey of a regional herbaceous flora from Central England (Thompson *et al*., 1997). In this ecological survey, leaves were sampled from 81 species assigned to 20 orders of angiosperms. One unassigned species was excluded from the analysis. Each species was sampled from at least five sites. The pH of soils within the survey ranged from 3.4 to > 7. Species were sampled from sites which differed in their underlying geology and land-use. The pH of the sites from where species were sampled, and the leaf concentrations of aluminium (Al), Ca, Cu, Fe, K, Mg, Mn, N, Na P, and Zn were measured. Data were obtained from the supplementary information cited in Thompson *et al*. (1997) (http://www.shef.ac.uk/uni/academic/N-Q/nuocpe/ucpe/nutrient.html). For each of the leaf mineral concentration traits, PICs were calculated using CAIC. As with the experimental data, potential evolutionary associations were explored using pair-wise regression analyses and PCA.

Results

The dominant mineral constituents of (mainly herbaceous) angiosperm shoots were quantified and the variation in shoot mineral concentration was partitioned within an angiosperm phylogeny. This was achieved using an experimental approach based on a pro rata sampling technique; species were selected in proportion to the number of species within each order. The phylogenetic variation in shoot mineral concentration was partitioned between and within orders. Correlations between the shoot concentration of different minerals were determined across species and compared with data from a published ecological survey.

Experimental variation in shoot mineral concentration traits

There was considerable variation in the shoot mineral concentration and shoot f. wt:d. wt ratio between the angiosperm species sampled (Table 1). Shoot C concentration, on a d. wt basis, was lowest in *Beta vulgaris* and

Table 1. *Phylogenetic classification, shoot mineral concentration (% d. wt) and shoot f. wt:d. wt ratio of up to 117 species, representing 24 orders and one unassigned family of angiosperms, grown hydroponically (sd=standard deviation*; n=*number of samples)*

Order	Family	Genus	Species	*n* (unless stated)	Ca		K		Mg		Na		P		Organic–N			Total–N			C			Shoot f. wt:d. wt		
					Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	*n*	Mean	sd	*n*	Mean	sd	*n*	Mean	sd	*n*
Alismatales	Araceae	*Zantedeschia*	*aethiopica*	2	0.89	0.16	7.75	0.90	0.15	0.02	0.090	0.085	0.84	0.04	4.14	0.66	2	5.57	–	1	43.7	–	1	13.8	2.3	2
Apiales	Apiaceae	*Apium*	*graveolens*	6	1.12	0.16	6.93	2.23	0.31	0.05	0.345	0.071	0.84	0.29	3.76	1.64	6	–	–	–	–	–	–	11.2	2.9	6
Apiales	Apiaceae	*Coriandrum*	*sativum*	4	0.64	0.12	7.21	0.59	0.29	0.02	0.018	0.013	0.57	0.10	4.24	0.55	4	5.73	–	1	42.5	–	1	8.8	0.4	3
Apiales	Apiaceae	*Daucus*	*carota*	6	1.77	0.26	5.67	0.98	0.50	0.05	0.052	0.065	0.86	0.12	4.51	0.19	6	5.41	0.26	4	43.8	0.9	4	8.1	0.7	5
Apiales	Araliaceae	*Fatsia*	*japonica*	3	1.06	0.32	3.42	1.18	0.18	0.02	0.047	0.015	0.41	0.14	2.96	0.67	3	–	–	–	–	–	–	7.0	1.0	3
Arecales	Arecaceae	*Phoenix*	*canariensis*	1	0.11	–	1.61	–	0.10	–	0.060	–	0.36	–	2.15	–	1	–	–	–	–	–	–	4.8	–	1
Asparagales	Agavaceae	*Agave*	*parryi*	3	0.99	0.72	3.54	2.62	0.32	0.13	0.153	0.075	0.51	0.08	3.75	0.36	3	–	–	–	–	–	–	17.1	3.0	3
Asparagales	Agavaceae	*Calibanus*	*hookeri*	1	0.91	–	3.99	–	0.21	–	0.020	–	0.41	–	1.67	–	1	–	–	–	–	–	–	11.0	–	1
Asparagales	Alliaceae	*Allium*	*cepa*	5	1.38	0.34	7.50	1.10	0.32	0.06	0.058	0.018	0.84	0.20	4.35	0.87	5	–	–	–	–	–	–	27.7	22.4	5
Asparagales	Alliaceae	*Allium*	*ampeloprasum*	4	0.84	0.06	7.21	0.91	0.23	0.02	0.043	0.013	0.76	0.06	4.14	0.51	4	4.78	–	1	45.5	–	1	14.1	0.4	4
Asparagales	Amaryllidaceae	*Narcissus*	*pallidiflorus*	1	0.33	–	3.89	–	0.18	–	0.020	–	0.89	–	4.05	–	1	–	–	–	–	–	–	5.4	–	1
Asparagales	Asparagaceae	*Asparagus*	*officinalis*	1	0.85	–	3.69	–	0.25	–	0.050	–	0.59	–	3.66	–	1	–	–	–	–	–	–	6.4	–	1
Asparagales	Asphodelaceae	*Aloe*	*pruinosa*	3	2.84	0.45	4.76	1.06	0.51	0.03	0.423	0.096	0.62	0.04	2.80	0.35	3	–	–	–	–	–	–	29.7	2.7	3
Asparagales	Asphodelaceae	*Asphodeline*	*lutea*	1	1.18	–	5.18	–	0.22	–	0.080	–	0.80	–	5.08	–	1	–	–	–	–	–	–	10.0	–	1
Asparagales	Hyancinthaceae	*Lachenalia*	*contaminata*	1	1.04	–	3.87	–	0.23	–	0.390	–	0.55	–	3.06	–	1	–	–	–	–	–	–	13.0	–	1
Asparagales	Iridaceae	*Crocosmia*	*masauorum*	5	0.98	0.12	3.20	0.47	0.28	0.01	0.122	0.037	0.56	0.03	4.67	1.54	5	–	–	–	–	–	–	8.7	0.9	5
Asparagales	Iridaceae	*Freesia*	*elimensis*	3	0.82	0.13	3.68	0.24	0.25	0.03	0.027	0.006	1.47	0.25	4.71	0.12	3	–	–	–	–	–	–	5.9	0.4	3
Asparagales	Iridaceae	*Gladiolus*	*blandus*	1	0.72	–	0.98	–	0.19	–	1.050	–	1.10	–	–	–	–	–	–	–	–	–	–	9.0	–	1
Asparagales	Iridaceae	*Iris*	*psuedocorus*	1	1.00	–	6.17	–	0.27	–	0.030	–	1.11	–	4.13	–	1	–	–	–	–	–	–	10.0	–	1
Asterales	Asteraceae	*Achillea*	*milleflorum*	6	0.83	0.11	8.35	0.74	0.28	0.03	0.025	0.008	1.00	0.22	3.98	0.42	6	–	–	–	–	–	–	14.0	1.2	6
Asterales	Asteraceae	*Aster*	*novibelgii*	4	0.99	0.05	6.49	0.70	0.26	0.03	0.028	0.015	1.40	0.18	5.04	0.36	4	–	–	–	–	–	–	10.2	1.3	3
Asterales	Asteraceae	*Bellis*	*perennis*	6	0.97	0.20	5.49	0.60	0.25	0.03	0.185	0.065	0.69	0.18	3.53	0.35	6	–	–	–	–	–	–	9.8	0.8	5
Asterales	Asteraceae	*Carthamus*	*tinctorius*	5	1.25	0.24	6.94	0.61	0.34	0.05	0.026	0.006	0.53	0.05	4.48	0.09	5	–	–	–	–	–	–	16.1	1.6	4
Asterales	Asteraceae	*Chrysanthemum*	*leucanthemum*	6	0.70	0.07	6.84	1.44	0.23	0.04	0.092	0.127	0.91	0.28	4.49	0.57	6	6.81	0.39	5	39.7	1.2	5	11.8	1.2	5
Asterales	Asteraceae	*Cichorium*	*intybus*	6	1.41	0.21	6.39	0.63	0.36	0.06	0.057	0.067	1.01	0.08	4.84	0.34	6	6.21	–	1	39.1	–	1	12.4	2.5	5
Asterales	Asteraceae	*Cynara*	*cardunculus*	1	1.36	–	6.15	–	0.36	–	0.280	–	0.63	–	3.02	–	1	5.93	–	1	43.3	–	1	12.3	–	1
Asterales	Asteraceae	*Dahlia*	spp.	6	1.95	0.35	4.97	0.44	0.51	0.04	0.017	0.005	0.80	0.09	4.32	0.30	6	–	–	–	–	–	–	14.0	0.6	5
Asterales	Asteraceae	*Helianthus*	*annuus*	6	2.61	0.29	6.25	0.63	0.54	0.07	0.010	0.000	0.96	0.20	6.40	0.47	6	7.47	0.21	6	39.5	0.3	6	13.4	0.2	5
Asterales	Asteraceae	*Lactuca*	*sativa*	6	0.96	0.11	6.26	1.27	0.40	0.04	0.027	0.005	0.83	0.19	5.04	0.50	6	6.06	0.64	6	43.1	2.0	6	15.6	2.7	5
Asterales	Asteraceae	*Pyrethrum*	*roseum*	6	0.63	0.23	7.46	1.68	0.21	0.07	0.027	0.010	0.85	0.26	4.29	1.21	6	4.73	–	1	46.5	–	1	10.0	4.5	6
Asterales	Asteraceae	*Taraxacum*	*officinale*	6	1.08	0.05	7.70	0.55	0.36	0.03	0.033	0.005	0.72	0.04	4.18	0.27	6	6.39	0.38	5	40.8	2.1	5	11.1	0.6	5
Asterales	Asteraceae	*Tragopogon*	*porrifolius*	6	1.09	0.22	5.28	0.96	0.31	0.07	0.092	0.109	0.62	0.12	5.08	1.10	6	5.65	0.71	3	44.6	3.2	3	9.1	1.3	5
Asterales	Brunoniaceae	*Brunonia*	*australis*	3	1.41	0.32	4.45	0.76	0.52	0.22	0.053	0.058	1.15	0.47	3.31	0.86	3	5.36	–	1	45.7	–	1	12.1	1.6	3
Asterales	Campanulaceae	*Campanula*	*rotundifolia*	6	1.02	0.21	4.75	0.78	0.44	0.07	0.025	0.019	1.04	0.23	3.83	0.77	6	5.45	0.91	2	46.7	2.7	2	7.8	1.0	6
Brassicales	Brassicaceae	*Arabidopsis*	*thaliana*	4	1.54	0.45	2.97	0.89	0.38	0.06	0.683	0.689	0.82	0.10	3.51	4.58	2	–	–	–	–	–	–	8.3	3.0	4
Brassicales	Brassicaceae	*Brassica*	*oleracea*	6	4.41	0.30	4.67	0.57	0.61	0.04	0.035	0.006	0.74	0.09	5.34	0.38	6	6.88	0.22	6	37.5	0.5	6	12.1	1.4	5
Brassicales	Brassicaceae	*Brassica*	*napus*	6	2.44	0.21	4.89	0.27	0.37	0.02	0.027	0.005	0.46	0.03	3.37	0.49	6	–	–	–	–	–	–	12.6	0.5	5
Caryophyllales	Aizoaceae	*Mesembry-anthemum*	*criniflorum*	5	0.79	0.28	9.01	2.71	0.40	0.14	2.472	0.187	0.61	0.14	5.19	0.67	5	7.43	0.16	2	39.7	5.2	2	33.0	4.6	4
Caryophyllales	Amaranthaceae	*Amaranthus*	*hypochon-driacus*	2	1.88	0.59	6.03	0.91	0.95	0.08	0.020	0.000	0.67	0.14	4.93	0.62	2	6.80	0.22	2	42.2	2.7	2	10.1	1.8	2
Caryophyllales	Cactaceae	*Echinofossulo-cactus*	spp.	3	1.38	1.07	8.12	1.75	0.92	0.30	1.760	1.050	1.42	0.40	5.75	1.32	3	–	–	–	–	–	–	21.7	19.1	3
Caryophyllales	Caryophyllaceae	*Dianthus*	*barbatus*	3	0.99	0.34	4.91	1.23	0.29	0.08	0.020	0.000	0.79	0.06	4.35	0.32	3	–	–	–	–	–	–	7.0	0.7	3
Caryophyllales	Chenopodiaceae	*Beta*	*vulgaris*	3	1.06	0.20	8.53	1.24	0.92	0.15	1.317	0.527	1.17	0.09	5.05	0.44	3	6.33	0.20	3	37.1	0.8	3	16.7	0.7	3
Caryophyllales	Chenopodiaceae	*Chenopodium*	*bonus-henricus*	6	1.01	0.07	7.45	0.35	0.91	0.11	0.018	0.008	0.55	0.04	4.70	0.81	6	6.38	–	1	43.0	–	1	10.4	0.8	5
Caryophyllales	Plumbaginaceae	*Armeria*	*maritima*	1	0.59	–	3.33	–	0.92	–	0.030	–	0.69	–	4.08	–	1	–	–	–	–	–	–	6.9	–	1
Celastrales	Celastraceae	*Celastrus*	*orbiculatus*	3	1.15	0.10	3.20	0.61	0.30	0.11	0.190	0.321	0.37	0.13	3.15	0.73	3	3.80	0.13	2	47.9	1.1	2	6.2	0.3	3

Table 1. *Continued*

Order	Family	Genus	Species	*n* (unless stated)	Ca		K		Mg		Na		P		Organic–N		Total–N			C			Shoot f. wt:d. wt		
					Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	*n*	Mean	sd	*n*	Mean	sd	*n*	Mean	sd	*n*
Cucurbitales	Cucurbitaceae	*Curcurbita*	*maxima*	6	3.26	0.51	6.25	0.50	0.57	0.08	0.018	0.004	1.28	0.12	5.83	0.46	6	6.96	0.12	6	38.0	0.6	6	15.3	0.8	5
Dipsacales	Valerianaceae	*Valeriana*	*officinalis*	3	0.78	0.21	3.55	2.00	0.32	0.11	0.013	0.006	0.54	0.24	3.55	0.80	3	3.89	0.61	2	47.5	1.7	2	7.7	1.3	3
Dipsacales	Valerianaceae	*Valerianella*	*locusta*	6	1.19	0.08	4.63	1.18	0.34	0.03	0.005	0.006	0.95	0.27	4.57	0.33	6	6.09	0.46	5	44.9	1.4	5	9.4	1.7	5
Ericales	Ericaceae	*Vaccinium*	*vitis-idaea*	4	1.07	0.32	1.81	0.52	0.43	0.12	0.790	0.499	0.52	0.11	2.94	2.62	4	–	–	–	–	–	–	5.1	3.3	4
Fabales	Fabaceae	*Anthyllis*	*vulneraria*	6	2.73	0.32	4.86	0.50	0.44	0.03	0.000	0.000	0.53	0.04	3.96	0.17	6	5.49	0.43	5	42.4	2.8	5	10.0	0.9	5
Fabales	Fabaceae	*Lens*	*culinaris*	6	1.58	0.16	3.07	0.21	0.32	0.02	0.015	0.006	0.62	0.04	3.91	0.26	6	–	–	–	–	–	–	6.2	0.3	5
Fabales	Fabaceae	*Lotus*	*coniculatus*	6	1.21	0.40	5.08	0.72	0.36	0.14	0.202	0.372	0.99	0.33	5.71	0.35	5	–	–	–	–	–	–	7.3	0.8	5
Fabales	Fabaceae	*Melitius*	*officinalis*	2	1.50	0.11	3.86	0.15	0.30	0.04	0.010	0.000	0.71	0.04	4.80	0.36	2	6.88	–	1	44.5	–	1	8.2	0.1	2
Fabales	Fabaceae	*Pisum*	*sativum*	6	1.41	0.09	3.78	0.21	0.23	0.01	0.015	0.006	0.71	0.03	5.79	0.32	5	6.26	0.23	6	44.5	0.6	6	8.3	0.3	5
Fabales	Fabaceae	*Trifolium*	*pratense*	6	1.75	0.13	3.93	0.40	0.42	0.06	0.013	0.005	0.66	0.08	4.75	0.53	6	5.61	0.08	2	43.0	0.3	2	7.8	0.8	5
Fabales	Fabaceae	*Trifolium*	*repens*	6	1.46	0.19	4.59	0.40	0.26	0.03	0.007	0.005	0.75	0.14	5.11	0.24	6	6.24	–	1	46.6	–	1	8.3	0.7	6
Fabales	Fabaceae	*Vicia*	*cracca*	4	1.61	0.40	2.92	0.63	0.22	0.03	0.198	0.264	0.56	0.13	4.79	0.30	4	–	–	–	–	–	–	5.2	1.3	3
Fabales	Fabaceae	*Vicia*	*sativa*	6	1.49	0.31	3.89	0.17	0.25	0.04	0.032	0.008	1.08	0.19	5.64	0.64	6	5.81	0.42	2	47.9	0.7	2	8.5	4.8	6
Gentianales	Apocynaceae	*Vinca*	*rosea*	6	1.42	0.11	2.27	0.72	0.32	0.03	0.028	0.004	0.52	0.03	4.02	0.49	6	6.05	–	1	47.7	–	1	6.5	0.9	5
Gentianales	Rubiaceae	*Asperula*	*odorata*	5	1.54	0.36	4.97	0.89	0.37	0.03	0.020	0.017	0.56	0.15	4.74	0.78	5	7.29	–	1	44.6	–	1	11.8	1.5	5
Gentianales	Rubiaceae	*Galium*	*verum*	6	1.18	0.19	4.93	0.35	0.30	0.03	0.030	0.028	0.71	0.11	5.06	0.66	6	5.91	0.42	2	46.9	1.2	2	6.4	0.7	5
Gentianales	Rubiaceae	*Gardenia*	*jasminoides*	1	0.90	–	2.98	–	0.33	–	0.230	–	0.60	–	4.04	–	1	–	–	–	–	–	–	4.1	–	1
Lamiales	Acanthaceae	*Hypoestes*	*sanguinolenta*	6	3.58	0.55	5.33	0.28	0.63	0.06	0.010	0.000	0.77	0.06	6.23	0.47	6	7.39	0.51	6	39.3	2.3	6	8.5	0.6	5
Lamiales	Lamiaceae	*Ocimum*	*basilicum*	5	1.58	0.39	5.91	1.82	0.32	0.05	0.024	0.009	1.01	0.31	3.77	0.49	5	5.31	–	1	44.8	–	1	13.7	6.0	5
Lamiales	Lamiaceae	*Origanum*	*virens*	2	1.15	0.08	4.20	0.35	0.46	0.05	0.130	0.156	0.57	0.06	3.91	0.68	2	5.87	–	1	44.7	–	1	9.6	3.4	2
Lamiales	Oleaceae	*Syringa*	*vulgaris*	1	0.57	–	2.15	–	0.26	–	0.090	–	0.71	–	–	–		–	–	–	–	–	–	4.7	–	1
Lamiales	Scrophulariaceae	*Antirrhinum*	*majus*	3	1.54	0.24	4.58	0.37	0.67	0.13	0.017	0.012	0.68	0.03	5.73	0.27	3	7.77	–	1	43.4	–	1	5.1	5.8	3
Lamiales	Scrophulariaceae	*Digitalis*	*purpurea*	6	0.94	0.12	4.73	0.89	0.26	0.06	0.027	0.010	0.69	0.14	3.33	0.78	6	5.60	0.47	3	43.3	0.9	3	9.9	1.2	5
Lamiales	Scrophulariaceae	*Rehmannia*	*angulata*	3	1.70	0.30	6.00	1.17	0.45	0.03	0.010	0.000	0.61	0.04	4.33	0.40	3	6.02	0.27	3	42.2	1.7	3	11.9	1.2	3
Malpighiales	Clusiaceae	*Hypericum*	*perforatum*	2	0.67	0.04	3.15	0.07	0.31	0.04	0.170	0.014	0.98	0.05	4.72	0.08	2	–	–	–	–	–	–	6.5	0.1	2
Malpighiales	Euphorbiaceae	*Euphorbia*	*lathyrus*	3	0.99	0.17	3.94	0.77	0.33	0.10	0.043	0.035	0.44	0.08	3.84	0.69	3	–	–	–	–	–	–	9.9	1.4	3
Malpighiales	Euphorbiaceae	*Ricinus*	*communis*	4	1.61	0.18	4.07	0.51	0.47	0.04	0.010	0.000	0.81	0.09	5.95	0.48	4	7.01	0.07	4	42.1	0.5	4	10.6	1.0	3
Malpighiales	Flacourtiaceae	*Passiflora*	*caerulea*	2	1.24	0.35	2.85	0.52	0.36	0.05	0.020	0.014	0.54	0.00	4.86	0.74	2	–	–	–	–	–	–	5.5	0.5	2
Malpighiales	Violaceae	*Viola*	*tricolor*	6	0.88	0.33	5.92	0.68	0.44	0.13	0.047	0.061	0.93	0.12	4.72	2.18	6	–	–	–	–	–	–	11.5	0.7	5
Malvales	Malvaceae	*Althaea*	*officinalis*	2	2.29	0.07	5.29	0.06	0.70	0.07	0.040	0.000	1.19	0.07	4.88	0.57	2	–	–	–	–	–	–	9.3	0.1	2
Malvales	Malvaceae	*Althaea*	*rosea*	3	2.26	0.19	5.47	0.39	0.66	0.03	0.013	0.023	0.46	0.06	4.09	0.20	3	5.73	0.13	2	40.9	0.3	2	9.6	1.2	3
Malvales	Malvaceae	*Gossypium*	*herbaceum*	1	3.19	–	4.01	–	0.57	–	0.030	–	0.81	–	5.02	–	1	6.77	–	1	39.3	–	1	9.6	–	1
Myrtales	Lythraceae	*Cuphea*	*ignea*	6	0.96	0.16	3.10	0.39	0.51	0.11	0.022	0.008	0.71	0.16	4.25	0.54	6	–	–	–	–	–	–	7.6	0.6	5
Myrtales	Melastomataceae	*Melastoma*	*sanguineum*	3	1.55	0.27	2.17	0.64	0.50	0.07	0.523	0.581	0.96	0.07	5.30	1.64	3	–	–	–	–	–	–	4.1	1.5	3
Myrtales	Myrtaceae	*Eucalyptus*	*stricklandii*	2	0.54	0.03	2.12	1.35	0.17	0.01	0.285	0.163	1.34	0.54	4.42	3.84	3	–	–	–	–	–	–	7.0	0.8	3
Myrtales	Onagraceae	*Clarkia*	*elegans*	6	1.19	0.20	5.23	0.83	0.36	0.04	0.018	0.004	0.80	0.17	5.54	0.53	6	6.57	0.28	6	44.0	1.9	6	11.0	0.4	5
Myrtales	Onagraceae	*Epilobium*	*hirsuteum*	6	1.10	0.05	5.22	0.33	0.42	0.06	0.032	0.004	0.97	0.17	4.21	0.39	6	6.12	0.53	6	43.4	2.1	6	12.0	0.6	5
Myrtales	Onagraceae	*Fuchsia*	spp.	5	1.24	0.20	6.39	1.40	0.32	0.03	0.012	0.005	0.91	0.11	4.52	0.53	5	6.04	0.69	3	43.1	3.1	3	14.0	2.0	5
not assigned	Boraginaceae	*Borago*	*officinalis*	2	1.62	0.14	9.15	0.00	0.26	0.05	0.285	0.092	1.32	0.19	6.27	0.54	2	6.73	0.03	2	37.5	0.4	2	17.2	0.8	2
not assigned	Boraginaceae	*Myosotis*	*arvensis*	4	1.05	0.20	7.75	0.66	0.22	0.04	0.128	0.202	0.85	0.15	3.99	0.79	4	–	–	–	–	–	–	12.3	1.9	2
Oxalidales	Oxalidaceae	*Oxalis*	*valdiviensis*	4	0.92	0.15	4.56	0.15	0.54	0.09	0.003	0.005	1.17	0.23	4.22	0.21	4	–	–	–	–	–	–	10.4	1.0	3
Poales	Cyperaceae	*Carex*	*psuedocyperus*	1	0.51	–	5.99	–	0.29	–	0.020	–	0.52	–	3.75	–	1	5.15	–	1	45.8	–	1	9.1	–	1
Poales	Cyperaceae	*Carex*	*nigra*	1	0.28	–	6.91	–	0.24	–	0.120	–	0.57	–	1.84	–	1	–	–	–	–	–	–	6.6	–	1
Poales	Juncaceae	*Luzula*	*nivea*	5	0.53	0.16	4.61	0.27	0.25	0.05	0.026	0.037	0.62	0.07	3.71	0.40	5	4.49	–	1	48.0	–	1	5.8	1.3	4
Poales	Poaceae	*Anthoxanthum*	*odoratum*	4	0.27	0.05	5.94	0.18	0.15	0.02	0.045	0.044	0.78	0.09	4.60	0.55	4	–	–	–	–	–	–	7.7	0.2	4
Poales	Poaceae	*Avena*	*sativa*	2	0.32	0.04	6.94	0.01	0.21	0.00	0.015	0.007	0.99	0.05	4.07	0.52	2	6.55	0.37	2	40.8	0.8	2	10.6	0.0	2
Poales	Poaceae	*Briza*	*media*	6	0.19	0.04	4.46	0.50	0.12	0.03	0.030	0.039	0.62	0.06	4.06	0.76	6	–	–	–	–	–	–	6.0	0.6	5
Poales	Poaceae	*Bromus*	*macrostachys*	6	0.51	0.07	7.85	0.45	0.20	0.02	0.022	0.004	0.85	0.06	4.95	0.28	6	6.88	0.07	5	41.6	0.6	5	11.5	0.3	5
Poales	Poaceae	*Calamagrostis*	*epigejos*	2	0.30	0.03	4.37	0.66	0.25	0.05	0.060	0.042	0.90	0.21	5.57	1.20	2	–	–	–	–	–	–	6.3	0.3	2

Table 1. *Continued*

Order	Family	Genus	Species	*n* (unless stated)	Ca		K		Mg		Na		P		Organic–N			Total–N			C			Shoot f. wt:d. wt		
					Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	*n*	Mean	sd	*n*	Mean	sd	*n*	Mean	sd	*n*
Poales	Poaceae	*Elymus*	*glaucus*	5	0.33	0.04	5.53	0.13	0.16	0.02	0.002	0.005	0.74	0.04	4.29	0.20	5	5.57	0.27	4	45.6	1.5	4	6.4	0.3	4
Poales	Poaceae	*Festuca*	*ovina*	2	0.28	0.08	3.13	0.60	0.17	0.02	0.020	0.000	0.69	0.19	3.52	1.29	2	–	–	–	45.6	1.5	4	6.4	0.3	4
Poales	Poaceae	*Koeleria*	*glauca*	6	0.35	0.05	4.11	0.24	0.17	0.02	0.015	0.008	0.82	0.06	5.05	0.34	6	5.93	0.40	4	–	–	–	4.8	0.2	2
Poales	Poaceae	*Lolium*	*perenne*	6	0.65	0.52	6.14	0.61	0.22	0.03	0.052	0.022	0.81	0.05	4.14	0.39	6	–	–	–	47.2	1.1	4	6.1	0.3	5
Poales	Poaceae	*Miscanthus*	*sinensis*	4	0.63	0.30	3.42	0.88	0.30	0.04	0.023	0.025	1.08	0.19	3.12	0.55	4	–	–	–	–	–	–	8.2	0.8	5
Poales	Poaceae	*Phleum*	*pratense*	6	0.35	0.09	3.94	0.20	0.18	0.02	0.007	0.010	0.72	0.07	3.92	0.14	6	3.87	–	1	–	–	–	7.2	0.9	3
Poales	Poaceae	*Poa*	*annua*	6	0.56	0.32	6.00	0.28	0.29	0.09	0.040	0.018	0.80	0.09	5.23	0.35	6	–	–	–	49.3	–	1	4.9	0.3	5
Poales	Poaceae	*Secale*	*cereale*	6	0.54	0.07	7.00	0.44	0.23	0.01	0.012	0.004	1.15	0.13	5.03	0.32	6	7.05	0.28	6	–	–	–	8.0	0.2	5
Poales	Poaceae	*Triticum*	*aestivum*	6	0.36	0.11	6.25	0.94	0.20	0.04	0.048	0.079	0.93	0.09	5.39	0.68	6	7.04	0.12	3	41.6	0.6	6	9.9	0.4	5
Poales	Poaceae	*Zea*	*mays*	4	0.45	0.04	5.44	0.28	0.23	0.02	0.010	0.000	1.02	0.13	3.55	0.04	4	–	–	–	41.9	0.5	3	8.2	0.9	5
Proteales	Proteaceae	*Grevillea*	*robusta*	3	0.96	0.37	1.69	0.66	0.26	0.03	0.030	0.010	0.72	0.29	3.47	0.83	3	–	–	–	–	–	–	14.5	0.6	3
Rosales	Moraceae	*Morus*	*alba*	3	0.98	0.34	3.11	1.29	0.34	0.13	0.040	0.027	0.96	0.25	1.27	0.96	3	–	–	–	–	–	–	4.2	0.5	3
Rosales	Rosaceae	*Fragaria*	*vesca*	6	1.42	0.16	3.51	0.25	0.42	0.02	0.012	0.016	0.73	0.06	3.66	0.35	6	4.33	0.08	2	–	–	–	7.3	0.7	3
Rosales	Rosaceae	*Potentilla*	*erecta*	1	0.89	–	1.86	–	0.35	–	0.010	–	0.36	–	2.72	–	1	–	–	–	47.0	0.9	2	6.0	0.4	5
Sapindales	Anacardiaceae	*Rhus*	*glabra*	3	1.36	0.09	2.66	0.11	0.25	0.03	0.010	0.000	0.58	0.14	3.38	0.25	3	5.49	–	1	–	–	–	4.8	–	1
Sapindales	Rutaceae	*Citrus*	*limettoides*	2	1.76	0.06	1.98	0.04	0.34	0.05	0.035	0.007	0.43	0.01	4.42	0.08	2	–	–	–	47.1	–	1	6.0	0.3	3
Sapindales	Rutaceae	*Ruta*	*graveolens*	5	1.09	0.24	3.65	0.61	0.28	0.05	0.088	0.164	0.60	0.11	4.35	0.88	5	4.87	–	1	–	–	–	6.8	3.7	2
Sapindales	Sapindaceae	*Cardio-spermum*	*halicacabum*	1	1.30	–	4.01	–	0.43	–	0.000	–	0.83	–	5.19	–	1	7.16	–	1	48.1	–	1	6.1	0.8	5
Saxifragales	Saxifragaceae	*Bergenia*	*cordiflora*	6	1.31	0.19	2.83	0.26	0.32	0.04	0.030	0.020	0.67	0.06	2.45	0.83	6	–	–	–	43.5	–	1	9.2	–	1
Solanales	Solanaceae	*Lycopersicon*	*esculentum*	6	1.96	0.14	4.81	0.77	0.42	0.02	0.022	0.004	0.76	0.07	6.69	0.42	6	7.13	0.31	6	–	–	–	7.9	0.6	6
Solanales	Solanaceae	*Nicotiana*	*tabacum*	4	1.55	0.33	6.84	1.55	0.53	0.08	0.018	0.010	0.59	0.08	5.40	0.39	4	6.60	0.14	4	41.1	1.1	6	15.3	2.0	5
Solanales	Solanaceae	*Petunia*	spp.	6	1.27	0.26	7.78	0.70	0.37	0.02	0.030	0.000	0.64	0.07	4.91	0.41	6	7.76	0.35	6	39.6	1.3	4	16.0	1.9	3
Solanales	Solanaceae	*Solanum*	*melongena*	6	1.56	0.12	5.79	0.45	0.35	0.03	0.002	0.004	0.52	0.06	4.40	0.38	6	6.98	0.22	6	39.5	2.6	6	18.7	1.3	5

Table 2. *Phylogenetic classification, mean shoot mineral concentration (% d. wt) and shoot f. wt:d. wt ratio of up to 24 orders and one unassigned family of angiosperms calculated from up to 117 species* (n) *grown hydroponically*

Informal group	Order	*n* (unless stated)	Ca (%)	K (%)	Mg (%)	Na (%)	P (%)	Organic-N		Total-N		C		Shoot f. wt:d. wt
								(%)	*n*	(%)	*n*	(%)	*n*	
Eudicot	Apiales	4	1.16	5.83	0.32	0.13	0.67	3.87	4	5.58	2	43.2	2	8.6
Eudicot	Asterales	15	1.23	6.28	0.36	0.06	0.88	4.38	15	6.00	10	42.9	10	11.9
Eudicot	Boraginaceae	2	1.41	8.64	0.25	0.22	1.13	5.16	2	6.72	1	37.5	1	15.0
Eudicot	Brassicales	3	2.80	4.23	0.45	0.25	0.69	4.09	3	6.88	1	37.5	1	10.9
Eudicot	Caryophyllales	7	1.13	6.75	0.76	0.82	0.86	4.84	7	6.77	4	40.3	4	15.1
Eudicot	Celastrales	1	1.15	3.13	0.30	0.16	0.37	3.09	1	–	–	–	–	6.2
Eudicot	Cucurbitales	1	3.29	6.26	0.58	0.02	1.27	5.74	1	6.96	1	38.0	1	15.3
Eudicot	Dipsacales	2	0.99	4.09	0.33	0.02	0.76	4.07	2	4.97	2	46.1	2	8.4
Eudicot	Ericales	1	1.04	1.67	0.42	0.79	0.51	2.99	1	–	–	–	–	5.0
Eudicot	Fabales	9	1.65	4.03	0.31	0.06	0.74	4.94	9	6.04	6	44.9	6	7.7
Eudicot	Gentianales	4	1.27	3.84	0.33	0.09	0.60	4.49	4	6.34	3	46.7	3	7.1
Eudicot	Lamiales	7	1.59	4.68	0.43	0.06	0.72	4.54	6	6.33	6	42.9	6	9.1
Eudicot	Malpighiales	5	1.11	3.95	0.38	0.06	0.75	4.80	5	7.01	1	42.1	1	8.7
Eudicot	Malvales	3	2.64	4.93	0.65	0.04	0.85	4.62	3	6.24	2	39.9	3	9.6
Eudicot	Myrtales	6	1.09	4.00	0.38	0.16	0.95	4.74	6	6.23	3	43.5	3	9.2
Eudicot	Oxalidales	1	0.87	4.49	0.53	-0.02	1.16	4.24	1	–	–	–	–	10.2
Eudicot	Proteales	1	0.99	1.57	0.26	0.05	0.72	3.51	1	–	–	–	–	3.9
Eudicot	Rosales	3	1.13	3.02	0.37	0.04	0.72	2.56	3	4.33	1	47.0	1	6.2
Eudicot	Sapindales	4	1.40	3.08	0.32	0.04	0.63	4.39	4	5.26	3	46.3	3	6.9
Eudicot	Saxifragales	1	1.32	2.80	0.32	0.03	0.66	2.42	1	–	–	–	–	7.9
Eudicot	Solanales	4	1.60	6.36	0.42	0.02	0.63	5.31	4	7.12	4	40.2	4	15.3
Commelinoid monocot	Poales	18	0.42	5.44	0.22	0.04	0.82	4.22	18	5.83	9	44.6	9	7.9
Commelinoid monocot	Arecales	1	0.07	1.59	0.10	0.06	0.40	2.25	1	–	–	–	–	5.1
Non-commelinoid monocot	Asparagales	13	1.10	4.43	0.27	0.21	0.80	3.82	12	5.13	2	46.0	2	13.1
Non-commelinoid monocot	Alismatales	1	0.93	7.82	0.15	0.09	0.86	4.23	1	5.58	1	43.4	1	13.8
P (orders within informal groups do not differ in trait means)	Within eudicots		***	***	***	*	ns	**		ns		***		**
(*** P <0.001; ** P <0.01; * P <0.05; ns P >0.05)	Within commelinoid monocots		*	*	*	ns	*	*		ns		ns		ns
	Within non-commelinoid monocots		ns	ns	ns	ns	ns	ns		ns		ns		ns

Brassica oleracea (*c*. 37%), and highest in *Phleum pratense* and *Ruta graveolens* (*c.* 49%). Shoot organic-N concentration was lowest in *Morus alba* (1.3%) and *Calibanus hookeri* (1.7%), and highest in *Lycopersicon esculentum* (6.7%) and *Helianthus annuus* (6.4%). Shoot Ca concentration was lowest in *Phoenix canariensis* (0.11%) and *Briza media* (0.19%), which were also the two species with the lowest shoot Mg concentration (0.10% and 0.12% respectively), and highest in *B. oleracea* (4.4%) and *Hypoestes sanguinolentua* (3.6%). Shoot Mg concentration was highest in *Amaranthus hypochondriacus* (0.95%) and *Echinofossulocactus* spp. (0.92%). Shoot K concentration was lowest in *Gladiolus blandus* (0.98%) and *Phoenix canariensis* (1.61%), and highest in *Borago officinalis* (9.2%) and *Mesembryanthemum criniflorum* (9.0%). Sodium was not detected in the shoots of *Cardiospermum halicacabum* and *Anthyllis vulneraria*, whilst Na was highest in the shoots of *Mesembryanthemum criniflorum* (2.5%) and *Echinofossulocactus* spp. (1.8%). Finally, shoot P concentration was lowest in *Potentilla erecta* and *Phoenix canariensis* (both species 0.36%) and highest in *Freesia elimensis* (1.47%) and *Echinofossulocactus* spp. (1.42%). Leaf and shoot f. wt:d. wt ratios on these young plants were highly correlated (r=0.98; d.f.=115). Shoot f. wt:d. wt ratio was lowest in *Gardenia jasminoides* and *Melastoma sanguineum* (*c.* 4.1), and highest in *Mesembryanthemum criniflorum* (33.0) and *Aloe pruinosa* (29.7).

The trait means differed between orders; those orders with extreme mineralogies often included species with

Table 3. *Trait variation and mean shoot mineral concentration and shoot f. wt:d. wt ratio of three informal groups of angiosperms, derived from up to 117 species* (n) *grown hydroponically*

Variations are expressed as proportions of the total trait variation (100%) for each informal group. Asterisks indicate where negative variation estimates, derived from residual maximum likelihood analyses, have been floored to zero.

Informal group	Hierarchical level	*n* (unless stated)	Trait variation (%) partitioned within each informal group											
			Ca	P	K	Na	Mg	C	*n*	Total-N	*n*	Organic-N	*n*	Shoot f. wt:d. wt
Angiosperm	Informal group	117	36.6	0.0	0.0*	0.0*	33.4	1.2	62	6.0	62	3.3	115	10.4
	Order		27.2	6.8	49.7	23.1	31.6	42.9		3.3		20.3		16.5
	Species		36.2	93.2	50.3	76.9	35.1	56.0		90.7		76.4		73.2
Eudicot	Order	84	38.3	9.2	53.8	19.9	41.7	54.2	50	13.2	50	25.7	83	28.0
	Species		61.7	90.8	46.2	80.1	58.3	45.8		86.8		74.3		72.0
Commelinoid monocot	Order	19	76.3	70.9	78.1	0.0*	70.9	na	9	na	9	na	19	8.8
	Species		23.7	29.1	21.9	100.0	29.1	na		na		na		91.2
Non-commelinoid monocot	Order	14	0.0*	0.0*	59.1	0.0*	36.5	64.7	3	0.0*	3	0.0*	13	0.0*
	Species		100.0	100.0	40.9	100.0	63.5	35.3		100.0		100.0		100.0*
			Trait means											
			Ca (%)	P (%)	K (%)	Na (%)	Mg (%)	C (%)	*n*	Total-N (%)	*n*	Organic-N (%)	*n*	Shoot f. wt:d. wt
Angiosperm		117	1.22	0.79	4.94	0.14	0.36	43.3	62	6.08	62	4.33	115	10.04
Eudicot		84	1.42	0.78	4.91	0.15	0.41	43.0	50	6.17	50	4.46	83	10.05
Commelinoid monocot		19	0.40	0.80	5.24	0.04	0.21	44.6	9	5.83	9	4.12	19	7.71
Non-commelinoid monocot		14	1.09	0.81	4.67	0.20	0.26	45.2	3	5.28	3	3.85	13	13.13
P (informal groups: eudicots, commelinoid and non-commelinoid monocots do not differ in trait means) (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns $P > 0.05$)			***	ns	ns	ns	***	ns		ns		ns		**

extreme trait means (Table 2). Shoot C concentration ranged from 37.5% (Boraginaceae and Brassicales) to 47% (Rosales). Shoot organic-N concentration ranged from 2.25% (Arecales) to 5.74% (Cucurbitales). Shoot Ca concentration ranged from 0.87% (Oxalidales) to 3.29% (Cucurbitales). Shoot Mg concentration ranged from 0.10% (Arecales) to 0.76% (Caryophyllales). Shoot K concentration ranged from 1.59% (Proteales) to 8.64% (Boraginaceae). Shoot Na concentration ranged from –0.02% (Oxalidales; a negative value resulted from an adjustment for environmental variation in the REML procedure) to 0.82% (Caryophyllales). Finally, shoot P concentration ranged from 0.37% (Celastrales) to 1.27% (Cucurbitales). One-way ANOVA between orders, within informal classification level (eudicot, commelinoid monocot and non-commelinoid monocot), revealed significant trait differences between eudicot orders for all traits except for shoot P and total-N concentration, despite several orders being represented by only one species.

Low shoot Ca and Mg concentration and low shoot f. wt:d. wt ratio was a general feature of the commelinoid monocots (Table 3). Thus, one-way ANOVA revealed significant differences in shoot Ca and Mg concentration and shoot f. wt:d. wt ratio between informal groups. Partitioning the variation in shoot C, Ca, K, Mg, Na, organic-N, P, and total-N concentration and shoot f. wt:d. wt ratio between and within each informal group, and estimating the variation between and within orders restricting for informal group, revealed that the proportion of variation at or above the level of the order was 65% for shoot Mg concentration and 64%, 50% and 44% for shoot Ca, K and C concentration, respectively. Little variation was accounted for at or above the level of the order for shoot P (7%) or for total-N (9%) concentration. Intermediate levels of variation occurred at or above the level of order for shoot Na (23%) and organic-N (24%) concentration, and for shoot f. wt:d. wt ratio (27%). Within the eudicots, >50% of the variation in shoot K and C concentration, and approximately 40% of the variation in shoot Ca and Mg concentration, occurred between orders.

Phylogenetically independent contrasts of experimental shoot mineral concentration traits

Pair-wise comparisons of PICs revealed positive correlations between the shoot concentration of Ca, K, Mg, Na, organic-N, P, and the shoot concentration of at least one other mineral element (Table 4). Leaf f. wt:d. wt ratio was positively associated with shoot Ca, K and Na concentration. Three loadings accounted for the majority of the variation in both the seven-trait and the 14-trait PIC matrices (data not shown). The summary correlation

Table 4. *Probability of a lack of association between phylogenetically independent contrasts (PICs) of seven shoot mineral concentration traits and leaf f. wt:d. wt ratio of 115 species sampled from up to 24 orders and one unassigned family of angiosperms, grown hydroponically*

Values are *F*-probabilities of the regression through the origin between pairs of PICs. The explanatory trait defines the phylogeny.

Response trait							
Leaf f. wt:d. wt	0.002	<0.001	0.173	<0.001	0.590	0.206	–
P	0.863	0.001	0.411	0.357	<0.001	–	0.278
Organic-N	0.001	0.007	0.012	0.994	–	<0.001	0.543
Na	0.507	0.052	0.288	–	0.974	0.376	<0.001
Mg	<0.001	0.729	–	0.286	0.011	0.413	0.177
K	0.267	–	0.578	0.036	0.007	<0.001	<0.001
Ca	–	0.299	<0.001	0.557	0.002	0.792	0.001
	Ca	K	Mg	Na	Organic-N	P	Leaf f. wt:d. wt
	Explanatory trait						

coefficients for the seven-trait data set revealed strong positive associations between shoot Ca and Mg concentration, between shoot K and Na concentration and leaf f. wt:d. wt, and between shoot organic-N and P concentration (Table 5). The summary correlation coefficients for the 14-trait data set revealed positive associations between shoot Ca and Mg concentration and shoot f. and d. wt, and between shoot Ca, Mg and organic-N concentration. There were positive associations between shoot K, Na, and total cation concentration and shoot and leaf f. wt:d. wt ratios. There were also positive correlations between shoot P and total cation concentration and between shoot P and organic-N concentration. There were strong negative associations between shoot C concentration and several traits including shoot K, total-N and total-cation concentration and between shoot C concentration and shoot f. and d. wt. The summary correlation coefficients were consistent with the pair-wise comparisons of PICs.

A survey of leaf mineral nutrient concentrations in a regional herbaceous flora

Thompson *et al*. (1997) determined the proportions of variation in the leaf Ca, K, N, Mg, and P concentration at different hierarchical levels within the angiosperms using a classical taxonomy. At the family level and above 73, 35, 51, 37, and 19% of the variation in leaf Ca, K, Mg, N, and P concentration was accounted for respectively. Of the 132 pair-wise comparisons of PICs for leaf Al, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, and Zn concentration and the mean soil pH, there were significant correlations between each of the traits and at least one other trait, with the exception of Al (data not shown). Leaf Al concentration did not associate with any other leaf mineral concentration trait and did not associate with soil pH. Four loadings accounted for the majority of the variation in the 12-trait PIC correlation matrix representing all traits from the survey (data not shown). The summary correlation coefficients (Table 6) revealed strong positive correlations between leaf N and P concentration, between leaf K, Mg, N, and P concentration, between leaf Fe and Zn concentration, between leaf Cu and Fe concentration, and between leaf Ca concentration and soil pH. There were strong negative associations between leaf Ca and Mn concentration, between leaf Mg and Zn concentration, and between leaf Mn concentration and soil pH.

Comparing experimental and survey data

The designed, phylogenetically balanced, experiment was performed under a single set of external conditions whilst the ecological survey represented a wide range of soil conditions. Species data were strikingly similar in the designed experiment and in the ecological survey (Figs 1, 2). Excluding Caryophyllales species, shoot Ca and Mg concentration regressed significantly across the angiosperms in both the designed experiment (y=0.13x+0.17; r^2=0.55; d.f. 1, 116; P <0.001) and in the ecological field survey (y=0.13x+0.14; r^2=0.62; d.f. 1, 80; P <0.001). This represents an identical Ca:Mg ratio of ~7.7:1 in both the designed experiment and in the ecological survey. Poales were consistently low in shoot Ca and Mg concentration; *Typha latifolia* was the only Poales species to exceed a leaf Ca concentration of 1% in either the designed experiment or in the survey. Caryophyllales species accumulated more Mg than other angiosperms, with the exception of a single species from the survey, *Minuartia verna* (Fig. 1b). To determine if a low shoot Ca concentration in the Poales was related to the soil conditions in which they were growing, the soil pH was plotted as a function of shoot Ca concentration. The Poales species were not constrained to soils of low pH, which, like Caryophyllales species, were sampled from a wide range of soil conditions (Fig. 1c). The dominant cation in the shoot tissues of Poales species was K, under both experimental and natural conditions (Fig. 2). Other species, notably those of the Caryophyllales, accumulated significant amounts of other cations.

Table 5. *Pair-wise correlation coefficients of the phylogenetically independent contrasts of (a) 14 shoot mineral concentration and weight traits of 62 plant species and (b) seven shoot mineral concentration and weight traits of of 115 plant species, representing 24 orders and one unassigned family of angiosperms, grown hydroponically (light-grey boxes, correlations >+0.6; dark-grey boxes, correlations <–0.6)*

Correlation coefficients were estimated using principal components analysis (PCA) and are the cosines of the mean angle between two traits (vectors) from the (a) 14 or (b) seven PCA configurations, with each trait in turn defining the phylogeny. The total range between the upper and lower 95% confidence intervals are presented alongside. For each PCA configuration, the angle between pairs of traits was calculated across three loadings using vector analysis (Smyrl, 1980).

(a)

Response trait	Mean correlation coefficient													
Ca	–													
Total cations	0.30	–												
Nitrate-N	–0.38	–0.14	–											
C	–0.37	–0.74	–0.44	–										
N	0.34	0.30	0.69	–0.85	–									
Organic-N	0.87	0.51	–0.71	–0.24	0.01	–								
K	–0.22	0.85	0.17	–0.62	0.22	0.01	–							
Mg	0.94	0.04	–0.14	–0.32	0.45	0.65	–0.43	–						
Na	–0.40	0.64	–0.27	–0.11	–0.32	0.04	0.81	–0.68	–					
P	0.49	0.77	–0.70	–0.28	–0.16	0.81	0.45	0.17	0.56	–				
Shoot f. wt	0.68	0.56	0.24	–0.88	0.86	0.51	0.25	0.67	–0.19	0.32	–			
Shoot d. wt	0.69	0.31	0.34	–0.78	0.90	0.40	0.02	0.76	–0.43	0.08	0.96	–		
Leaf f. wt:d. wt	–0.35	0.77	0.15	–0.52	0.14	–0.07	0.97	–0.56	0.88	0.42	0.15	–0.07	–	
Shoot f. wt:d. wt	–0.28	0.82	0.01	–0.49	0.07	0.04	0.97	–0.52	0.91	0.53	0.15	–0.10	0.99	–
	Ca	Total cations	Nitrate–N	C	N	Organic–N	K	Mg	Na	P	Shoot f. wt	Shoot d. wt	Leaf f. wt:d. wt	Shoot f. wt:d. wt
	Explanatory trait													

Response trait	Total range covered by 95% confidence interval													
Ca	–													
Total cations	0.08	–												
Nitrate-N	0.19	0.22	–											
C	0.09	0.10	0.08	–										
N	0.25	0.19	0.15	0.05	–									
Organic-N	0.10	0.17	0.07	0.13	0.21	–								
K	0.13	0.09	0.24	0.10	0.16	0.20	–							
Mg	0.03	0.13	0.16	0.13	0.16	0.12	0.11	–						
Na	0.09	0.13	0.10	0.11	0.06	0.13	0.15	0.06	–					
P	0.18	0.13	0.16	0.21	0.32	0.11	0.24	0.21	0.19	–				
Shoot f. wt	0.14	0.18	0.18	0.07	0.11	0.17	0.11	0.08	0.14	0.28	–			
Shoot d. wt	0.19	0.21	0.20	0.12	0.07	0.20	0.16	0.11	0.12	0.28	0.02	–		
Leaf f. wt:d. wt	0.06	0.07	0.07	0.10	0.07	0.07	0.05	0.08	0.03	0.20	0.10	0.08	–	
Shoot f. wt:d. wt	0.06	0.06	0.07	0.10	0.08	0.06	0.06	0.07	0.03	0.15	0.11	0.09	0.01	–
	Ca	Total cations	Nitrate–N	C	N	Organic–N	K	Mg	Na	P	Shoot f. wt	Shoot d. wt	Leaf f. wt:d. wt	Shoot f. wt:d. wt
	Explanatory trait													

Across the 17 orders of angiosperms common to both the designed experiment and the ecological survey, shoot K concentration correlated (r=0.47; P <0.05). In the designed experiment, shoot K concentration correlated strongly with shoot f. wt:d. wt ratio at both the order level and at the species level to an asymptote of 9% K (Fig. 3). In the designed experiment, shoot K and total cation concentration was inversely correlated with shoot C concentration (Fig. 4). Data for shoot f. wt:d. wt ratio and shoot C concentration were not reported in Thompson *et al*. (1997) and thus cannot be compared between the designed experiment and the ecological survey. Shoot organic-N and P concentration, and to a lesser extent shoot K concentration, was higher in the designed experiment than in the ecological survey, however, shoot organic-N, P and K concentration correlated in both the experiment and in the ecological survey at the order level (Fig. 5).

Discussion

Quantifying the phylogenetic variation in the shoot mineral concentration of angiosperms

An experiment designed to determine the shoot mineral concentration of angiosperms was conducted. A pro rata

Table 5b.

(b)

Response trait	Mean correlation coefficient						
Ca	–						
K	0.04	–					
Leaf f. wt:d. wt	0.35	0.72	–				
Mg	0.97	–0.09	0.12	–			
Na	–0.15	0.72	0.87	–0.38	–		
Organic-N	0.23	0.43	–0.16	0.34	–0.30	–	
P	–0.27	0.57	–0.14	–0.18	–0.02	0.85	–
	Ca	K	Leaf f. wt:d. wt	Mg	Na	Organic-N	P
	Explanatory trait						
Response trait	Total range covered by95% confidence interval						
Ca	–						
K	0.10	–					
Leaf f. wt:d. wt	0.07	0.21	–				
Mg	0.02	0.18	0.10	–			
Na	0.11	0.26	0.08	0.07	–		
Organic-N	0.14	0.30	0.07	0.15	0.18	–	
P	0.10	0.24	0.14	0.12	0.15	0.05	–
	Ca	K	Leaf f.:d. wt	Mg	Na	Organic-N	P
	Explanatory trait						

sampling technique was adopted to ensure that species were represented in proportion to their distribution within the angiosperms (Broadley *et al*., 2003). The proportion of variation in shoot mineral concentration and shoot f. wt:d. wt ratio assigned to the order level and above decreased in the sequence Mg ⩾Ca >K ⩾C >shoot f. wt:d. wt ratio ⩾organic-N ⩾Na >total-N ⩾P in the designed experiment. In the ecological survey (Thompson *et al*., 1997), the proportion of variation in shoot mineral concentration assigned to the family and above decreased in the sequence Ca >Mg >organic-N ⩾K >P, assuming that nitrate-N accumulation was minimal and that shoot N concentration reflected organic-N concentration. Thus, data from the designed experiment is consistent with data from the ecological survey (Thompson *et al*., 1997) and both approaches indicate that shoot P and organic-N concentration is a species level trait, that shoot C, Ca, and Mg concentration is influenced by more ancient evolutionary processes, and that shoot K concentration is intermediary.

Phylogenetically dependent shoot mineral characteristics

A large proportion of the variation in shoot Ca and Mg concentration occurred at or above the level of the order in both the designed experiment and in the ecological survey. Although it is not yet possible to propose a mechanism to explain this phenomenon, two intriguing correlates have been observed at the order level, both of which warrant further study and could subsequently be tested through physiological experiments (Broadley *et al*., 2003). The first correlate is that shoot Ca and Mg concentration is positively correlated with the cation exchange capacity (CEC) of plant roots. Monocot orders in the commelinoid clade, with low shoot Ca and Mg concentration, have lower root CEC due to the lower pectin concentration of their cell walls. The second correlate is that the shoot concentration of Ca and Mg may be inversely related to shoot silicon (Si) concentration. Inverse correlations between shoot Ca/Mg and shoot Si concentration in monocot species could be confirmed either using a designed experiment or by sampling shoot tissues from botanical collections growing on similar substrates.

Shoot Mg and Ca concentration correlated at the order level in the designed experiment. Two of the three orders with the highest shoot Mg concentration were the eudicot orders Malvales and Cucurbitales, which were also amongst the orders with the highest shoot Ca concentration. The three orders with the lowest shoot Mg concentration were the monocot orders Alismatales, Arecales and Poales, which were amongst the orders with the lowest shoot Ca concentration. Since *c.* 40% of the variation in shoot Ca and Mg concentration occurred between different eudicot orders, a more focused sampling strategy could be used to resolve which eudicot clades differ in their shoot Ca and Mg concentration. There was a positive correlation between shoot Ca and Mg concentration across species in

Table 6. *Pair-wise correlation coefficients of the phylogenetically independent contrasts of 12 leaf mineral content traits of 81 species representing 20 orders of angiosperms (light-grey boxes, correlations >+0.6; dark-grey boxes, correlations <–0.6)*

Leaf mineral content data are from Thompson *et al*. (1997). Correlation coefficients were estimated using principal components analysis (PCA) and are the cosines of the mean angle between two traits (vectors) from 12 PCA configurations, with each trait in turn defining the phylogeny. The total range between the upper and lower 95% confidence intervals are presented alongside. For each PCA configuration, the angle between pairs of traits was calculated across four loadings using vector analyses (Smyrl, 1980).

Response trait	Mean correlation coefficient											
Al	–											
Ca	–0.503	–										
Cu	0.203	0.024	–									
Fe	0.266	–0.207	0.875	–								
K	0.077	–0.045	0.582	0.458	–							
Mg	0.237	0.162	0.323	0.084	0.815	–						
Mn	–0.022	–0.695	–0.426	–0.127	0.142	–0.063	–					
Na	0.343	0.141	0.441	0.224	0.379	0.566	–0.433	–				
N	–0.363	0.147	0.486	0.385	0.872	0.587	0.162	0.200	–			
P	–0.445	0.209	0.528	0.424	0.769	0.496	0.061	0.304	0.968	–		
Soil pH	0.144	0.695	0.124	–0.199	–0.127	0.285	–0.888	0.476	–0.225	–0.177	–	
Zn	–0.097	–0.153	0.361	0.639	–0.298	–0.672	–0.067	–0.239	–0.122	–0.003	–0.344	–
	Al	Ca	Cu	Fe	K	Mg	Mn	Na	N	P	Soil pH	Zn
	Explanatory trait											
Response trait	Total range covered by 95% confidence interval											
Al	–											
Ca	0.248	–										
Cu	0.222	0.274	–									
Fe	0.140	0.298	0.096	–								
K	0.128	0.225	0.225	0.209	–							
Mg	0.258	0.250	0.225	0.177	0.121	–						
Mn	0.160	0.322	0.217	0.184	0.123	0.226	–					
Na	0.466	0.573	0.388	0.294	0.424	0.362	0.484	–				
N	0.164	0.208	0.199	0.155	0.120	0.129	0.096	0.310	–			
P	0.219	0.201	0.233	0.244	0.151	0.214	0.109	0.177	0.035	–		
Soil pH	0.101	0.211	0.118	0.138	0.075	0.168	0.105	0.313	0.110	0.089	–	
Zn	0.142	0.430	0.133	0.074	0.328	0.156	0.212	0.469	0.134	0.092	0.299	–
	Al	Ca	Cu	Fe	K	Mg	Mn	Na	N	P	Soil pH	Zn
	Explanatory trait											

both the experiment and in the survey (Fig. 1). Across all species, the ratio of Ca:Mg approximated 7.7:1 in both the experiment and in the survey which is, remarkably, identical to the Ca:Mg ratio of 7.7:1 reported from an ecological survey of vascular, non-vascular and aquatic plants (Garten, 1976). General correlations in tissue Ca:Mg ratios across species are likely to be due to the chemical similarities between Ca^{2+} and Mg^{2+} and a lack of selectivity during ion uptake and transport by plants (White, 2001). In Caryophyllales species, however, shoot Mg concentration was often high, whilst shoot Ca concentration was no greater than other eudicots. Elevated shoot Mg concentration in the Caryophyllales warrants further investigation, for example, through a comparative phenomenological, physiological and molecular dissection of Ca^{2+} and Mg^{2+} transport in the Caryophyllales and model Brassicales species (e.g. *Arabidopsis, Brassica*). Caryophyllales species generally accumulated more cations than species from other orders (Fig. 2). For example, even the single Caryophyllales species with a low shoot Mg concentration in the survey, *Minuartia verna*, is a species characteristic of Zn-rich habitats and had a high shoot Zn concentration (Thompson *et al*., 1997). It has been hypothesized that ancestral Caryophyllales evolved in dry, mineral-rich environments (Ehrendorfer, 1976; Cuénoud *et al*., 2002). This heritage may, in part, explain the frequency of halophytes found in this clade. However, halophytes are distributed throughout the angiosperms, and include many monocot species. It would be interesting (1) to determine the phylogenetic distribution of halophyte angiosperms, and (2) to determine if the comparative shoot Ca and Mg concentration of glycophyte and halophyte monocots is consistent with the observation that many monocots seem to be phylogenetically constrained to low shoot Ca and Mg concentration.

More than 50% of the variation in shoot K and C concentration occurred between different eudicot orders under experimental conditions. At a species and order level, shoot K concentration (expressed on a d. wt basis) was positively related to shoot f. wt:d. wt ratio (Fig. 3).

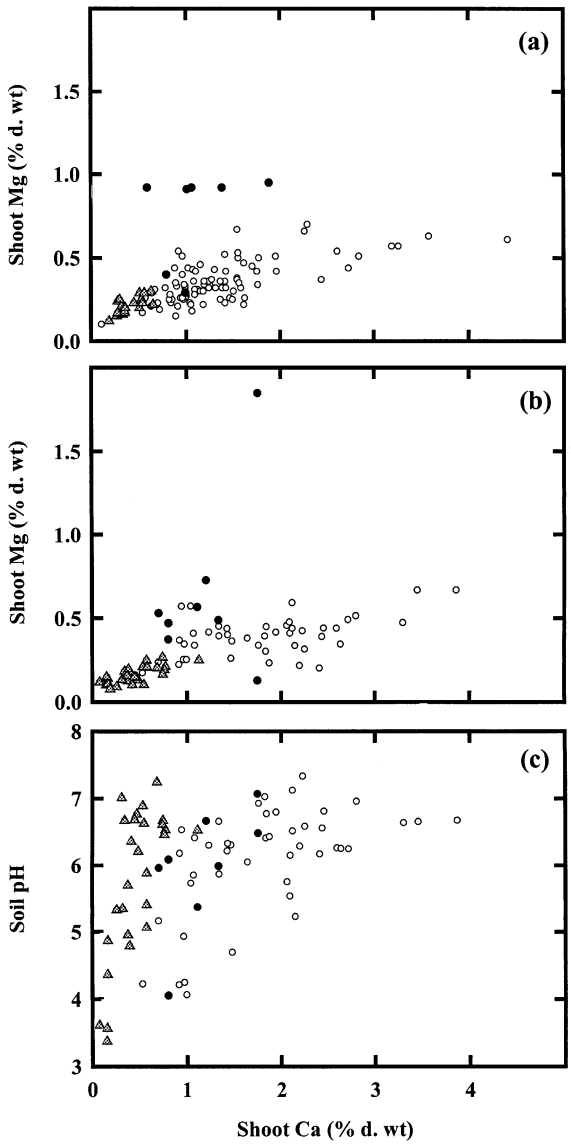

Fig. 1. (a) Mean shoot Mg and Ca concentration of 117 species from 24 orders and one unassigned family of angiosperms grown hydroponically. Mean shoot Mg concentration (b) and the mean soil pH of sites sampled for each species (c) and mean shoot Ca concentration of 81 species from 20 orders of angiosperms reported in an ecological survey (Thompson *et al.*, 1997). Filled circles are species of Caryophyllales; grey triangles are species of Poales; open circles are all other species.

Order means for K concentration, expressed on a tissue water basis, were calculated from species means of shoot f. and d. wt ratio reported by Broadley *et al.* (2003). Tissue water K concentration was approximately constant across orders, ranging between *c.* 100 and 170 mM, although the mean tissue K concentration within the orders Saxifragales (on one replicate) and Malvales (on three replicates) was higher (*c.* 220 mM and 230 mM, respectively). Thus, it is possible to predict some of the variation in shoot K concentration of eudicot species from the f. wt:d. wt ratio of closely related species. This positive association of shoot K and water concentration is consistent with the hypothesis that K^+ has a major biophysical role in plants as the dominant cationic osmoticum in vacuoles (Leigh and Wyn Jones, 1984). Further, the positive association between shoot K and water concentration is also consistent with the inverse relationship between shoot K and C concentration observed under experimental conditions, since more organic solutes are produced when insufficient K^+ or other inorganic cations are available as osmotica (Leigh and Wyn Jones, 1984).

Phylogenetically independent shoot mineral characteristics

Analyses of PICs from experimental and survey data suggest that many shoot mineral concentration traits did not evolve independently. For example, with the exception of Al, a non-essential mineral nutrient for most plant species (Jansen *et al.*, 2002), all shoot mineral concentration and f. wt:d. wt ratio PICs correlated with PICs for at least one other trait. These results are consistent with observations that shoot organic-N and P concentration and shoot Ca, Mg and K concentration are positively associated across a range of vascular, non-vascular and aquatic plant species (Garten, 1976). There are two explanations for the non-independent evolution of shoot mineral concentrations across species. The first explanation is based on the 'critical' mineral concentration concept and is pertinent to plants growing in nutrient-limited environments. The second explanation is applicable to species which can accumulate 'luxury' amounts of minerals in their shoots in nutrient-rich environments and is driven by the osmotic capacity of a plant cell and by the need to maintain electrical neutrality.

A plant requires a critical minimum concentration of each essential mineral in its shoot tissues to grow at its maximum rate, assuming that other resources are non-limiting (Marschner, 1995). This critical concentration represents the metabolic, structural and osmotic requirements of a cell, integrated for the whole shoot. In environments where mineral nutrients limit growth, species will not tend to accumulate luxury amounts of minerals and, in particular, shoot organic-N and P concentrations are likely to be below critical levels. Assuming that the ratios of shoot organic-N:P concentration cannot differ too greatly between species below critical levels, shoot organic-N:P concentrations will often be positively associated across species in nutrient-limited environments. This assumption is consistent with ecological surveys of species where shoot organic-N:P concentrations often approximate 10:1 (Garten, 1976; Thompson *et al.*, 1997; Tessier and Raynal, 2003), which is similar to the maximum critical organic-N:critical P concentration ratio observed for a range of agricultural crop species (Greenwood *et al.*, 1980). It is therefore

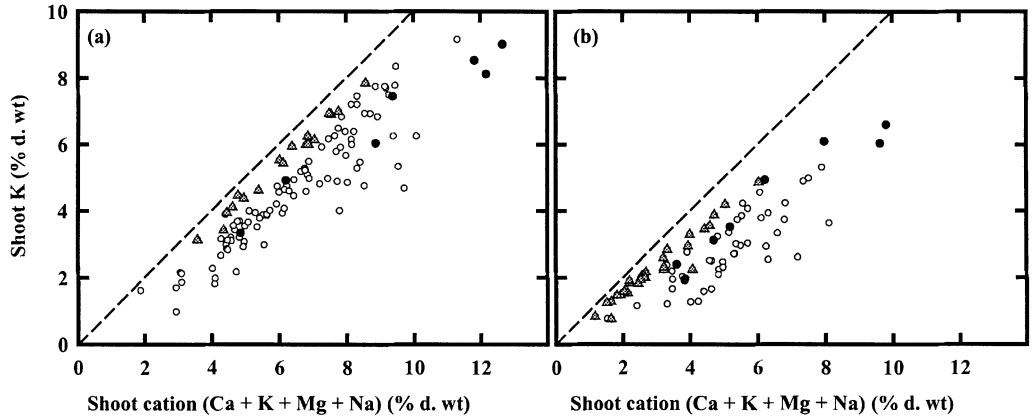

Fig. 2. (a) Mean shoot K concentration as a function of mean shoot cation (Ca+K+Mg+Na) concentration of 117 species, from 24 orders and one unassigned family of angiosperms grown hydroponically, (b) mean shoot K concentration as a function of mean shoot cation (Ca+K+Mg+Na) concentration of 81 species from 20 orders of angiosperms reported in an ecological survey (Thompson *et al.*, 1997). Filled circles are species of Caryophyllales; grey triangles are species of Poales; open circles are all other species. The dashed line indicates unity between shoot K and cation concentration.

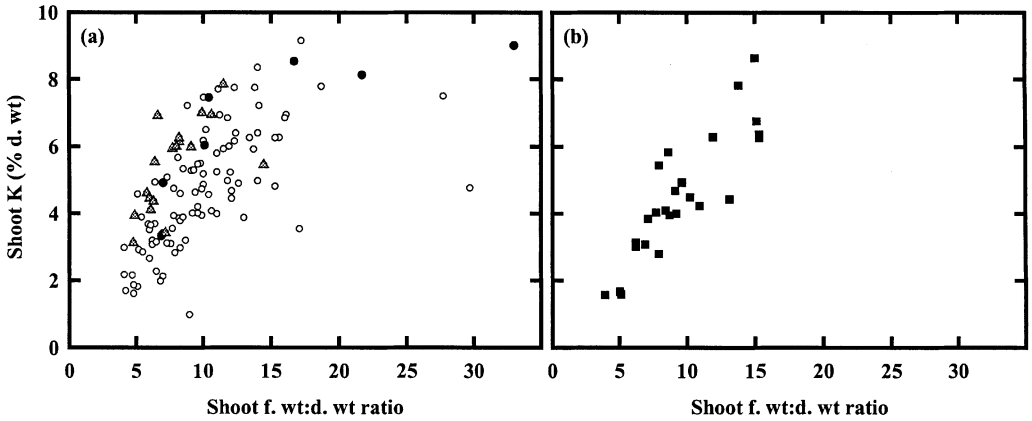

Fig. 3. Shoot K concentration and shoot f. wt:d. wt ratio of (a) 117 species, and (b) 24 orders and one unassigned family of angiosperms grown hydroponically. Filled circles are species of Caryophyllales; grey triangles are species of Poales; open circles are all other species.

plausible that positive associations between PICs of shoot minerals reflect relatively constant, subcritical, mineral ratios. However, since the ratios of critical N, P and K differ between agricultural crop species (Greenwood *et al.*, 1980), this assumption can only be a partial explanation.

If several minerals are at, or below, critical concentration levels in shoots, and if the ratios of different minerals are relatively constant in different species, positive associations in the shoot concentration of different minerals would occur *across* species, irrespective of the morphology or the relative growth rate (RGR) of a species. Conversely, *between* species differences in the shoot concentration of a single mineral would arise through differences in morphology or RGR between species. For example, interactions between RGR and the shoot organic-N concentration of plants can be conceptualized in scaling terms. During plant growth, the volume of non-photosyn-

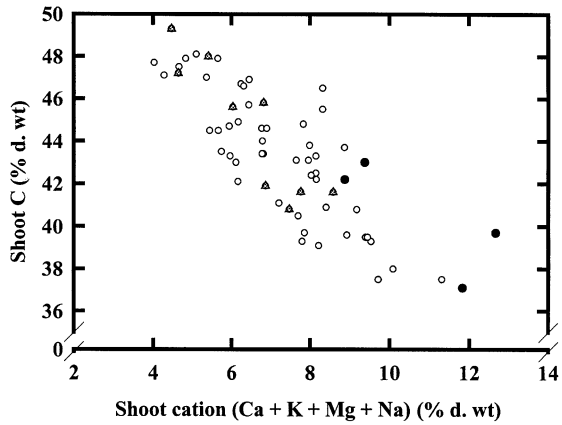

Fig. 4. Shoot C concentration and shoot cation (Ca+K+Mg+Na) concentration of 62 species of angiosperms grown hydroponically. Filled circles are species of Caryophyllales; grey triangles are species of Poales; open circles are all other species.

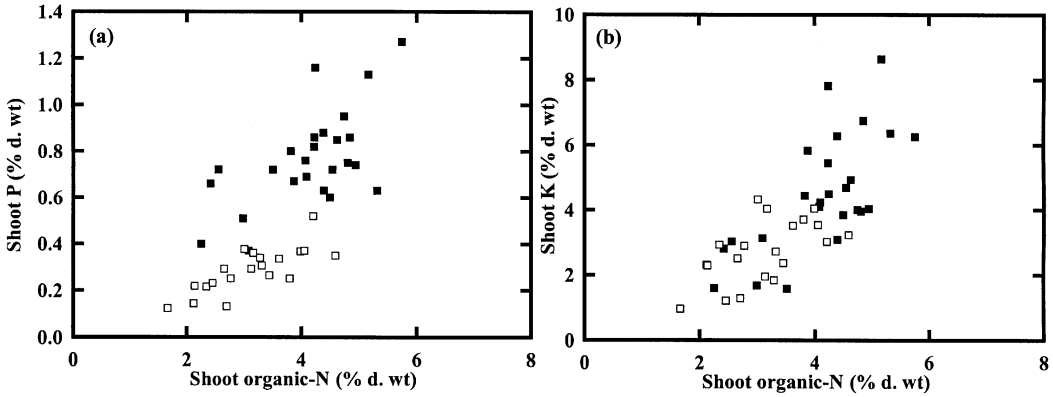

Fig. 5. (a) Mean shoot P concentration and (b) mean shoot K concentration and mean shoot organic-N concentration. Filled squares represent 24 orders and one unassigned family of angiosperms, grown hydroponically; open squares represent 20 orders of angiosperms reported in an ecological survey (Thompson *et al.*, 1997).

thetic materials increases faster than photosynthetically-active surface areas (Hardwick, 1987; Greenwood *et al.*, 1990). Since non-photosynthetic materials contain less organic-N than photosynthetically-active materials, critical organic-N concentration declines during the growth of a plant (Greenwood *et al.*, 1990). Thus, positive associations between the shoot organic-N concentration and RGR *within a species* are routinely observed when plants are supplied with sub-optimal N (Ågren, 1988). This logic implies that, in the absence of luxury N accumulation, positive associations between shoot organic-N concentration and RGR should also occur *across species* with different RGRs. This hypothesis is consistent with independent field surveys (Field and Mooney, 1986; Hunt and Cornelissen, 1997).

Shoot tissue longevity and nutrient retention are important traits that allow certain plant species to tolerate mineral stresses in natural habitats where soil nutrient supplies are limiting (Chapin, 1980; Grime, 2001). Such stress-tolerant species contain lower amounts of N and P in their shoot tissues (Grime *et al.*, 1997). However, potentially fast-growing annual species characteristic of disturbed, nutrient-rich sites ('ruderals'), have the capacity to accumulate luxury quantities of minerals in their shoot tissues under certain conditions. The capacity to accumulate 'luxury' quantities of minerals in shoots maximizes the potential of a plant to exploit other resources (e.g. light and water) more effectively in temporally and spatially heterogeneous or unpredictable environments and can thus confer a selective advantage (Grime, 2001). Thus, shoot mineral concentration can exceed the mineral concentration required for immediate growth in some species. Luxury quantities of minerals will often be stored as inorganic ions in cell vacuoles (e.g. NO_3^-, polyphosphates and P-esters, K^+, Ca^{2+}, and Mg^{2+}) as a more energy-efficient method for generating an osmotic potential for rapid cell expansion and growth than the biochemical synthesis of organic ions. Since a vacuole must maintain electrical neutrality, inorganic ions will be co-localized. For example, polyphosphates in vacuoles may function as cation exchangers for K^+ or other cations (Peverley *et al.*, 1978). Thus, positive correlations between the shoot concentration of different minerals under conditions of higher nutrient supply (Fig. 5) may occur because the mineral concentration of the vacuole dominates the mineral concentration of the shoot.

Perspective

This study is a first attempt to quantify the phylogenetic variation in the shoot mineral concentration of angiosperms. There are systematic differences in the shoot concentration of certain minerals between angiosperm clades whilst certain minerals and shoot f. wt:d. wt ratios are correlated across species. Differences between angiosperm clades, and correlations between phylogenetically independent traits, can be tested and explored using a more detailed experimental strategy that focuses on different regions of the angiosperms (Broadley *et al.*, 2003).

Determining the shoot mineral concentration of different angiosperm clades has several uses. Firstly, in agriculture, knowledge of the shoot mineral concentration of different angiosperm clades can be used to optimize fertilizer applications to different crop species and to identify potential dietary deficiencies in humans and livestock reliant on certain crop types. For example, diets that are reliant on crops from commelinoid monocot clades may contain insufficient Ca. Secondly, knowledge of the shoot mineral concentration of different angiosperm clades can be used to improve descriptions of the cycling of mineral elements and contaminants in the environment. For example, strontium-90 (^{90}Sr) is a radioisotope of radiological concern in the environment due to its high bioavailability and its relatively long half-life (*c.* 28 years). Since ^{90}Sr behaves almost identically to Ca in soils and

plants (White, 2001), knowing how Ca accumulation differs between species from different plant clades could therefore improve predictions of ^{90}Sr cycling in the environment. Thirdly, knowledge of the shoot mineral concentration of different angiosperm clades can be used to improve understanding of plant community structure and function. For example, since mineral nutrients are '… the primary limiting currency of vegetation and ecosystem processes' (Grime, 2001; p. xiv), and since the evolution of stress-tolerance traits such as high shoot tissue longevity and nutrient retention, and low shoot nutrient requirement and concentration, is causally associated with low mineral nutrient supply (Grime, 2001), it is possible to use shoot mineral concentration traits to improve predictions of how the species composition of plant communities might change in response to environmental perturbation. Although the high species-level variation in shoot organic-N and P concentration indicates that ancient evolutionary histories will not define how a species will respond to environmental perturbations such as N or P pollution, shoot C, Ca, Mg, and K, concentration are influenced by more ancient evolutionary effects which should not be ignored in ecological studies.

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