# Testing the distinctness of shoot ionomes of angiosperm families using the Rothamsted Park Grass Continuous Hay Experiment

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## Summary

- The ionome is the elemental composition of a tissue or organism. Phylogenetic variation in the ionomes of plant shoots has been widely reported based on controlled experiments, vegetation surveys and literature meta-analyses. However, environmental effects on phylogenetic variation in shoot ionomes have not been quantified. This study tests the hypothesis that phylogenetic variation in shoot ionomes is robust to environmental perturbation and that plant families can be distinguished by their shoot ionomes.
- Herbage was sampled from six subplots of the Rothamsted Park Grass Experiment. Subplots had received contrasting fertilizer treatments since 1856. Herbage was separated into its constituent species ($n = 21$) and concentrations of eleven mineral elements were determined in dried shoot material.
- Shoot concentrations of calcium (Ca), zinc (Zn), manganese (Mn), magnesium (Mg) and sodium (Na) showed significant variation associated with plant species, and responded similarly to fertilizer treatments in diverse plant species. Species × treatment interactions were indicated for phosphorus (P), potassium (K), nickel (Ni), copper (Cu) and iron (Fe). Plant families could be distinguished by their shoot ionomes. The most informative elements for discriminant analysis were Ca > Mg > Ni > S > Na > Zn > K > Cu > Fe > Mn > P.
- Whilst shoot ionomes were sensitive to fertilizer treatment, phylogenetic variation in a subset of the shoot ionome (Ca, Zn, Mn, Mg) was robust to this environmental perturbation.

## Introduction

The elemental composition of a subcellular compartment, cell, tissue or organism is termed its ionome (Salt *et al.*, 2008). The ionome includes all mineral elements, whether essential or non-essential for life, in whatever chemical form these occur. Many studies indicate that the concentrations of mineral elements in shoot tissues differ between plant species growing in the same environment. Such studies have been combined using residual maximum likelihood (REML) procedures and employed in meta-analyses to determine phylogenetic effects on shoot concentrations of mineral elements (Broadley *et al.*, 1999, 2001, 2003, 2004, 2007; White *et al.*, 2004; Hodson *et al.*, 2005; Willey & Wilkins, 2006). Knowledge of the systematic variation in shoot concentrations of mineral elements can be used both in agriculture, to optimize the application of fertilizers to crops (Broadley *et al.*, 2004) and the delivery of mineral elements to the diets of humans and livestock (White & Broadley, 2005, 2009), and in ecology, to improve our understanding of the structure and function of plant communities (Thompson *et al.*, 1997; Broadley

*et al.*, 2004; Kerkhoff *et al.*, 2006; White & Hammond, 2008; Fyllas *et al.*, 2009; Elser *et al.*, 2010; Kattge *et al.*, 2011; Zhang *et al.*, 2012) and the cycling of natural and anthropogenic mineral elements in the environment (Broadley *et al.*, 2001, 2004; Beresford *et al.*, 2004; Reich, 2005; Kerkhoff *et al.*, 2006; Watanabe *et al.*, 2007; Amatangelo & Vitousek, 2008, 2009; Kattge *et al.*, 2011). In addition, information from these meta-analyses can provide insight into the evolution of differences in shoot tissue concentrations of mineral elements between plant taxa (Broadley *et al.*, 2001, 2004, 2007; White & Broadley, 2003; Hodson *et al.*, 2005; Kerkhoff *et al.*, 2006; White *et al.*, 2007; Kattge *et al.*, 2011; Metali *et al.*, 2012; Zhang *et al.*, 2012).

It has long been recognized that shoot mineral concentrations are also influenced by environmental conditions and, in particular, fertilizer applications (Marschner, 2012). The aim of this study was to test whether the relative differences in shoot concentrations of mineral elements between plant species were consistent under contrasting fertilization regimes. The study used data on the concentrations of mineral elements in shoots of 21 plant

species growing in six subplots of the Park Grass Continuous Hay Experiment at Rothamsted, which have had contrasting fertilizer treatments for many decades (Warren & Johnston, 1964; Crawley *et al.*, 2005; Silvertown *et al.*, 2006). These data suggest that, although fertilizer practice influences shoot concentrations of mineral elements profoundly, the rank order in shoot concentrations of many mineral elements among plant species is consistent under contrasting fertilization regimes and that little variation in the concentrations of many mineral elements in plant shoots can be attributed to genotype × environment interactions. Thus, conclusions pertaining to the phylogenetic effects on shoot concentrations of mineral elements derived from meta-analyses of combined literature data are robust for these elements.

## Materials and Methods

The Park Grass Experiment was established by Lawes and Gilbert at Rothamsted in 1856 on *c.* 2.8 ha of parkland that had been permanent pasture for at least two centuries (Warren & Johnston, 1964; Crawley *et al.*, 2005; Silvertown *et al.*, 2006). The treatments imposed in 1856 included control plots, with no additions of either fertilizer or manure, and plots receiving annual applications of various combinations of phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), sulphur (S) and nitrogen (N) fertilizers, the latter being supplied as either sodium nitrate or ammonium salts. In 1903 many plots were halved, to test the effects of liming every 4 yr, and a further modification was made in 1965 when most plots were divided into four subplots. Three of these subplots have been limed to first establish and then maintain pHs of 7, 6 and 5 on Subplots a, b and c, respectively. The fourth subplot, Subplot d, receives no lime and soil pHs now range from 5.7 to 3.5 depending on the fertilizer treatment and natural acidifying inputs (Warren & Johnston, 1964; Johnston *et al.*, 1986). The calcium carbonate ($CaCO_3$) required to maintain plots at a given pH is substantially less for those receiving nitrate than for those receiving ammonium (Warren & Johnston, 1964; Silvertown *et al.*, 2006). The herbage on each plot is cut each year, usually in June, and made into hay on the plot. A second cut is taken in the autumn and the produce is weighed green and removed from the plot. Each year from 1991 to 2000 the herbage on each plot was sampled immediately before being cut for hay, separated into individual plant species, and the contribution of each plant species to dry matter yield was determined as described by Crawley *et al.* (2005). Both the biomass attributed to individual plant species and the contributions of different plant species to the total dry matter yield of a plot varied greatly between plots (Supporting Information Table S1).

The annual fertilizer applications to the six subplots used in this study are described in Table 1. The soil pH of the six subplots is intended to be pH 7.0 and, when sampled in 1991, their soil pH ranged from 6.2 to 7.0. Herbage from the subplots was separated into shoot material from individual plant species by Crawley and co-workers in their field campaign of 1993 (Crawley *et al.*, 2005). These subplots were selected by maximizing three criteria: (1) the number of plant species in common between the

**Table 1** Fertilizer treatments, number of plant species present and (in parentheses) the number of species contributing 10% or more to the total dry matter yield over the period 1991–2000, and the annual yield of subplots of the Park Grass Continuous Hay Experiment

| Subplot | Fertilizer treatment[a] | Number of species[b] | Mean annual yield, 2000–2004 (t DM ha$^{-1}$)[c] |
|---------|-------------------------|----------------------|--------------------------------------------------|
| 3a | | 39 (5) | 3.3 |
| 4/2a | N2, P | 22 (3) | 5.5 |
| 7a | P, K, Na, Mg | 27 (4) | 7.4 |
| 9/2a | N2, P, K, Na, Mg | 22 (5) | 7.8 |
| 14/2a | N*2, P, K, Na, Mg | 24 (3) | 7.5 |
| 18a | N2, K, Na, Mg | 30 (5) | 3.9 |

[a]Fertilizer treatments: N2 = 96 kg N y$^{-1}$ as $(NH_4)_2SO_4$; N*2 = 96 kg N y$^{-1}$ as $NaNO_3$; P = 35 kg P y$^{-1}$ as triplesuperphosphate; K = 225 kg K y$^{-1}$ as $K_2SO_4$; Na = 15 kg Na y$^{-1}$ as $Na_2SO_4$; Mg = 10 kg Mg y$^{-1}$ as $MgSO_4$.
[b]Data from Crawley *et al.* (2005).
[c]Data from Rothamsted Research (2006).

subplots, (2) the number of plant species in common with previous studies of phylogenetic effects on shoot concentrations of mineral elements, and (3) the number of plant families represented in the subplots. The six selected subplots covered 21 plant species representing seven plant families. The Poaceae were represented by *Agrostis capillaris* L., *Alopecurus pratensis* L., *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Dactylis glomerata* L., *Festuca rubra* L., *Holcus lanatus* L., *Poa pratensis* L., and *Poa trivialis* L., the Ranunculaceae by *Ranunculus acris* L., the Fabaceae by *Lathyrus pratensis* L., *Lotus corniculatus* L., and *Trifolium pratense* L., the Polygonaceae by *Rumex acetosa* L., the Plantaginaceae by *Plantago lanceolata* L., the Asteraceae by *Achillea millefolium* L., *Centaurea nigra* L., *Leontodon hispidus* L., *Taraxacum officinale* F.H. Wigg, and *Tragopogon pratensis* L., and the Apiaceae by *Anthriscus sylvestris* Hoffm., and *Heracleum sphondylium* L.

Dried herbage samples from each plant species in each subplot were milled to a powder using a ball-mill. Accurately weighed powdered subsamples (*c.* 50 mg DW) were digested in closed vessels using a microwave digester (MARS Xpress, CEM Microwave Technology, Buckingham, UK). Samples were first digested with 10 ml concentrated nitric acid ($HNO_3$), before 3 ml of 30% hydrogen peroxide ($H_2O_2$) was added to each vessel and digestion completed. Digested samples were diluted with milliQ (sterile, 18.2 MΩ cm) water before elemental analyses. Total K, calcium (Ca), Mg, P, S, Na, iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and nickel (Ni) concentrations were determined on digested material by inductively-coupled plasma mass spectrometry (ICP-MS, ELAN DRCe, PerkinElmer, Waltham, MA, USA). For these analyses, a tomato leaf standard (Reference 1573a; National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) was used as an internal control. Duplicate subsamples of herbage exhibiting high concentrations of Ca, S, Fe or Ni were processed and analysed to confirm that outliers in these data were not attributed to sample preparation or analytical process.

Estimates of variation in concentrations of mineral elements in shoots were assigned to species, treatment and species–treatment

interactions using residual maximum likelihood (REML) procedures. Spearman rank correlation analysis was used to determine whether the shoot concentration of a mineral element responded similarly to fertilizer treatments in all plant species and discriminant analysis was used to investigate whether shoot mineral element composition was characteristic of particular plant families. All statistical analyses were performed using GenStat (v12, VSN International, Oxford, UK).

## Results

Different fertilizer treatments produced different amounts of herbage with contrasting concentrations of mineral elements in the subplots of the Park Grass Experiment (Table 2, Fig. 1). The application of P-fertilizer increased mean herbage P concentrations of plant species in Subplots 4/2a, 7a, 9/2a and 14/2a, and the application of K-fertilizer has increased mean herbage K concentrations of plant species in Subplots 7a, 9/2a, 14/2a and 18a (Table 2, Fig. 1). Mean Na concentrations in herbage of plant species from Subplots 3a, 4/2a, 9/2a and 14/2a were higher than those from Subplots 7a and 18a. The highest mean S and Mn concentrations were found in Subplots 4/2a, 9/2a and 18a, which were supplied with ammonium sulphate $((NH_4)_2SO_4)$. A lower mean Zn concentration was found in herbage from Subplot 7a than in herbage from other subplots, although the range of Zn concentrations in shoots of different plant species was comparable. Differences in mean Ca, Mg and Ni concentrations in herbage from different subplots can also be observed.

Within-plot variation in the concentrations of mineral elements in shoots of diverse plant species appears to differ between subplots in an element-specific manner (Fig. 1). For example, subplots to which P or K fertilizer have been applied have greater variation in shoot concentrations of the element supplied than unfertilized plots, shoot Na concentrations are less variable in plots whose mean herbage Na concentrations are low, and shoot Mg concentration in the unfertilized plot shows more variation than in all other subplots. *Plantago lanceolata* harvested from Subplot 9/2a had exceptionally high shoot S and Ca concentrations. *Lathyrus pratensis* and *Taraxacum officinale* had exceptionally high shoot Ni concentrations in Subplots 9/2a and 18a, respectively. *Agrostis capillaris*, *Taraxacum officinale* and *Arrhenatherum elatius* had exceptionally high shoot Fe concentrations in subplots 7a, 14/2a and 18a, respectively. However, the concentration of no element in the herbage sampled from the Park Grass Experiment exceeded that thought to be toxic to plants (White & Brown, 2010).

In order to quantify the sources of variation affecting each mineral element a REML analysis, with treatment (subplot) and species as random effects, was performed (Table 3, Fig. S1). The coefficient of variation (CV = SD/mean) for shoot concentrations of mineral elements increased in the order Zn (0.35) < Cu < P < S < Mg < K < Ni < Mn < Ca < Fe << Na (1.28). The CV for the residual variation, excluding the major factors of treatment and species, increased in the order Zn (0.17) < P < K < Ca < Cu < S < Mg < Mn < Ni < Fe < Na (0.78). Thus, shoot Zn concentrations had both the least variation across the experiment and the least variation attributed to residual variation.

**Table 2** Concentrations of mineral elements in the dry matter (DM) of shoots of plant species harvested from six subplots of the Park Grass Experiment, expressed as mean ± SE of *n* species

| Element | | Subplot 3a (*n* = 15) | Subplot 4/2a (*n* = 11) | Subplot 7a (*n* = 15) | Subplot 9/2a (*n* = 13) | Subplot 14/2a (*n* = 14) | Subplot 18a (*n* = 13) |
|---|---|---|---|---|---|---|---|
| P (mg g⁻¹ DM) | Mean | 1.16 | 4.66 | 3.67 | 3.81 | 3.39 | 1.44 |
| | SE | 0.09 | 0.31 | 0.28 | 0.20 | 0.22 | 0.10 |
| K (mg g⁻¹ DM) | Mean | 15.06 | 11.46 | 40.35 | 40.18 | 39.91 | 36.56 |
| | SE | 1.39 | 1.01 | 3.30 | 5.04 | 3.70 | 3.29 |
| Na (mg g⁻¹ DM) | Mean | 5.91 | 8.80 | 0.60 | 1.87 | 4.91 | 0.53 |
| | SE | 1.17 | 1.69 | 0.16 | 0.96 | 1.37 | 0.17 |
| S (mg g⁻¹ DM) | Mean | 1.85 | 3.22 | 2.09 | 3.70 | 2.27 | 3.97 |
| | SE | 0.17 | 0.35 | 0.26 | 0.77 | 0.29 | 0.30 |
| Mn (µg g⁻¹ DM) | Mean | 46.35 | 87.82 | 58.32 | 86.88 | 54.91 | 106.96 |
| | SE | 7.48 | 12.60 | 12.71 | 12.67 | 7.73 | 12.62 |
| Zn (µg g⁻¹ DM) | Mean | 29.41 | 30.79 | 24.69 | 34.25 | 30.77 | 36.30 |
| | SE | 2.63 | 2.43 | 2.51 | 2.98 | 2.94 | 2.88 |
| Ca (mg g⁻¹ DM) | Mean | 11.30 | 10.43 | 7.54 | 8.60 | 6.57 | 6.16 |
| | SE | 1.60 | 1.80 | 1.36 | 2.41 | 1.28 | 1.41 |
| Mg (mg g⁻¹ DM) | Mean | 2.93 | 2.38 | 1.54 | 1.76 | 1.58 | 1.48 |
| | SE | 0.48 | 0.24 | 0.18 | 0.16 | 0.16 | 0.24 |
| Ni (µg g⁻¹ DM) | Mean | 0.43 | 0.62 | 0.35 | 0.48 | 0.35 | 0.53 |
| | SE | 0.04 | 0.06 | 0.05 | 0.10 | 0.06 | 0.12 |
| Fe (µg g⁻¹ DM) | Mean | 76.24 | 63.94 | 64.83 | 94.62 | 89.15 | 95.85 |
| | SE | 5.63 | 6.09 | 11.77 | 10.23 | 16.15 | 28.94 |
| Cu (µg g⁻¹ DM) | Mean | 6.74 | 8.55 | 5.89 | 7.75 | 8.14 | 8.83 |
| | SE | 0.61 | 1.00 | 0.65 | 1.28 | 0.95 | 0.94 |
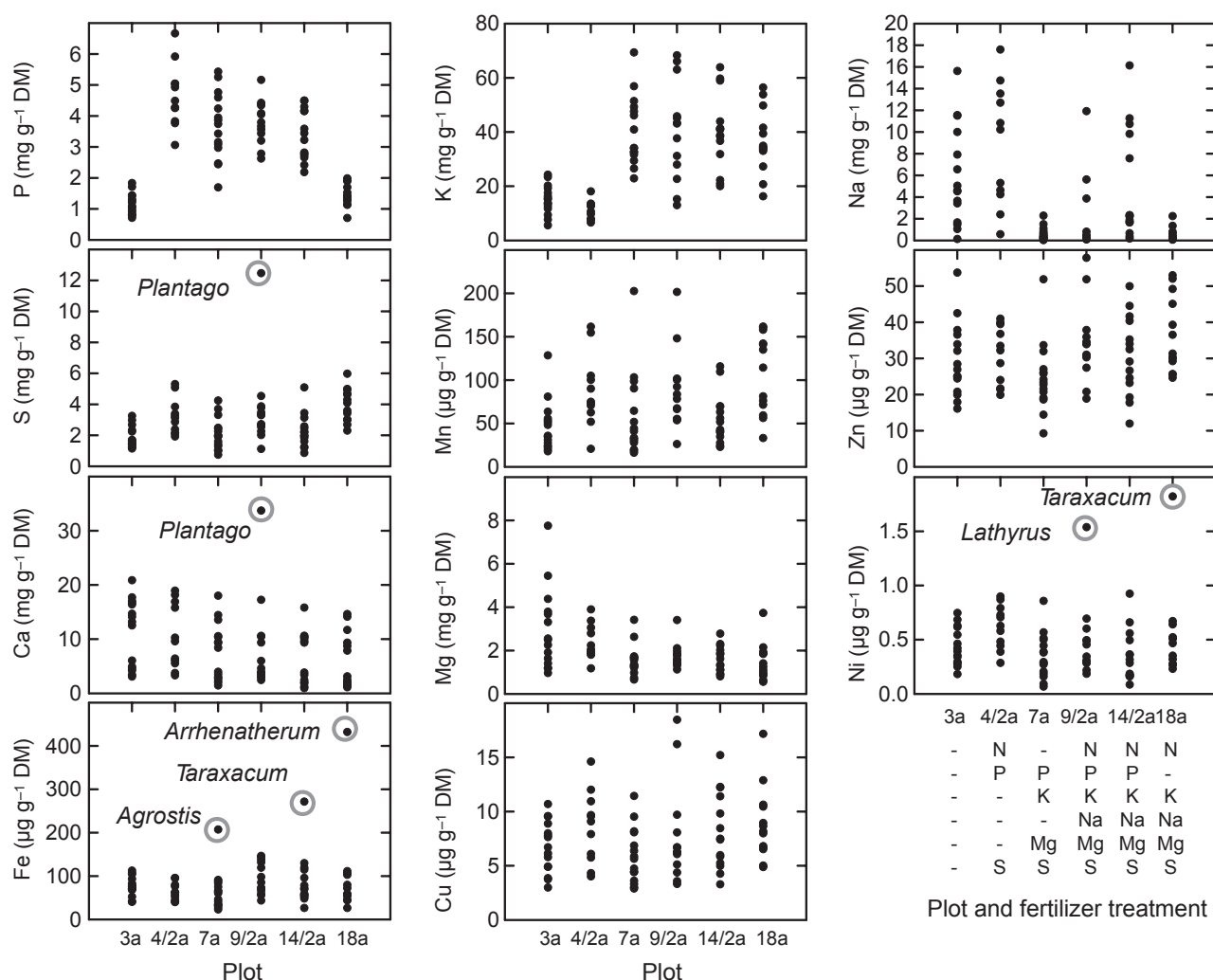
**Fig. 1** Concentrations of mineral elements in shoots of diverse plant species from six subplots of the Park Grass Experiment. Each datum indicates the concentration of an element in the shoot of an individual species in a particular subplot. Outliers are indicated by their genus.

**Table 3** Estimated trait means and variances obtained from a residual maximum likelihood (REML) analysis of 81 observations for concentrations of each of 11 mineral elements in shoots of plant species sampled from six subplots of the Park Grass Experiment

|  | Mean | Variance | Species (%) | Treatment (%) | Residual (%) |
|---|---|---|---|---|---|
| Calcium | 9.4 | 40.5 | 70.8 | 8.2 | 21.0 |
| Zinc | 31.5 | 124.2 | 64.3 | 13.4 | 22.3 |
| Manganese | 68.9 | 2096.2 | 36.3 | 23.4 | 40.3 |
| Magnesium | 2.0 | 1.3 | 32.8 | 19.9 | 47.3 |
| Copper | 8.0 | 11.5 | 30.8 | 6.6 | 62.6 |
| Nickel | 0.5 | 0.09 | 29.6 | 12.6 | 57.8 |
| Sodium | 3.8 | 23.7 | 24.8 | 37.8 | 37.4 |
| Sulphur | 2.8 | 2.6 | 24.8 | 27.5 | 41.3 |
| Potassium | 30.9 | 326.0 | 19.6 | 53.9 | 26.5 |
| Phosphorus | 3.1 | 2.6 | 10.7 | 77.0 | 12.3 |
| Iron | 79.4 | 3097.0 | 2.3 | −0.7 | 98.4 |

Data are expressed as mg g$^{-1}$ DM for Ca, Mg, Na, S, K and P, and as µg g$^{-1}$ DM for Zn, Mn, Cu, Ni, and Fe. Variances are partitioned as percentages of the total variance attributable to plant species, fertilizer treatment (subplot) and residual components. The residual component includes species × treatment interactions due to lack of plot replication.

Most of the variance in shoot P, K, Ca and Zn concentrations in the Park Grass Experiment can be attributed to a combination of species and treatment effects, with relatively little residual variation. Most of the variance in shoot P and K concentrations can be attributed to fertilizer treatment, whereas most of the variance in shoot Ca and Zn can be attributed to characteristic differences between plant species. By contrast, a large proportion of the variation in shoot concentrations of other elements was attributed to residual variation, which includes species × treatment interactions. For several elements, such as Mn, Mg, Cu and Ni, the variance attributed to characteristic differences between species is higher than that attributed to fertilizer treatments, whereas the variance attributed to fertilizer treatments is greater than that attributed to differences between species for S and Na. Using the residual variation to test the significance of the additional variation associated with the treatment term, then P, K, Na, S, Zn, Mn ($P < 0.1\%$), Mg, Ca ($P < 1\%$), and Ni ($P < 5\%$) concentrations all showed significant variation among treatments. Similarly, using the residual variation to test the significance of the additional variation associated with the species term, then Ca,

Zn ($P < 0.1\%$), Mn, P ($P < 1\%$), K, Mg and Na ($P < 5\%$) concentrations showed significant variation among plant species.

In order to investigate further whether the shoot concentration of a mineral element responded similarly to fertilizer treatments in all plant species, a Spearman rank correlation analysis was performed (Table 4). Pairwise Spearman rank correlations of plant species between fertilizer treatments were significant for the shoot concentrations of most mineral elements (Table 4). This suggests that the shoot concentration of a mineral element generally behaves similarly in the plant species studied in response to fertilizer treatments imposed here (Table 4). In particular, this analysis indicated that shoot concentrations of Ca, Zn, Mg, S, Na, Mn and Ni behaved similarly in the plant species studied in response to the fertilizer treatments imposed, implying substantial effects of plant genotype on the relative shoot concentrations of these mineral elements. Fewer pairwise Spearman Rank Correlations were significant for shoot concentrations of Fe, Cu, P and K, suggesting significant species × treatment interactions for these elements in some circumstances.

In order to identify those aspects of shoot mineral element composition characteristic of particular plant families a discriminant analysis was performed on 81 observations of the concentrations of 11 mineral elements in shoots of plant species sampled from the six subplots of the Park Grass Experiment using the estimated trait means obtained from the REML analysis (Figs 2, S2). The discriminant analysis was performed in two dimensions using plant family as the grouping of each training set. The discriminant analysis separated data from the seven plant families studied, suggesting that plant families have characteristic shoot ionomes. The 95% confidence intervals of the mean scores for each plant family indicated that data for the Poaceae, Polygonaceae and Plantaginaceae were statistically unique (Fig. S2). However, the 95% confidence interval for the Asteraceae
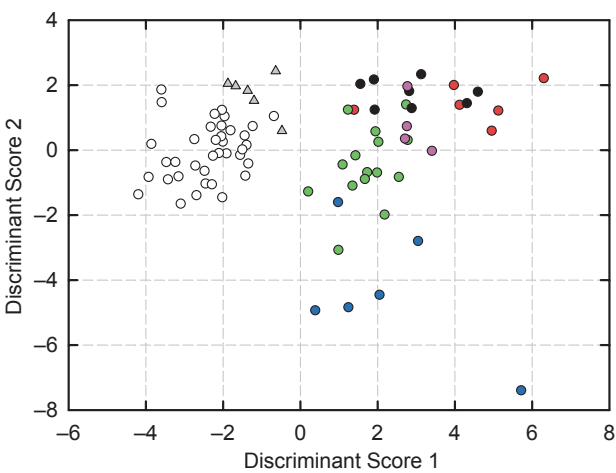


**Fig. 2** Discriminant analysis of the shoot mineral composition of diverse plant species. Discriminant analysis was performed on 81 observations of the concentrations of 11 mineral elements in shoots of plant species sampled from six subplots of the Park Grass Experiment using the estimated trait means obtained from a REML analysis of the raw data (Fig. 1). Symbols represent individual data, from species in the Poaceae (white circles), Polygonaceae (grey triangles), Plantaginaceae (blue circles), Asteraceae (green circles), Ranunculaceae (pink circles), Fabaceae (black circles) and Apiaceae (red circles). Score 1 accounts for 62% of the variation and Score 2 accounts for 22% of the variation.

overlapped that for the Ranunculaceae, the 95% confidence interval for the Fabaceae overlapped those for the Apiaceae and the Ranunculaceae, and the 95% confidence interval for the Apiaceae overlapped with those of the Fabaceae and Ranunculaceae (Fig. S2). The most informative mineral elements were Ca > Mg > Ni > S > Na > Zn > K > Cu > Fe > Mn > P. In this analysis, Scores 1 and 2 accounted for 84% of the variation in shoot mineral element concentrations. Score 1, which accounted for 62% of the variation in shoot mineral element concentrations, separated data from species in the Poaceae (*Agrostis capillaris*, *Alopecurus pratensis*, *Arrhenatherum elatius*, *Dactylis glomerata*, *Festuca rubra*, *Holcus lanatus*, *Poa pratensis*, *Poa trivialis*) and Polygonaceae (*Rumex acetosa*) from those of other angiosperm families (Figs 2, S2). The driving element in Score 1 was Ca, which is consistent with previous studies indicating that species from the Poales have consistently lower shoot Ca concentrations than those from eudicot orders (Broadley *et al.*, 2003, 2004; White & Broadley, 2003). Score 2, which separated data for *Plantago lanceolata* (Plantaginaceae) from those of other angiosperm species, was not dominated by any single mineral element.

In general, species from the Poaceae had lower REML-estimated shoot Ca and Mg concentrations than the other angiosperm species studied irrespective of the fertilizer treatment (Table 5; Fig. 3). This is consistent with previous studies (Broadley *et al.*, 2003, 2004; White & Broadley, 2003). Species from the Poaceae also had higher shoot Mg/Ca quotients than most other angiosperm species, with the exception of *Rumex acetosa* (Fig. 3). This is consistent with previous studies indicating that members of the Caryophyllales often have exceptionally high

**Table 4** Significance of the Spearman rank correlations in 15 pairwise comparisons of the rankings of shoot concentrations of each mineral element in 21 plant species across the six subplots of the Park Grass Experiment

| | $P < 0.0001$ (****) | $P < 0.001$ (***) | $P < 0.01$ (**) | $P < 0.05$ (*) | ns |
|---|---|---|---|---|---|
| Ca | 15 | 0 | 0 | 0 | 0 |
| Zn | 13 | 2 | 0 | 0 | 0 |
| Mg | 10 | 4 | 1 | 0 | 0 |
| S | 11 | 2 | 1 | 1 | 0 |
| Na | 6 | 4 | 5 | 0 | 0 |
| Mn | 6 | 3 | 5 | 1 | 0 |
| Ni | 4 | 6 | 2 | 3 | 0 |
| Fe | 5 | 5 | 2 | 2 | 1 |
| Cu | 5 | 3 | 4 | 1 | 2 |
| P | 5 | 3 | 3 | 3 | 1 |
| K | 1 | 3 | 5 | 4 | 2 |

Rank orders for 21 plant species were based on the estimated trait means obtained from a residual maximum likelihood (REML) analysis of 81 observations for concentrations of each of the 11 mineral elements in shoots of plant species sampled from six subplots of the Park Grass Experiment.

**Table 5** Concentrations of mineral elements in the shoot dry matter (DM) of seven plant families calculated from estimated trait means of species obtained from a residual maximum likelihood (REML) analysis of 81 observations for concentrations of each of the 11 mineral elements in shoots of plant species sampled from six subplots of the Park Grass Experiment

| | | $n$ | Ca mg g$^{-1}$ DM | Cu µg g$^{-1}$ DM | Fe µg g$^{-1}$ DM | K mg g$^{-1}$ DM | Mg mg g$^{-1}$ DM | Mn µg g$^{-1}$ DM | Na mg g$^{-1}$ DM | Ni µg g$^{-1}$ DM | P mg g$^{-1}$ DM | S mg g$^{-1}$ DM | Zn µg g$^{-1}$ DM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Asteraceae | Mean | 30 | 12.33 | 10.53 | 94.29 | 36.57 | 2.59 | 51.96 | 6.07 | 0.57 | 3.61 | 3.42 | 36.61 |
| | SE | | 0.58 | 0.44 | 8.58 | 3.06 | 0.24 | 5.11 | 0.94 | 0.05 | 0.27 | 0.20 | 2.06 |
| Poaceae | Mean | 48 | 3.33 | 6.83 | 65.67 | 24.19 | 1.33 | 93.59 | 2.61 | 0.36 | 2.63 | 2.43 | 26.95 |
| | SE | | 0.25 | 0.37 | 9.20 | 1.92 | 0.07 | 6.82 | 0.53 | 0.03 | 0.20 | 0.15 | 0.89 |
| Apiaceae | Mean | 12 | 15.41 | 7.02 | 79.17 | 48.30 | 2.50 | 67.60 | 1.33 | 0.47 | 3.58 | 2.31 | 37.15 |
| | SE | | 1.00 | 1.05 | 8.80 | 4.70 | 0.35 | 10.77 | 0.73 | 0.06 | 0.43 | 0.30 | 5.33 |
| Fabaceae | Mean | 18 | 13.75 | 8.31 | 93.46 | 26.89 | 2.43 | 47.08 | 2.57 | 0.85 | 3.31 | 2.50 | 30.83 |
| | SE | | 0.87 | 0.69 | 5.31 | 3.14 | 0.22 | 5.24 | 0.72 | 0.06 | 0.34 | 0.20 | 1.45 |
| Plantaginaceae | Mean | 6 | 17.38 | 9.13 | 88.18 | 32.35 | 1.69 | 33.92 | 8.41 | 0.34 | 3.08 | 5.80 | 43.25 |
| | SE | | 3.59 | 1.02 | 11.31 | 9.52 | 0.13 | 9.38 | 2.39 | 0.04 | 0.68 | 1.43 | 3.14 |
| Ranunculaceae | Mean | 6 | 13.50 | 8.52 | 77.18 | 33.35 | 2.25 | 62.31 | 5.82 | 0.56 | 3.23 | 2.34 | 32.96 |
| | SE | | 1.39 | 0.56 | 7.68 | 7.33 | 0.40 | 9.01 | 1.47 | 0.08 | 0.66 | 0.37 | 3.20 |
| Polygonaceae | Mean | 6 | 5.90 | 3.85 | 66.91 | 28.73 | 2.87 | 65.77 | 3.49 | 0.27 | 2.32 | 1.68 | 19.63 |
| | SE | | 1.23 | 0.38 | 13.92 | 4.70 | 0.41 | 20.86 | 1.61 | 0.04 | 0.45 | 0.40 | 1.50 |

Data are expressed as mean ± SE of $n$ species × subplot combinations.
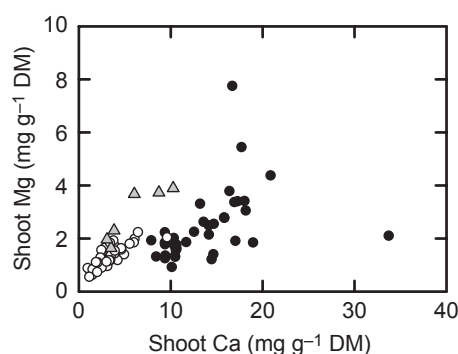


**Fig. 3** Relationship between shoot Ca concentration and shoot Mg concentration among 81 observations of plant species from the Poaceae (white circles), Polygonaceae (grey triangles) and other plant families (black circles) sampled from six subplots of the Park Grass Experiment.

shoot Mg/Ca quotients (White & Broadley, 2003; Broadley *et al.*, 2004, 2008; Karley & White, 2009). Data from the Poaceae can be separated from data from *Rumex acetosa* using Score 2 (Fig. 2). It is noteworthy that shoots of the Poaceae generally have higher REML-estimated Cu, Ni, Zn and S concentrations than *Rumex acetosa* (Table 5). Data from *Plantago lanceolata* (Plantaginaceae) suggest that this species has higher REML-estimated shoot Zn and S concentrations and lower REML-estimated shoot Mg and Mn concentrations than other angiosperm species (Table 5). The Apiaceae (*Anthriscus sylvestris*, *Heracleum sphondylium*) are characterized by high REML-estimated shoot K and Ca concentrations and low REML-estimated shoot Na concentrations, the Fabaceae (*Lathyrus pratensis*, *Lotus corniculatus*, *Trifolium pretense*) by high REML-estimated shoot Ni and Fe concentrations, and the Asteraceae (*Achillea millefolium*, *Centaurea nigra*, *Leontodon hispidus*, *Taraxacum officinale*, *Tragopogon pratensis*) by high REML-estimated shoot Cu and Fe concentrations (Table 5).

## Discussion

For the five subplots for which it is possible to make comparisons – Subplots 3a, 4/2a, 7a, 9/2a and 14/2a – shoot P, K, Ca and Mg concentrations averaged across plant species for each subplot (Table 2) were similar to values in the hay samples reported by Warren & Johnston (1964) of 1.6, 3.6, 3.0, 2.7, 2.5 mg P g$^{-1}$ dry matter (DM), 13.4, 6.4, 31.7, 27.6, 26.6 mg K g$^{-1}$ DM, 10.3, 6.1, 7.0, 3.6, 3.6 mg Ca g$^{-1}$ DM, and 3.3, 2.8, 1.8, 1.3, 1.3 mg Mg g$^{-1}$ DM, respectively. However, shoot Na concentrations averaged across plant species for each of the five subplots (Table 2) were consistently higher than the values reported by Warren & Johnston (1964) of 3.4, 3.4, 0.3, 0.2, 2.1 mg Na g$^{-1}$ DM for hay samples from Subplots 3a, 4/2a, 7a, 9/2a and 14/2a, respectively. Nevertheless, shoots of plants grown on Subplots 7a and 9/2a had low Na concentrations, as was observed previously (Warren & Johnston, 1964). Zhao *et al.* (1998) reported concentrations of 1.1 mg P g$^{-1}$ DM and 2.4 mg S g$^{-1}$ DM in herbage sampled from the unlimed control Subplot 3d between 1993 and 1995, which are comparable with the data obtained here for Subplot 3a (Table 2).

The shoot concentrations of other mineral elements averaged across plant species for the subplots sampled here (Table 2) were generally lower than those for composite herbage samples from subplots of the Park Grass Experiment reported previously (Blake *et al.*, 1994; Blake & Goulding, 2002). Although the range of shoot Zn (16–53 µg g$^{-1}$ DM), Cu (3.0–10.7 µg g$^{-1}$ DM) and Mn (18–130 µg g$^{-1}$ DM) concentrations for plant species growing in Subplot 3a fall within the ranges reported previously for herbage from this subplot, values for shoot Ni concentrations (0.29–0.75 µg g$^{-1}$ DM) are conspicuously low. Blake & Goulding (2002) reported concentrations of 37–50 µg Zn g$^{-1}$ DM, 9–13 µg Cu g$^{-1}$ DM, 66–140 µg Mn g$^{-1}$ DM and 1.6–2.7 µg Ni g$^{-1}$ DM in herbage sampled from subplot 3a between 1856 and 1991. Blake *et al.* (1994) reported

concentrations of 55 µg Zn g$^{-1}$ DM, 13 µg Cu g$^{-1}$ DM, 650 µg Mn g$^{-1}$ DM and 9 µg Ni g$^{-1}$ DM in herbage sampled from Subplot 3d between 1986 and 1989. The reasons for these discrepancies are unclear, but they might be attributed to differences in analytical techniques, differences in concentrations of elements in herbage between years or between subplots, or related to differences in shoot mineral concentrations between species and the contribution of each to the harvested herbage.

Spearman rank correlation analysis indicated that plant species generally followed the same rank order for the shoot concentration of a particular mineral element in subplots of the Park Grass Experiment (Table 4). According to this analysis, plant species responded similarly in their shoot concentrations of Ca, Zn, Mg, S, Na, Mn and Ni to the fertilizer treatments imposed in the Park Grass Experiment, although some species × treatment interactions were indicated in the responses of shoot Fe, Cu, P and K concentrations to these fertilizer treatments. This is consistent with a smaller proportion of the variation in shoot concentrations of Ca, Zn, Na, S, Mn and Mg being attributed to residual variation, which includes species × treatment interactions, than in shoot concentrations of Ni, Cu and Fe (Table 3, Fig. S1).

The analysis of variation (Table 3), Spearman rank correlation analysis (Table 4) and the discriminant analysis (Figs 2, S2) all indicate that the relative concentration of many mineral elements in the shoots of plants growing in the same environment is determined, to an extent, by the species and/or family to which they belong. These observations are consistent with previous reports of large phylogenetic effects on shoot concentrations of several mineral elements. Experiments in hydroponics (Broadley et al., 2003, 2004), meta-analyses of comparative studies in the literature (Broadley et al., 2001, 2003, 2007) and ecological surveys (Thompson et al., 1997; Watanabe et al., 2007; Zhang et al., 2012) have often indicated that a large proportion (> 40%) of the variation in shoot Ca, Mg, K, Ni and Zn concentrations can be attributed to phylogenetic effects at the level of the family or above. Linear regressions indicated significant correlations between REML-means for shoot Ca concentrations of plant species reported by Broadley et al. (2003) and those reported for the Park Grass Experiment ($R^2$ = 0.772, $n$ = 10 common species), but not for REML-means for shoot Zn concentrations of plant species reported by Broadley et al. (2007) and those reported for the Park Grass Experiment ($R^2$ = 0.066, $n$ = 11 common species). There were insufficient species in common for comparison of REML-means for shoot Mg, K or Ni concentrations from previous meta-analyses with data from the Park Grass Experiment. It is noteworthy that the most informative mineral elements in the discriminant analysis (Ca, Mg, Ni) were elements for which considerable variation in leaf element concentrations has been attributed above the family level (Thompson et al., 1997; Broadley et al., 2001, 2003, 2004; Watanabe et al., 2007), whilst the least informative mineral elements in the discriminant analysis (Cu, Fe, Mn, P) were those elements for which least phylogenetic variation in leaf element concentrations has been attributed above the family level (Broadley et al., 2001, 2004; Watanabe et al., 2007; Zhang et al., 2012).

Aspects of the ionome have previously been observed to differentiate angiosperm taxa (e.g. Markert, 1992; Jansen et al., 2002; Broadley et al., 2004; Shtangeeva et al., 2009), fern taxa (Amatangelo & Vitousek, 2008) and lichen taxa (Bennett, 2008). The discriminant analysis reported here (Figs 2, S2) separated plant families on the basis of their shoot ionomes even when plant species were grown in contrasting environments, which confirms its potential as a taxonomic character. The uniqueness of the ionomes of angiosperm taxa could have far reaching consequences. For example, the biofortification of edible produce or the adaptation of species to metalliferous soils might be restricted by inherited constraints (Broadley et al., 2001, 2004; White & Broadley, 2009). In addition, the general similarity of the ionomes of species from a particular angiosperm family, but the distinctness of the ionomes of different families, has important implications for the design of ecological studies of plant composition, which cannot now assume that plant species provide independent samples. However, grouping plants into families might simplify incorporating the effects of biodiversity into models that predict the cycling of natural and anthropogenic mineral elements in the environment (Beresford et al., 2004; Kattge et al., 2011).

In summary, residual maximum likelihood (REML) analyses indicated that variation associated with species was significant for shoot Ca, Zn ($P < 0.1\%$), Mn, P ($P < 1\%$), K, Mg and Na ($P < 5\%$) concentrations, but that a large proportion of the variation in shoot Ni, Cu and Fe concentrations was attributed to residual variation, which includes species × treatment interactions. Spearman rank correlation analyses indicated that shoot concentrations of Ca, Zn, Mn, Mg, Na, S and Ni behaved similarly in diverse species in response to fertilizer treatments, but that some species × treatment interactions occurred for P, K, Cu and Fe. Thus, meta-analyses that combine data from experiments performed in contrasting environments will be most robust for shoot Ca, Zn, Mn and Mg concentrations and least robust for shoot Cu and Fe concentrations. Discriminant analysis indicated that plant families could be distinguished by their unique shoot ionomes. The most informative mineral elements in this analysis were Ca > Mg > Ni > S > Na > Zn > K > Cu > Fe > Mn > P. Thus, shoot Ca and Mg concentrations have the potential to discriminate between plant families and these traits are robust to combining experiments performed in different environments. Whilst shoot Zn and Mn concentrations are less informative in discriminating between plant families, these traits are still robust to combining experiments performed in different environments.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** The coefficients of variation for shoot concentrations of mineral elements and residual variation in shoot concentrations of mineral elements excluding the major factors of treatment and species.

**Fig. S2** Discriminant analysis of the shoot mineral composition of diverse plant species sampled from six subplots of the Park Grass Experiment.

**Table S1** Fertilizer treatments, number of plant species present, annual yield over the period 2000–2004, and the percentage contribution of plant species studied in this paper to the total dry matter yield over the period 1991–2000 of subplots of the Park Grass Continuous Hay Experiment

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