Biogeosciences Discuss., doi:10.5194/bg-2016-432, 2016
Manuscript under review for journal Biogeosciences
Published: 4 November 2016
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Biogeosciences Discussions Open Access EGU

Leaf trait variation and field spectroscopy of generalist tree species on contrasting soil types

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Summary

Understanding the causes of variation in plant functional traits is a central issue in ecology, particularly in the context of global change. Analyses of the drivers of traits variation based on thousands of tree species are starting to unravel patterns of variation at the global scale, but these studies tend to focus on interspecific variation, and the contribution of intraspecific changes remains less well understood. Hyperspectroscopy is a recently developed technology for estimating the traits of fresh leaves. Few studies have evaluated its potential for assessing inter- and intra-specific trait variability in community ecology. Working with 24 leaf traits for European tree species on contrasting soil types, found growing on deep alluvial soils and nearby shallow chalk soils, we ask: (i) What contribution do soil type and species identity make to trait variation? (ii) When traits are clustered into three functional groups (light capture and growth, leaf structure and defence, as well as rock-derived nutrients), are some groups more affected by soil than others? (iii) What traits can be estimated precisely using field spectroscopy? (iv) Can leaf spectra be used to detect inter-soil as well as inter-specific variation in traits? The contribution of species and soil-type effects to variation in traits were evaluated using statistical analyses. Foliar traits were predicted from spectral reflectance using partial least square regression, and so inter- and intra-specific variation. Most leaf traits varied greatly among species. The effects of soil type were generally weak by comparison. Macronutrient concentrations were greater on alluvial than chalk soils while micronutrient concentration showed the opposite trend. However, structural traits, as well as most pigments and phenolic concentrations varied little with soil type. Field spectroscopy provided accurate estimates of species-level trait values, but was less effective at detecting subtle variation of rock-derived nutrients between soil types. Field spectroscopy was a powerful technique for estimating cross-species variation in foliar traits and Si predictions using spectroscopy appear to be promising. However, it was unable to detect subtle within-species variation of traits associated with soil type.

Key-words Inter-specific variation; Partial least-squares regression; Plant traits; Reflectance spectroscopy; Soil variation; Temperate forests; Within-species variation.

1 Introduction

There is currently great interest in using plant traits to understand the influences of environmental filtering and species identity on the functioning of plant communities, and to model community responses to environmental change (MacGillivray et al., 1995; McGill et al., 2006; Green et al., 2008; Funk et al., 2016). Traits vary at multiple scales within individuals, within populations, among populations, and among species (Albert et al., 2011), and analysis of this variation is key to evaluating the strength of various filtering processes on

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communities growing along environmental gradients (Violle et al., 2012). For example, intraspecific variation in traits may reflect differences in microclimate driven by competition, disturbance, environmental conditions and age (Funk et al., 2016), whereas inter-specific and inter-site variation may reflect both genetic variation and phenotypic plasticity in response to environment (Sultan, 2001; Donohue et al., 2005). Despite substantial advances in trait-based community ecology over the past decade (Funk et al., 2016), the importance of environmental filters is still debated, especially at small scales where biotic factors may prevail over abiotic environmental constraints (Vellend, 2010). Global analyses of leaf nitrogen, phosphorus and leaf mass per unit areas (LMA) indicate that about half of all variation occurs within communities (Wright et al., 2004), underscoring the importance of community-level variation in traits.

An increasing number of leaf traits are being measured routinely in plant communities (Asner et al., 2011; Asner et al., 2015), and these traits can be placed with three functional groups involved in shaping plant performance (Asner, 2014): (i) light capture and growth traits which include pigments, C isotope discrimination, N isotope discrimination, N content, which constitutes on average 19% of protein mass (Milton and Dintzis, 1981), soluble C compounds and leaf water content; (ii) defence and structural traits include Si, cell wall constituents (cellulose, hemicellulose and lignin), that are associated with leaf toughness, longevity and defence capability (Hikosaka, 2004), polyphenols that are associated with defence against herbivores (Mithöfer and Boland, 2012), and LMA, a primary axis of specialization among plants (Grime et al., 1997; Lambers and Poorter, 1992), that plays a crucial role in herbivore defence as well as leaf longevity (Wright et al., 2004); (iii) rock-derived nutrients include phosphorus, which is involved in many enzymatic, genetic and epigenetic processes (Schachtman et al.,1998), and calcium, magnesium, potassium, zinc, manganese, boron and iron, which are involved in signalling pathways and/or cofactors of enzymes (Marschner, 2012). Analyses involving this large suite of traits are so far restricted to comparisons of tropical forests, and emphasize cross-site and cross-species differences with little consideration on within-species variation (Asner et al., 2011; Asner et al., 2015). Placing traits into functional groups, and analysing intraspecific variation, may help understand trade-offs and plant strategies along environmental change.

Remote sensing has increasingly emerged as a promising tool for studying plant chemistry (Ustin et al., 2004; Asner and Martin, 2009; Ustin et al., 2009). Rapid, non-destructive determination of leaf traits *in vivo* and *in situ* using spectroscopy reduces the need to collect large amounts of material in the field, decreases processing time, lessens costly chemical analyses, and eliminates sampling that could itself alter experimental conditions (Couture et al., 2013). Spectroscopy can provide estimates of a range of foliar properties at the leaf and canopy scales within diverse tropical ecosystems (Asner et al., 2011; Doughty et al., 2011). However, spectral and chemical properties may be uncoupled if intraspecific variation in foliar traits is high and/or phenotypic plasticity exceeds phylogenetic patterns among leaf properties (Asner and Martin, 2011). Bolster, Martin and Aber (1996) demonstrated that equations for estimating leaf properties from one site were unable to predict leaf properties for other sites, due to variability in the magnitudes of foliar traits levels between data sets and environmental influences. To our knowledge, the link between foliar traits and spectral properties of trees has not been broadly demonstrated for temperate forests and the capacity of measuring inter-specific trait variability and environmental variation using spectroscopy is relatively unknown.

This paper examines the drivers of leaf trait variation in temperate woodlands growing on the chalklands of southern England compared with woodlands growing on nearby alluvial soils. Several studies have evaluated change in species composition among British semi-natural habitats that differ markedly in soils

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(Haines-Young et al., 2003; Smart et al., 2003), but few have compared within- versus between-species variation of leaf traits in this context. The alkalinity of calcareous soils gives rise to phosphorus limitation, preventing short-term responses to nitrogen addition (Grime et al., 2000), so comparisons of chalklands with less-alkaline soils nearby provide strong edaphic contrast. We investigated leaf property on these contrasting soil types and examined the ability of reflectance spectroscopy to quantify leaf chemical and structural traits. Our specific questions were: (i) what is the relative contribution of soil type and species to leaf trait variation? (ii) does the importance of the three functional groups (light capture and growth, leaf structure and defence, as well as rock-derived nutrients and secondary elements) change due to soil or more due to species variation? (iii) What traits can be accurately and precisely estimated using spectroscopy in temperate woodlands? (iv) To what extent can leaf spectra be used to detect inter-soil and inter-specific variation in traits?

2 Material and methods

2.1 Field site and sampling

Leaves were collected from trees growing on deep alluvial soils and shallow chalk soils, near Mickleham in Surrey (Latitude = 51.26, Longitude = 0.32). The alluvial soil, along the banks of the river Mole, was a loam of several metres depth. The chalk soil was located on a steep south-facing escarpment into which the river was cutting; the top soil was a few centimetres deep, underlain by solid chalk (i.e. a typical rendzina soil). The chalk soils were alkaline with a pH of 7.9 $\pm$ 1.0 (n = 10), whereas the alluvial was near neutral having a pH of 6.7 $\pm$ 0.2 (n = 10). Phosphorus becomes unavailable to plants in alkaline chalk soil (Gerke, 1992), and much greater depth of loamy soil on the alluvial surfaces must result in much greater availability of nutrients to plants.

Leaves of 66 trees of six species were collected from the two contrasting soil types. The six species were in common to both sites: *Acer campestre* L. (Field Maple), *Acer pseudoplatanus* L. (Sycamore), *Corylus avellana* L. (Hazel), *Crataegus monogyna* Jacq. (Hawthorn), *Fraxinus excelsior* L. (Ash) and *Sambucus nigra* L. (Elder). Two fully sunlit branches were selected, were cut and placed on ice in a cool box, and transported to a lab for processing within 2 hours (and often within 30 minutes). For each branch, ten mature leaves were selected. Three samples of 15 leaf disks were cored from these leaves using a 6 mm corer, wrapped in aluminium foil and frozen in liquid N for later chemical analyses. Leaf areas were measured from fixed-height photos against a white background analysed in *imageJ*. The scanned leaves were weighed to give hydrated mass, then dried at 70 °C for a minimum of 72 h to obtain dry mass. Leaf mass per area (LMA) was calculated as dry mass per unit of fresh leaf area. A further 23 leaf chemical traits were measured on these samples (see below).

2.2 Chemical assays

Protocols for chemical assays are adapted from those developed by the Carnegie Airborne Observatory (see http://spectranomics.ciw.edu). They are outlined here, with full details available in Supplementary Information. Oven dried leaves were ground and analysed for a variety of elements and carbon fractions. Concentration of elements (B, Ca, K, Mg, Mn, P, Si, Fe, Zn) were determined by ashing samples in a muffle furnace, digesting them in nitric acid, then running them through an inductively-coupled plasma mass spectrometry (Perkin Elmer SCIEX, Elan DRCII, Shelton, CT, USA). Nitrogen and carbon concentrations were determined using a Thermo Finnigan 253 with elemental analyser using a gas chromatographic separation column linked to a continuous

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flow isotope ratio mass spectrometer. This technique provided foliar concentrations of the stable isotopes of N and C. Carbon fractions, including hemicellulose, cellulose, lignin and soluble carbon (mainly carbohydrates, lipids, pectin and soluble proteins), were determined by sequential digestion of increasing acidity (Van Soest, 1994) in an Ankom fiber analyzer (Ankom Technology, Macedon, NY, USA). These carbon fractions are presented on an ash-free dry mass basis. Concentrations of photosynthetic pigments (chlorophyll *a*, *b*, anthocyanins and total carotenoids) were measured by spectroscopy of solution derived from frozen leaf disks on area basis. Absorbance values of the supernatant were measured at wavelengths 470 nm, 649 nm and 665 nm for chlorophyll *a*, *b* and total carotenoids determination and published equations used to calculate pigment concentrations as in Lichtenthaler (1987). Absorbance values were also measured at wavelengths 530 nm and 650 nm for anthocyanins determination and published equations used as per Giusti et al. (1999), but corrected for possible chlorophyll contamination as per Sims and Gamon (2002). The maximum efficiency of photosystem II (PSII) was calculated according to Genty et al. (1989) by measuring the maximum fluorescence (Fm) and the yield of fluorescence in the absence of an actinic (photosynthetic) light (Fo) using a PAM fluorometer. Total phenolic concentration of the upper methanol/water layer was determined colorimetrically using the Folin-Ciocalteau method, based on absorbance at 760 nm on a spectrophotometer, and quantified using tannic acid equivalents with water serving as a blank as per Davey et al. (2007).

2.3 Leaf and canopy spectroscopy

The remaining leaves were detached from the branches, and 10 leaves selected at random, avoiding damaged and soft/young leaves. These leaves were laid on a matt black surface. Reflectance within bands ranging from 400–2500 nm was measured using a FieldSpec 4, produced by Analytical Spectral Devices (ASD). The spectrometer's contact probe was mounted on a clamp and firmly pushed down onto the sample, so that no light escaped through the sides. The spectral measurements were taken at the mid-point between the main vein and the leaf edge, approximately half-way between the petiole and leaf tip, with the abaxial surface pointing towards the probe. The readings were calibrated against a Spectralon white reference every 5 samples. In all statistical analyses, the mean reflectance values of the 10 measurements per branch were used.

2.4 Statistical analyses

Analyses were performed within the R statistics framework (R Core Team 2014). Analyses of variance (ANOVA) were used to examine the influences of species and soil type on each of the 26 leaf traits. Species, soil and soil x species terms were included in the model, and the ratio of sum of squares of these terms versus the total sum of squares was used as an index of species- versus site-level variation. This partition of variance represent the variation between species, the influence of soil, the interaction between soil and species, and the unexplained variance referred as to residual variance, which is a combination of intraspecific variation, micro-site variability, canopy selection and analytical error. Where necessary, variables were log transformed to meet assumptions of ANOVA.

To evaluate the influence of soil and species on allocation of traits associated with (a) light capture and growth, (b) defence and structure and (c) rock-derived nutrients and secondary elements, permutational non-parametric multivariate analysis were performed (Anderson, 2001). This is an analysis of variance using distance matrices calculated using the *adonis* function in the *vegan* package of R. We recognise that grouping leaf properties into functional classes can be controversial, given that a single leaf property can contribute to

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more than one class (e.g. LMA is related to growth but also to defence). We also performed principal component analysis (PCA) for each functional class using the function *prcomp* in R. The principal components for the variables were obtained by the correlation matrix modelling *in lieu* of covariance matrix modelling, and then we used the unit variance scaling as in van den Berg et al. (2006) to avoid the effects of variables with high variance.

Partial least squares regression (PLSR) was used to evaluate whether field spectroscopy can reliably estimate leaf properties (Haaland and Thomas, 1988). There is strong co-llinearity in spectral reflectance data. PLSR involves dimensionality reduction, producing orthogonal uncorrelated latent vectors containing the maximum explanatory power in relation to the trait data (Wold et al., 2001). The number of latent variables (nL) used in the PLSR analysis was estimated by minimising the Prediction Residual Error Sum of Squares (PRESS) statistic to avoid overfitting (Chen et al. 2004), however was set from 1 to 10 to avoid over-fitting (Zhao et al., 2015). We adopted a leave-one-out cross-validation for each PLSR model and evaluated the model performance using coefficient of determination (R^2) and root mean square error (RMSE). We also standardised RMSE to the percentage of the response range (RMSE%) by dividing each RMSE by the maximum and minimum values of each leaf trait, as in Feilhauer et al., 2010.

The spectral reflectance curve of each sample was transformed into pseudo-absorption (log [1/ R]), where R is reflectance, based on previous studies (Bolster et al., 1996; Gillon et al., 1999; Richardson and Reeves, 2005; Petisco et al., 2006; Kleinebecker et al., 2009; Serbin et al., 2014). We reviewed past studies (Curran, 1989; Elvidge, 1990; Kokaly et al. 2009) to select well documented regions of the spectrum for absorption features as a basis for predicting each leaf trait. The visible (VIS, 400-700 nm), near infra-red (NIR, 700-1500) and shortwave infra-red I (SWIR I, 1500-1900), shortwave infra-red II (SWIR II, 1900-2500) regions, as well as combinations of the regions (700-1100 nm, 700-1900 nm, 700-2500 nm, 1100-1500 nm, 1100 -1900 nm, 1100-2500 nm, 1500-2500 nm and 400-2500 nm) were tested and selected based on the model that minimised RMSE.

3 Results

3.1 Soil and species controls on leaf properties

Relative foliar concentrations of the macronutrients N, P and K were 17 %, 43 % and 24 % higher on alluvial compared to chalk soils (Table 1). Nitrogen isotope discrimination ($\delta^{15}N$) varied greatly between the two soils, from -3.8 ‰ in the chalk soil to 3.4 ‰ in the alluvial. However, foliar concentrations of nutrients required in smaller quantities (Si, Ca, Mg, B, Mn and Zn) showed the opposite trend: they were higher in chalk soils (by 22%, 37%, 50%, 19%, 23% and 49%, respectively). Fe was the only mineral nutrient unaffected by soil type. The percentage contribution of soluble C was affected by soil, with an increase in soluble C of 9 % in the alluvial soil, whereas hemicellulose, cellulose, lignin and LMA were completely unaffected by location. Carotenoids had 25 % higher concentration in alluvial soil; however other pigments and traits related to water status ($\delta^{13}C$ and water content) varied little with soil type. The efficiency of PSII, which is related to carbon fixation under controlled conditions, showed a slight increase of 4 % in alluvial soil.

Most traits varied greatly among species and that variation was far greater than the soil effects (Fig. 1). Interspecific variation (Green, Fig. 1) accounted for $\geq$ 60% of the variation of eight traits (in descending order Si, water content, B, soluble C, N, LMA, K and cellulose concentrations), and $\geq$ 40% of the variation of another

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six traits (in descending order, lignin, hemicellulose, Mg, Zn, phenolics and Fe). Species exerted little or no influence on pigment concentrations, efficiency of PSII, $\delta^{13}C$, $\delta^{15}N$, P, Ca and Mn concentrations. The interaction between species and soil (Blue, Fig. 1) explained little variation and were significant for $\delta^{15}N$, P, Mn and Zn, but for no other traits. The pigments, efficiency of PSII and $\delta^{13}C$ represented the largest unexplained variance.

3.2 Variation among functional groups of traits

Species identity explained 59% of the investment in traits related to defence and structure and 31% of variation in investment in rock-derived nutrients and secondary elements altogether, but exerted no influence on the investment in light capture and growth (expressed as R^2 values in Table 2). By contrast, soil type explained 6% of the variation in the rock-derived nutrients with no influences on other functional group. There was an interaction between soil and species for properties related to the latter group only, which explained 19% of the total variability in the foliar properties. These results indicate that some species have to invest more in defence than others regardless of the soil type, whereas soil is an important modifier of traits related to allocation of macro and micronutrients to the leaves, even though species identity still play an important role in foliar traits variation for this group.

For leaf properties associated with light capture and growth, the first principal component (PC1) represents the variation in pigments and investment in light capture, and explains 38% of the total variability, whereas the second principal component (PC2) represents the variation in water, N, $\delta^{15}N$ and soluble C, which is related to investment in growth, and explains 25% the variability (Fig. 2). The heterogeneity within species along the PC1 axis tends to be large for all the species, whereas the variation within species along the PC2 tends to be considerably smaller. Investment in light capture is not species-oriented and also unaffected by soil variation.

For defence and structure, PC1 represents the lignocellulosic biomass explaining 51% of the total variability, whereas the PC2 represents LMA and phenolics explaining 21% the variability. Thus, it is possible to observe a separation of species into two main defensive strategies based on the type of defence. The PC1 distinguish, regardless of the soil type, the species into groups regarding the concentration of lignocellulosic biomass and Si. The PC2 distinguishes another two groups of species that are also not separated into soil type regarding the phenolic concentration and LMA.

For macro and micronutrients variation, PC1 represents the mineral nutrients required in greater amounts explaining 27% of the total variability of leaf properties, whereas the PC2 represents some micronutrients required in smaller quantities explaining 25% the variability. These 2 axes together explain 52% of the total variation and can be used to cluster soil into 2 groups: alluvial soils with high P and K concentration and chalk soil with high B, Mn and Zn concentrations. The inter-specific variation is greater along the PC1 related to Ca, Mg, Fe and B concentrations and can be used to group species.

3.3 Spectroscopy of leaf properties

Ability to predict leaf traits from hyperspectral reflectance varied greatly among the 24 traits fitted using the 6 species (Table 3). The number of latent variables ranged from 3 to 9. The R^2 obtained varied between 0.16 and 0.92, and RMSE% between 6.9% and 28.7%. PLSR modelling for LMA, water, Si, phenolics, carotenoids, K, B, efficiency of PSII, N, chlorophyll *a* and chlorophyll *b* were in descending order the best performing in terms

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of R^2. The highest RMSE % values were for LMA, water, phenolics, carotenoids, K, lignin, B, Si, chlorophyll *a* and efficiency of PSII. Some minerals, such as P, Zn and Mn, as well as $\delta^{13}C$ and $\delta^{15}N$ showed low R^2 and RMSE% values.

The majority of leaf properties showed higher goodness-of-fit using the regions of the spectrum between 1100 and 2500 nm. Pigments were the only traits that predictions were more accurate when using the visible region (400 – 700 nm). Predictions of phenolics, B, Zn and Mn were more accurate with the use of the region in the SWIR I between 1500 and 1900 nm, whilst Mg needs the use of SWIR II (1900-2500 nm) only. LMA showed higher R^2 and lower RMSE when using the spectrum region between 1100 and 2500 nm, as did Si, N, soluble C, hemicellulose, cellulose, lignin, $\delta^{13}C$ and $\delta^{15}N$. When using both SWIR regions, higher goodness-of-fit were obtained for K, Ca and P. Fe was the only foliar property that required the spectrum region between 700 and 2500 nm.

There were strong correlations among some of the leaf properties (Fig. 3) that can be potentially leveraging the estimation of other leaf traits from the use of PLSR. The correlation graphic also shows the similarity among variables through cluster analysis.

3.4 Use of spectroscopy to distinguish environmental and inter-specific variation

PLSR models of reflectance data were able to estimate differences in traits among species and detect intra-specific variation (Fig. 4). In general, inter-specific variation estimated foliar traits quantities reasonably well, as did for the unexplained variance of most traits. The soil importance was precise for the majority of leaf properties, but PLSR did not detect precisely the variation of rock-derived nutrients concentration in the leaves due to soil differences. The use of PLSR also considerably underestimated the importance of soil (~ 37 %) on the $\delta^{15}N$ variation, but the result was not shown in the graphic (see in *soil*, Fig. 4) due to visual aspects. The species x soil interaction effects were detected by PLSR modelling, except for traits that showed strong interaction (Mn, P and $\delta^{13}C$).

4 Discussion

Some leaf traits were strongly influenced by both species and soil type, while others were hardly affected by soil and only varied with species. Soil had a strong influence on concentrations of mineral nutrients in the leaves. Other foliar properties – mostly those involved in structure, defence and growth - varied among species but soil had little detectable effect. It is important to emphasize that only fully sunlit leaves were included in the analyses; as LMA, protein and pigment concentrations are strongly influenced by light environment, sampling understory leaves would have given a different result.

4.1 Phenotypic variation associated with soil

Our findings that trees growing on the chalk soils had relatively low concentrations of N, P and K in their leaves, and relatively high concentrations of Ca, Mg, B, Mn, Si and Zn, is consistent with previous analyses of mineral nutrition in calcareous soils. Thin chalk soils contain small quantities of macronutrients needed by plants, and are unproductive for growing crops unless heavily fertilized; however, cation exchange sites in the soil contain high concentrations of calcium and magnesium (Hillier et al., 1990). Soil pH has a strong influence on the plant-availability of many micronutrients: for instance, Zn is readily adsorbed at high pH and forms organic Zn-ligand complexes at low pH (Broadley et al., 2007). Species that specialize on chalks (so-called

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Manuscript under review for journal Biogeosciences
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calcicole species) have developed mechanisms for tolerating alkaline soils, associated with low phosphorus availability and excessive Ca and Mg supply (Misra and Tyler, 2000; Tyler, 2002).

$\delta^{15}N$ discrimination was strongly influenced by soil type, increasing from -3.83 in the chalk soil to 3.43 in the alluvial soil, resulting as the most sensitive foliar trait to soil changes. Although the species *Alnus glutinosa* (L.) Gaertn. was not included in the field measurements for trait determination, this species was restricted to alluvial soils in our study area and may help explain some differences in leaf traits between soils. The species *Alnus glutinosa* is an N fixing plant and is known to be dependent on mycorrhizal fungi (Hall et al., 1979) and the most important benefit of mycorrhizae is an increase in the efficiency of nutrient uptake by plants, especially phosphorus. Variation in $\delta^{15}N$ among plants within an ecosystem has been interpreted as representing differences in fixation, mycorrhizal dependence, depth of acquisition within the soil profile, utilization of depositional N and the form of N that plants predominantly acquire (Vallano and Sparks, 2013).

The discovery that structural and defensive traits do not vary with soil is consistent with a previous study in New Zealand's lowland temperate rain forests (Wright et al., 2010). That study compared traits of trees growing on phosphorus rich alluvium versus phosphorus-depleted marine terraces. Foliar phosphorus concentrations of species were halved on the marine terraces, but there was no detectable variation in structural traits, phenolic or tannin concentrations.

4.2 Inter-specific and residual variation

Species had a greater influence on trait values than soils for all traits, except P. The traits most influenced by species (in descending order) were Si, water, B, soluble C, N, LMA, K, cellulose, lignin, hemicellulose, magnesium, Zn, phenolics and Fe. It is interesting to note that two trace elements were near the top of this list; it is likely that strong differences in B and Si concentrations among species reflect differences in ion channel activity in roots (Ma and Yamaji, 2006). Previous studies have also shown Si to be under strong phylogenetic control, and to be little affected by environmental conditions (Hodson et al., 2005). We also found Si and B concentrations to be positively correlated, which might ameliorate the effects on B toxicity as Si can increases B tolerance of plants (Gunes et al., 2007). High Zn organization at the species level corroborates earlier analysis that show more than 70% of Zn variation occurs between and within species (Broadley et al., 2007). Structural foliar traits and more expensive compounds were also found to have high interspecific variation, such as cellulose and lignin, suggesting that even on a strong soil filtering, species play the crucial role to invest in these specific traits.

The residual variation is a combination of intraspecific variation, micro-site variability, canopy selection and measurement error. The residual variation was high for $\delta^{13}C$ and pigments, greatly exceeding soil and species effects, as also reported for pantropical trait studies (Asner and Martin, 2011). Low coefficient of variation in $\delta^{13}C$ among samples, and high residual variation, suggest that the efficiency of C fixation is maintained among species and soil. $\delta^{13}C$ is known to vary strongly with light condition and relative humidity (Yan et al., 2012), but their study sampled only from fully sunlit leaves.

4.3 Functional groups on contrasting soils

We investigated how traits in generalist species are responding to different soil conditions and the factors most contributing to changing leaf properties. The investment in light capture had high intra-specific variation, and neither species nor soil accounted for variation in foliar properties. The investment in growth showed relative

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high inter-specific variation separating out some species. Investment in traits related to defence and leaf structure is species-mediated, and may be separated into two defensive strategies. Considering these traits, some species invest more in LMA and phenolics and other species invest more in lignocellulosic biomass and Si regardless of soil type. The allocation of rock-derived nutrients to leaves is highly dependent on soil as environmental filter.

Traits favouring high photosynthetic rate and growth are considered to be advantageous in rich-resource soil environments, whereas expressions of traits favouring resource conservation are considered advantageous in low-resource environments (Aerts and Chapin, 1999, Westoby et al., 2002). Nevertheless, Fine et al. (2006) found similar results to ours with seedlings transplantation for 6 species into different soil types, concluding that investment in defence is due to genetically based, fixed traits, and defence differences are not just passive responses to differences of available nutrients in the soils.

4.4 Predictions of foliar traits using spectroscopy

Several leaf chemical traits and LMA could be estimated accurately using visible-to-shortwave infrared spectroscopy. Previous studies have also shown that leaf spectra can be used to predict leaf chemical properties (Asner and Martin, 2008, Asner and Martin, 2009; Asner et al., 2015). Doing so revealed that LMA, water, Si, total phenolics, carotenoids and K produced the most consistent and accurate calibrations.

The locations of important wavelengths in our PLSR models match the locations of known spectral absorption features related to proteins, starch, lignin, cellulose, hemicellulose and leaf water content (Kokaly et al., 2009). In the region between 700 and 2500 of the electromagnetic spectrum, absorption features are commonly the result of overtones and combinations of fundamental absorptions at longer wavelengths. The visible region was useful to predict pigments concentrations and the efficiency of PSII only, whereas the infra-red region was associated with most traits. The region of importance with correlated wavelengths with nitrogen varies between 1192 nm in deciduous forest (Bolster et al., 1996) to 2490 for forage matter (Marten et al., 1983), which results directly from nitrogen in the molecular structure. Although chlorophylls also contain nitrogen, the spectra of chlorophylls differ greatly from proteins because of their dissimilar chemical structures, showing strong absorption due to C-H bonds in the phytol tail of the molecule (Katz et al., 1966), also confirmed in this work when visualizing the regions of importance for predictions. The 1500-1900 nm region was also important for phenolic compounds prediction, which includes the 1660 nm feature across a variety of species and phenolic compounds (Windham et al., 1988; Kokaly and Skidmore, 2015).

A review in the literature suggests that the use of dry leaves may improve predictions of lignocellulosic biomass in the leaves with the use of spectroscopy (Richardson and Reeves, 2005; Asner et al., 2011; Serbin et al., 2014), as the strong water absorption features mask most of the biochemical absorption features (Fourty and Baret, 1998). On the other hand, the use of spectroscopy on fresh leaves is particularly better for LMA predictions, given the strong coupling between water content, leaf structure and LMA (Asner et al., 2011). The primary and secondary effects of water content on leaf reflectance are greatest in spectral bands centred at 1450, 1940, and 2500 nm (Carter, 1991), but has also been predicted using bands between 1100-1230 nm absorption features (Ustin et al., 1998; Asner et al., 2004).

The use of spectroscopy for Si predictions on fresh leaves appears to be promising considering our accurate results. The data available in the literature show that the ecological functions of Si have generally been poorly studied, and that there are almost no data about the role of Si structures in the reflection and transmission

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Published: 4 November 2016
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spectra of short-wave or photosynthetically active radiation in plants. Silicon is absorbed by plants from the soil solution in the form of silicic acid (H4SiO4) being translocated to the aerial parts of the vegetal through xylema, and then deposited along the plant as phytoliths (silicified bodies) (Tripathi, 2011). Smis et al. (2014) showed for the first time the potential use of NIR spectroscopy to predict Si concentration. Si shows strong interactions with plant biomolecules such as phenol- or lignin-carbohydrate complexes (Inanaga et al., 1995), cellulose (Law and Exley, 2011), and proteins (Perry and Keeling-Tucker, 2003). Predictions of Si concentrations, and other traits, from leaf spectra reflectance can be stronger than expected likely because leaf spectra integrate information on several foliar traits simultaneously.

Galvez-Sola et al. (2015) showed that near-infrared spectroscopy can constitute a feasible technique to quantify several macro and micronutrients such as N, K, Ca, Mg, Fe and Zn in citrus leaves of different leaves with coefficient of determination (R^2) varying between 0.53 for Mn and 0.99 for N, whereas B showed less accurate results with the use of spectroscopy. The regions of importance for prediction were relatively similar to all the mineral nutrients analysed in this study, except for B that had the band between 1500 and 1900 as the best predictive region. Similar to Si, the relative high precisions for K, Fe and B predictions can be stronger due to the integrating information on several foliar traits simultaneously.

4.5 Consideration on the use of spectroscopy to quantify patterns of foliar traits

The range of variation within species for most predicted traits tend to be smaller with the use of PLSR on reflectance, resulting in consistent slight overpredictions of the inter-specific variance. The interrelationships between foliar chemical and spectral properties for each species help to explain the successful results reported in developing species-level variation from leaf spectral data (Asner et al., 2009). In general, the residuals variation was lower for most leaf traits with the use of spectroscopy, possibly because the use of spectroscopy affects the ability to quantify measurement error, one of the residual variation components.

The variation caused by soil on mineral nutrients and $\delta^{15}N$ allocated to the leaves remained unchanged with the use of spectroscopy, possibly because structural leaf traits, such as LMA, cellulose, water, as well as pigments, contribute more to leaf reflectance. As these structural traits remained unchanged between soil types for the six species, it possibly explains why the analyses were not able to detect the mineral nutrients and $\delta^{15}N$ effects on reflectance, considering that spectroscopy sensitivity to these properties are an artefact of traits correlation rather than a real feature. The same occurs when accounting for variation related to the interaction between soil and species. The soil component in the interaction tends to be underestimated for rock-derived and $\delta^{13}C$.

This study particularly provides findings for a large range of traits that indicate that the use of spectroscopy may be useful to quantify structural traits but can be misleading to measure the environmental filtering on traits that are indirectly predicted, such as macro- and micronutrients. While remote sensing is not a direct replacement of field sampling, the ability of remote sensing platforms to assess biological phenomena at large spatial scales is unparalleled.

5 Conclusions

Analyses of trait variation shows that the identity of the species has a much stronger influence on most traits than the substrate upon which the tree grows. Traits associated with light capture, cell wall structure and defence were particularly uninfluenced by substrate, while rock-derived nutrients are strongly influenced by the soil

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409 characteristics. This study also demonstrates the potential for estimating foliar traits by field spectroscopy and
410 its promising use to predict Si. LMA, water, N, pigments, phenolics, K, B and hemicellulose were also
411 accurately estimated at the species level. However, subtle changes in traits associated with soil type were not
412 generally detectable, possibly because the spectroscopy sensitivity to these traits is an artefact of correlation
413 with other traits that did not change due to soil type.
414
415 **Authors' Contributions**
416 MHN participated in the chemical analysis, analysed the data and wrote the manuscript; MPD led the chemical
417 analysis and wrote the manuscript; DAC conceived the ideas, designed methodology, collected the data and led
418 the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for
419 publication
420
421 **Acknowledgments**
422 We thank undergraduate students Thomas Hitchcock, Lilian Halstead, Matt Chadwick and Connor
423 Willmington-Holmes for helping with field work, and Alexandra Jamieson for measuring phenolics. We are
424 grateful to David Burslem for providing access to carbon fractions analyser at the University of Aberdeen. The
425 field spectrometer was hired from the NERC Field Spectroscopy Facility; we are grateful to Alasdair MacArthur
426 for his training and advice. Matheus H. Nunes is supported by a PhD scholarship from the *Conselho Nacional*
427 *de Pesquisa e Desenvolvimento* (CNPq).
428
429 **Competing interests**
430 The authors declare that they have no conflict of interest.
431
432 **References**

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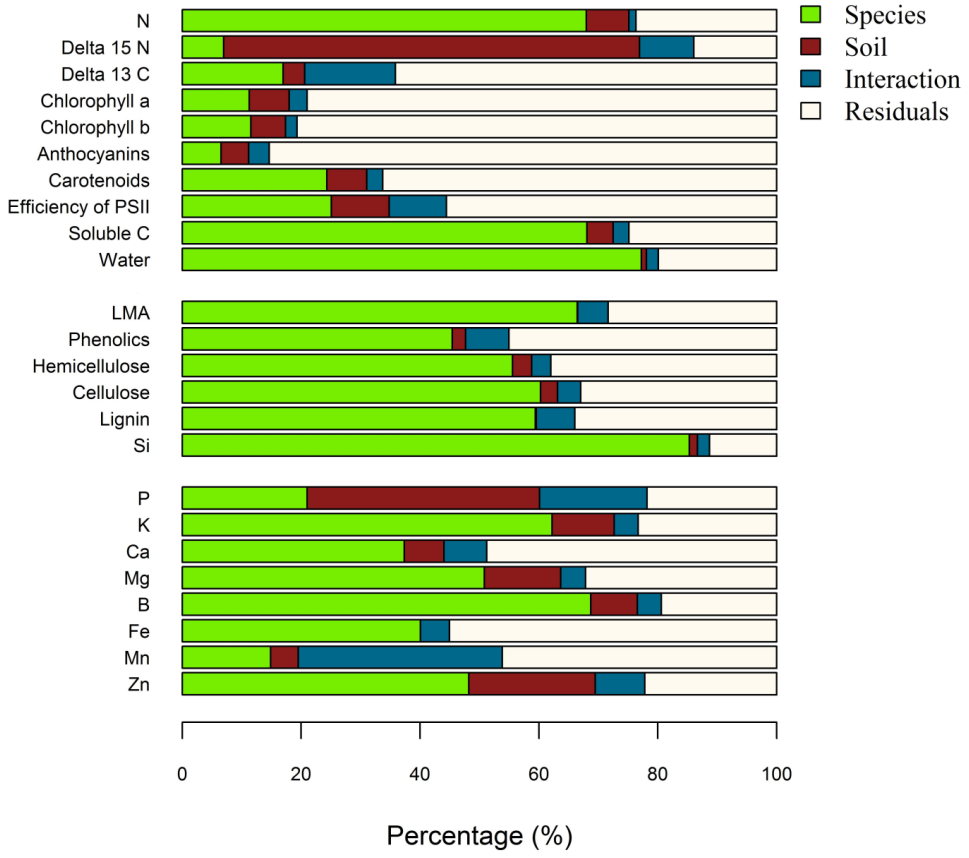

649
650 **Figure 1.** Partitioning of variance of foliar properties between species, soil, species x soil interaction and
651 residual components for six generalist species found on both chalk and alluvial soils. Residual variation arises
652 from within-site intraspecific variation, micro-site variability, canopy selection and measurement error variance.

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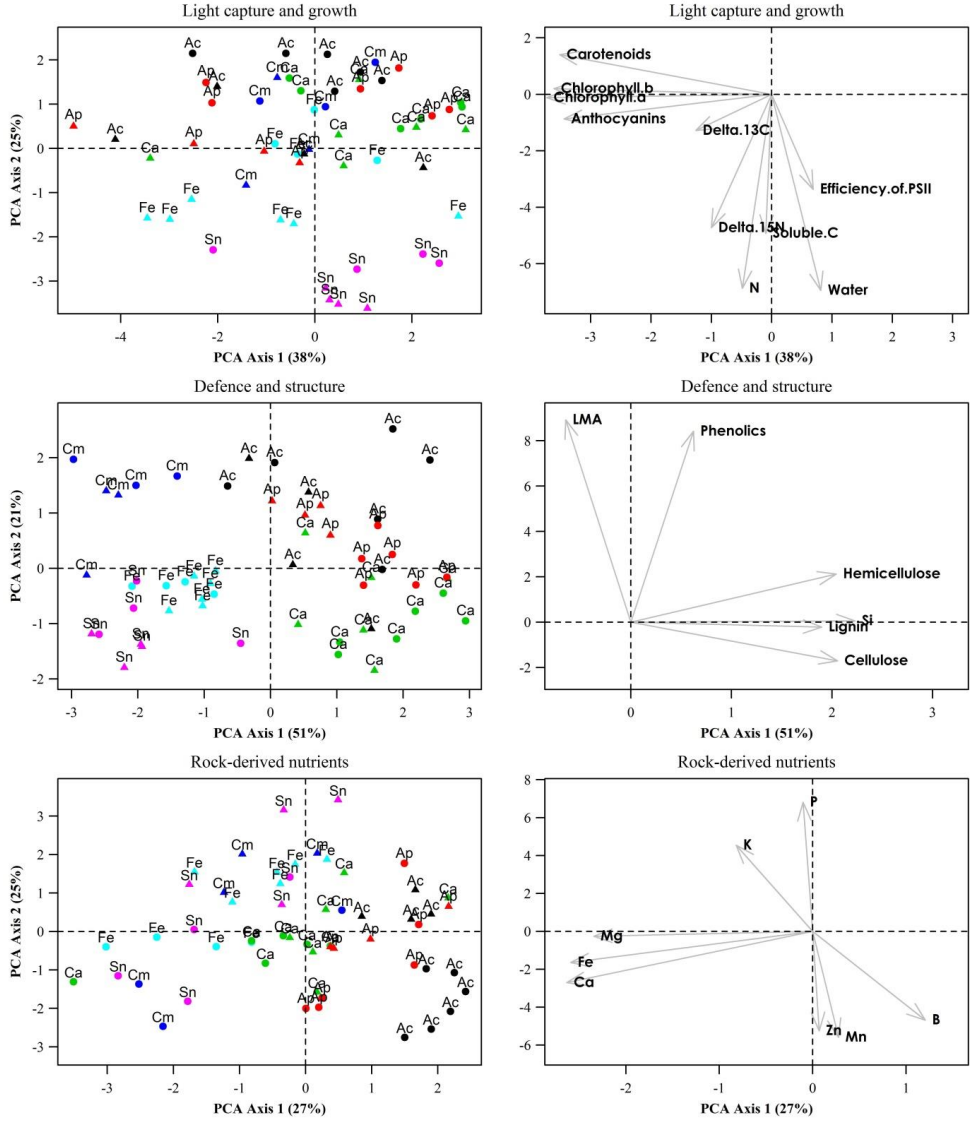

Figure2. Principal component analysis of traits related to light capture and leaf hydraulic, defence and leaf structure, and metabolism and maintenance. Fe = *Fraxinus excelsior*; Sn = *Sambucus nigra*; Ac = *Acer campestre*; Cm = *Crataegus monogyna*; Ca = *Corylus avellana*; Ap = *Acer pseudoplatanus*; Δ = alluvial soils; and ○ = chalk soils.

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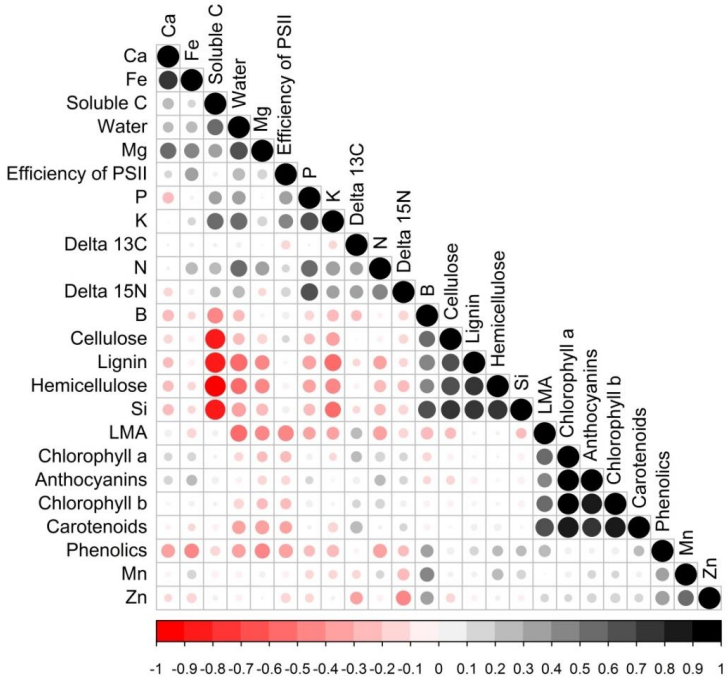

Figure 3. Spearman correlation rank test among leaf traits of 6 species growing on both soil types. Red and black circles mean negative and positive correlations. Foliar traits were organised using cluster analysis.

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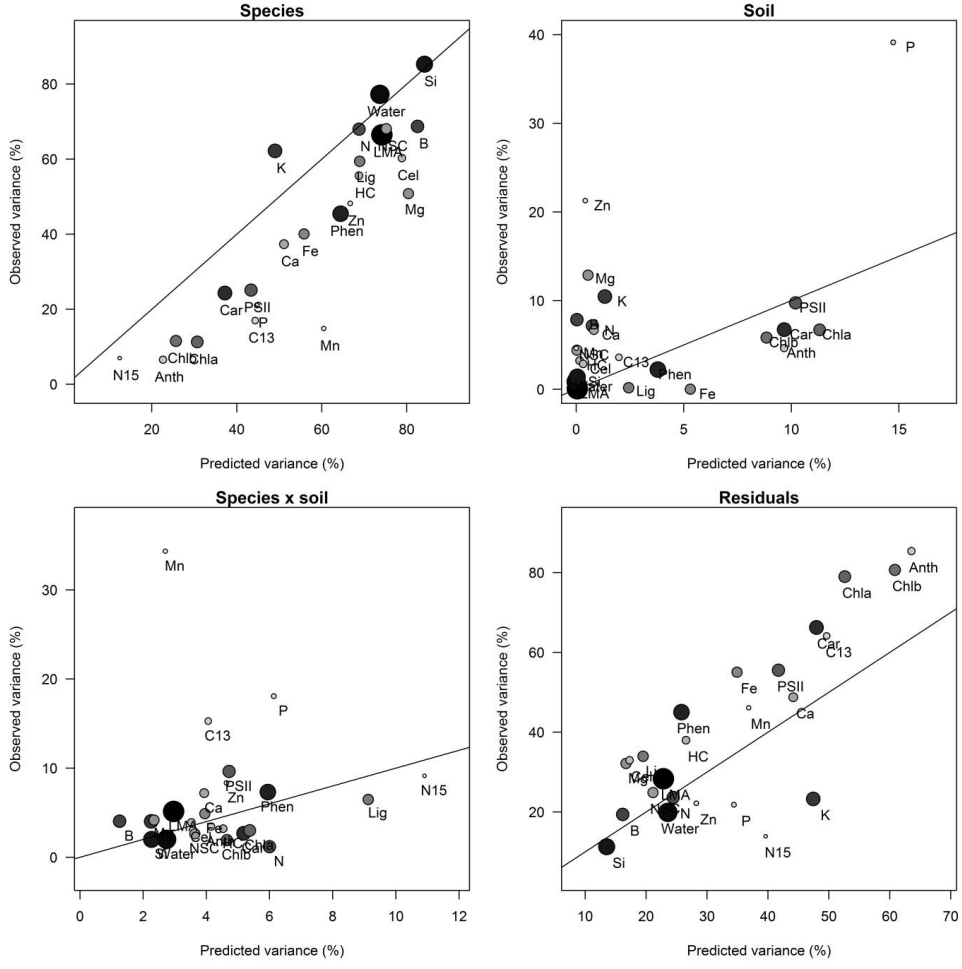

Figure 4. Predicted values from PLSR on reflectance versus actual partitioning of variance in foliar properties between species, soil, species x soil interaction and residual (intraspecific variation, micro-site variability, canopy selection and measurement error) variance, for six generalist species found on both chalk and alluvial soils. The greyness and size of each dot reflects the goodness-of-fit of the PLSR for each foliar trait, with darker and bigger points representing the most accurate PLSR predictions.

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675 **Table 1.** Average, standard deviation (SD) and coefficient of variation (CV) in percentage for leaf traits of six
676 generalist species growing on alluvial and chalk soils. Foliar property was statistically different between soil
677 types with *P*-value < 0.05 *, < 0.01 ** and < 0.001 ***.

Properties	Alluvial		Chalk	
	Mean ± SD	CV	Mean ± SD	CV
Light capture and growth				
N (%) ***	2.53 ± 0.81	32.1	2.16 ± 0.73	34.0
$\delta^{15}N$ (‰) ***	3.43 ± 2.65	77.3	-3.83 ± 2.01	52.3
$\delta^{13}C$ (‰)	-28.2 ± 1.2	4.5	-28.7± 1.0	3.6
+Chlorophyll a (mg m^{-2})	338.8 ± 116.0	34.2	279.6 ± 89.2	31.9
Chlorophyll b (mg m^{-2})	78.6 ± 27.6	35.1	64.7 ± 22.4	34.7
Anthocyanins (mg m^{-2})	423.3 ± 143.8	33.9	362.8 ± 121.6	33.5
Carotenoids (mg m^{-2}) *	110.5 ± 40.4	36.5	88.2± 35.5	40.2
Efficiency of PSII **	0.74 ± 0.05	7.1	0.71 ± 0.06	9.8
Soluble C (%) **	73.6 ± 6.5	8.8	70.3 ± 7.5	10.6
Water (%)	59.1 ± 8.2	14.0	58.5 ± 7.9	13.5
Defence and structure				
+LMA (g cm^{-2})	60.8 ± 24.0	39.4	60.6 ± 23.6	38.9
Phenolics (%)	83.7 ± 64.1	76.5	84.3 ± 49.7	59.0
+Hemicellulose (%)	10.9 ± 3.2	29.8	12.5 ± 3.6	29.4
Cellulose (%)	10.1 ± 1.8	18.6	11.0 ± 2.1	19.3
Lignin (%)	3.9 ± 1.9	49.8	4.7 ± 3.1	64.8
+Si (%) *	0.91 ± 0.56	62.2	1.11 ± 0.79	71.5
Rock-derived nutrients				
+P (%) ***	0.20 ± 0.05	25.5	0.14 ± 0.03	26.8
K (%) ***	0.98 ± 0.49	50.0	0.79 ± 0.50	64.4
+Ca (%) *	1.67 ± 0.75	45.1	2.29 ± 1.24	54.1
+Mg (%) ***	0.24 ± 0.11	47.1	0.36 ± 0.15	43.8
+B (µg g^{-1}) ***	29.0 ± 8.7	30.1	34.5 ± 12.4	36.0
+Fe (µg g^{-1})	122.3 ± 24.6	20.1	125.4 ± 32.0	25.5
+Mn (µg g^{-1}) *	84.7 ± 64.3	75.9	103.8 ± 69.5	66.9
+Zn (µg g^{-1}) ***	22.9 ± 12.6	55.0	34.1 ± 18.7	54.9

678 +log transformed prior to ANOVA.
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683 **Table2**. Permutational multivariate analysis to calculate the partitioning of variance in set of foliar traits related
684 to each functional class between species, soil, species x soil interaction and residual variance for six generalist
685 species found on both chalk and alluvial soils. All differences were significant (*P*-value < 0.05 *, < 0.01 ** and
686 < 0.001 ***) unless indicated as not significant (NS).

Component	Light capture and growth		Defence and structure		Rock-derived nutrients	
	F-test	R^2	*F*-test	R^2	*F*-test	R^2
Species	1.48^{ns}	0.13	14.9***	0.59	6.1***	0.31
Site	2.96^{ns}	0.05	0.84^{ns}	0.00	5.6**	0.06
Interaction	0.43^{ns}	0.04	1.23^{ns}	0.05	3.8***	0.19
Residuals		0.78		0.34		0.41

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717 **Table3.** Partial Least Squares Regression (PLSR) on spectral data and leave-one-out cross-validation for 24 leaf
718 traits of 6 species occurring on both alluvial and chalk soils. The model calibration and validation performance
719 was evaluated for each leaf property by calculating the coefficient of determination (R^2), root mean square error
720 (RMSE) and the percentage root mean square error (%) based on the given number of latent variables (nL) for
721 each PLS model.

Leaf property	Spectrum range (nm)	nL	R2		RMSE		RMSE%	
			Cal	Val	Cal	Val	Cal	Val
Light capture and growth								
N (%)	1100 – 2500	3	0.61	0.55	0.49	0.52	15.0	16.0
$\delta^{15}N$ (‰)	1100 – 2500	9	0.41	0.16	3.28	4.01	23.5	28.7
$\delta^{13}C$ (‰)	1100- 2500	6	0.46	0.30	0.85	0.96	16.1	18.2
$^{+}$Chlorophyll *a* (mg m^{-2})	400-700	7	0.65	0.53	60.05	69.62	13.5	15.7
Chlorophyll *b* (mg m^{-2})	400-700	4	0.59	0.50	16.48	18.57	15.2	17.1
Anthocyanins (mg m^{-2})	400-700	4	0.45	0.33	99.20	110.70	18.0	20.1
Carotenoids (mg m^{-2})	400-700	7	0.75	0.62	19.31	23.54	11.0	13.4
Efficiency of PSII	400-2500	6	0.68	0.55	0.03	0.04	13.4	15.9
Soluble C (%)	1100 – 2500	4	0.54	0.46	4.76	5.15	18.1	19.6
Water (%)	1100 – 1500	5	0.87	0.83	2.89	3.29	9.0	10.1
Defence and structure								
$^{+}$LMA (g cm^{-2})	1100 – 2500	6	0.94	0.92	1.09	1.12	6.1	6.9
Phenolics (%)	1500 – 1900	6	0.78	0.70	26.20	30.48	9.7	11.3
$^{+}$Hemicellulose (%)	1100-2500	4	0.44	0.35	1.28	1.30	18.4	19.8
Cellulose (%)	1100-2500	4	0.44	0.34	1.52	1.66	17.0	18.6
Lignin (%)	1100-2500	4	0.57	0.47	1.72	1.89	13.0	14.2
$^{+}$Si (%)	1100 – 2500	4	0.77	0.72	1.50	1.55	14.4	15.5
Rock-derived nutrients								
$^{+}$P (%)	1500-2500	7	0.43	0.22	1.26	1.30	17.8	20.2
K (%)	1500 – 2500	7	0.70	0.61	0.27	0.31	11.9	13.6
$^{+}$Ca (%)	1500-2500	7	0.53	0.40	1.40	1.47	15.9	17.9
$^{+}$Mg (%)	1900 – 2500	3	0.54	0.46	1.39	1.42	15.2	16.5
$^{+}$B (µg g^{-1})	1500-1900	6	0.66	0.56	1.24	1.28	13.6	15.2
$^{+}$Fe (µg g^{-1})	700 – 2500	5	0.56	0.46	1.17	1.19	15.6	17.2
$^{+}$Mn (µg g^{-1})	1500-1900	6	0.35	0.20	1.83	1.95	20.5	22.7
$^{+}$Zn (µg g^{-1})	1500-1900	7	0.41	0.21	1.50	1.60	19.5	22.4

722 $^{+}$ Trait values were natural log-transformed for PLSR.

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