On the challenges of using field spectroscopy to measure the impact of soil type on leaf traits

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Abstract

Understanding the causes of variation in plant functional traits is a central issue in ecology, particularly in the context of global change. Spectroscopy is increasingly used for rapid and non-destructive estimation of foliar traits, but few studies have evaluated its accuracy when assessing phenotypic variation in multiple traits. Working with 24 chemical and physical leaf traits of six European tree species growing on strongly contrasting soil types (i.e. deep alluvium versus nearby shallow chalk), we asked (i) whether variability in leaf traits is greater between tree species or soil type; and (ii) whether field spectroscopy is effective at predicting intraspecific variation in leaf traits as well as interspecific differences. Analysis of variance showed that inter-specific differences in traits were generally much stronger than intraspecific differences related to soil type, accounting for 25% versus 5% of total trait variation, respectively. Structural traits, phenolic defences and pigments were barely affected by soil type. In contrast, foliar concentrations of rock-derived nutrients did vary: P and K concentration were lower on chalk than alluvial soils, while Ca, Mg, B, Mn and Zn concentrations were all higher, consistent with the findings of previous ecological studies. Foliar traits were predicted from 400-2500 nm reflectance spectra collected by field spectroscopy using partial least square regression, a method that is commonly employed in chemometrics. Pigments were best modelled using reflectance data from the visible region (400 - 700 nm), whilst all other traits were best modelled using reflectance data from the shortwave infrared region (1100 - 2500 nm) region. Spectroscopy delivered accurate predictions of species-level variation in traits. However, it was ineffective at detecting intraspecific variation in rock-derived nutrients (with the notable exception of P). The explanation for this failure is that rock-derived elements do not have absorption features in the 400-2500 nm region, and their estimation is indirect, relying on elemental concentrations co-varying with structural traits that do have absorption features in that spectral region ("constellation effects"). Since the structural traits did not vary with soil type, it was impossible for our regression models to predict intraspecific variation in rock-derived nutrients via constellation effects. This study demonstrates the value of spectroscopy for rapid, non-destructive estimation of foliar traits across species, but highlights problems with predicting intraspecific variation indirectly. We discuss the implications of these findings for mapping functional traits by airborne imaging spectroscopy.

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, between populations and between species (Albert et al. 2011), and analysis of this variation is key to evaluating the strength of various filtering processes on communities growing along environmental gradients (Davey et al. 2009; 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 (Davey et al. 2009; Sultan 2001; Donohue et al. 2005). Despite substantial advances in trait-based community ecology over the past decade (Kunin et al. 2009; 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 measured routinely in plant communities and global tradeoffs among these traits are often interpreted in terms of life history of different species (Adler et al. 2014; Pillar et al. 2003; Aubin et al. 2009; Fry et al. 2014). In this study we measured 24 traits which we organise into three functional groups (Asner 2014, Asner et al. 2014; Asner et al. 2015): (i) *light capture and growth traits* include pigments, the maximum efficiency of photosystem II (PSII), nitrogen concentration which is closely related to protein concentration (Milton & Dintzis 1981), soluble C compounds and leaf water content, C isotope discrimination (δ^{13}C), N isotope discrimination (δ^{15}N); (ii) *defence and structural traits* include silicon (Si) organic 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 & Boland 2012), and LMA, a primary axis of specialization among plants (Grime et al. 1997; Lambers & Poorter 1992), that plays a crucial role in herbivore defence as well as leaf longevity (Wright et al. 2004); finally, (iii) *rock-derived nutrients* include phosphorus (P), which is involved in many enzymatic, genetic and epigenetic processes (Schachtman et al. 1998), and calcium (Ca), magnesium (Mg), potassium (K), zinc (Zn), manganese (Mn), boron (B) and iron (Fe), which are involved in signalling pathways and/or cofactors of enzymes (Marschner 2012). We recognise that leaf traits can contribute to more than one class (e.g. LMA is related to growth but also to defence, P is a rock-derived nutrient also associated with growth). Many analyses of traits have focussed on interspecific variation, but there is recognition that intraspecific variation can strongly influence species and community responses to environmental change (e.g. Weiner 2004; Funk et al. 2016).

There is currently great interest in using hyper-spectroscopy as a tool for studying the chemical and structural traits of leaves, particularly because improved airborne sensors and faster computing make it possible to map functional traits from the air (Ustin et al. 2009; Asner & Martin 2016b; Jetz et al. 2016; Asner et al. 2017). Plans to put hyperspectral sensors into space (e.g. DRL plan to launch EnMAP in 2018; Guanter et al. 2015) will soon enable spectral response curves of vegetation communities to be assessed at the global scale. 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 predictions of a range of foliar traits at the leaf and canopy scales within diverse tropical ecosystems (Asner et al. 2011a; Doughty et al. 2011) and temperate forests (Wessman et al. 1988; Serbin et al. 2014). However, some traits do not have absorption features within the visible and shortwave infrared spectral range of spectrometers conventionally used for vegetation analyses, but can be estimated indirectly through their covariance with traits that do have absorption features in the visible-to-shortwave-infrared region ("constellation effects" *sensu* Dana Chadwick & Asner 2016). These traits include elemental concentrations and isotope ratios (e.g. Serbin et al. 2014). In addition, structural differences (i.e., leaf thickness, number of air water interfaces, cuticle thickness, and pubescence) between leaves may have significant effects on the relationship between leaf reflectance and traits, and can complicate interpretation of data (Sims & Gamon 2002; Wu et al. 2016). The ability of spectroscopy to measure intraspecific variation in multiples traits between soil types, particularly when some of those traits are indirectly determined through constellation effects, has not been critically evaluated.

This paper examines the drivers of leaf trait variation in temperate woodlands growing on chalk in 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 soil type (Haines-Young et al. 2003; Smart et al. 2003), but none to our knowledge 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 24 leaf traits on these contrasting soil types and examined the ability of reflectance spectroscopy to quantify these leaf chemical and structural traits. We place these traits into groups based on ordination analyses, rather than working with pre-defined functional groups, and evaluate the functional significance of these groups. Our specific questions were: (i) is variability in leaf traits greater between tree species or soil type? (ii) is field spectroscopy effective at predicting intraspecific variation in leaf traits between soil types, as well as interspecific differences?

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, UK (latitude = 51°16'N, longitude = 0°19'W). 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 an average pH and standard deviation of 7.9 ± 1.0 ($n = 10$), whereas the alluvial was near neutral having a pH of 6.7 ± 0.2 ($n = 10$). Phosphorus becomes unavailable to plants in alkaline chalk soil (Gerke 1992), and greater depth of loamy soil on the alluvial surfaces must result in much greater availability of nutrients to plants.

Across both sites, leaves were collected from 66 trees, representing six species. The six species common to both sites were: *Acer campestre* (field maple), *Acer pseudoplatanus* (sycamore), *Corylus avellana* (hazel), *Crataegus monogyna* (hawthorn), *Fraxinus excelsior* (ash) and *Sambucus nigra* (elder). Two fully sunlit branches were selected, cut and placed in a cool box, and subsequently transported to a laboratory for processing within two hours. 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 area was 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. Leaf water content was computed as the ratio between the quantity of water (fresh weight – dry weight) and the fresh weight. A further 22 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). Briefly, 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 followed by digesting them in nitric acid and analysis on 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 flow isotope ratio mass spectrometer. This technique also 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 & Gamon (2002). The maximum efficiency of photosystem II (PSII) was calculated according to Genty et al. (1989) by measuring the maximum fluorescence (F_m) and the yield of fluorescence in the absence of an actinic (photosynthetic) light (F_o) 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 or 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, Boulder, Colorado, USA). 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 Team 2014). To evaluate the correlation among traits, Spearman rank correlation coefficient was calculated between all trait pairs and the variables were ordered in the figure by hierarchical clustering. Analyses of variance (ANOVA) were used to examine the influences of species identity and soil type on each of the 24 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 partitioning of variance quantifies the variation between species, between soil types, the interaction between soil and species, and the unexplained variance (residual variance). The residual variance comprises analytical error and various types of intraspecific variation including micro-site and within-canopy variation. Where necessary, variables were log transformed to meet assumptions of ANOVA (see Table 1 for details). In addition, permutation-based multivariate analysis of variance (PERMANOVA; Anderson 2001) was applied to the matrix of dissimilarity among traits to evaluate the importance of soil type, species identity and the interaction soil-species as a source of variation in the 24 traits simultaneously. The non-parametric permutation-based analysis of variance (PERMANOVA) was then performed on the resulting distances (10000 permutations). An alpha level of 0.05 was used for all significance tests, and no effort was made to test for or address non-normal data distributions. The PERMANOVA used distance matrices calculated using the *adonis* function in the *vegan* package of R.

Leaf traits were grouped using principal component analysis (PCA) using Simca-P (2016) software (Umetrics MKS Data Analytics Solutions, Sweden). The principal components for the variables were obtained by the correlation matrix modelling *in lieu* of covariance matrix modelling. We used the unit variance scaling (van den Berg et al. 2006) to avoid the effects of variables with high variance. The PCA was used to obtain score scatter and loadings plots to show the relatedness of all leaf traits in the dataset. R^2 and Q^2 overview plots were computed from the cumulated PCA axes 1-5. R^2 values denote how well a trait can be explained in the model and Q^2 denote how well a trait can be predicted from the dataset. The traits are ranked in descending R^2 order of how well they correlate with the other traits in the data set. These plots were used to evaluate whether traits clustered into functional groups. .

Partial least squares regression (PLSR) was used to evaluate whether field spectroscopy can reliably predict leaf traits (Haaland and Thomas, 1988). The spectral reflectance values of each sample were transformed into pseudo-absorption values, that is log [1/ R]) where R is reflectance (see Bolster et al. 1996; Gillon et al. 1999; Richardson & Reeves III 2005; Petisco et al. 2006; Kleinebecker et al. 2009). There is strong autocorrelation in pseudo-absorption values, so 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 predicted by minimising the Prediction Residual Error Sum of Squares (PRESS) statistic (Chen et al. 2004; Zhao et al. 2015). We adopted a leave-one-out cross-validation for each PLSR model. Model accuracy and precision were expressed by the 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). RMSE and R^2 were acquired during both model calibration and after model validation. PLSR was conducted initially using all available wavelengths (i.e. 400-2500 nm), but we then evaluated whether models based on smaller regions of the spectrum performed any better (see Serbin et al. 2014), based on comparisons of RMSE. The smaller regions were selected from absorption features recognised in previous papers (Curran 1989; Elvidge 1990; Kokaly et al. 2009). The visible (VIS, 400-700 nm), near infra-red (NIR, 700-1500 nm) and shortwave infra-red I (SWIR I, 1500-1900 nm), shortwave infra-red II (SWIR II, 1900-2500 nm) 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 the best-supported model selected based on minimisation of RMSE. To evaluate the effectiveness of field spectroscopy at measuring variation in traits related to soil type and species identity, we partitioned variance in model-predicted trait values using exactly the same approach as we used with lab-measured traits (i.e. first paragraph of methods).

3 Results

3.1 Soil and species controls on leaf traits

Foliar concentrations of rock-derived nutrients varied with soil type, but few other traits varied strongly with soil. 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 (δ^{15}N) varied greatly between the two soils, from -3.8 ‰ in the chalk soil to 3.4 ‰ in the alluvial. 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 rock-derived mineral nutrient that was unaffected by soil type. In contrast, hemicellulose, cellulose, lignin and LMA were completely unaffected by soil type, and pigments and traits related to water status (δ^{13}C and water content) varied little with soil type, with the exception of carotenoids concentration, which was 25 % higher in alluvial soil. The efficiency of PSII showed only a slight increase of 4 % in alluvial soil. The percentage contribution of soluble C was affected by soil, with an increase in soluble C of 9 % in the alluvial soil.

Most traits varied greatly between species and that variation was far greater than the soil effects (Fig. 1). Interspecific variation (green bars, 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 six traits (in descending order, lignin, hemicellulose, Mg, Zn, phenolics and Fe). Species identity exerted little or no influence on pigment concentrations, efficiency of PSII, δ^{13}C, δ^{15}N, P, Ca or Mn concentrations. The interactions between species and soil (blue bars, Fig. 1) explained little variation and were significant for δ^{15}N, P, Mn and Zn, but for no other traits. The pigments, efficiency of PSII and δ^{13}C had the largest unexplained variance. PERMANOVA analyses showed that, overall, species identity accounted for 25% of the variation in leaf traits, soil type accounted for 5%, while the interaction between species and soil accounted for virtually no variation (i.e. the traits of different species responded similarly to soil type).

The Principal Component Analysis (PCA) was able to distinguish species across component 1 and 2 (Fig. 2A), with less separation of species within the same genus (i.e. *A. campestre* and *A. pseudoplatanus*). The first two components of PCA explain 45% of the total variance. Separation of individuals between the soil types was weak. Growth vs structural/defence traits were separated in its first axis and area-based vs concentration-based traits in its second axis. The first two components of PCA explain 46% of the total variance. Considering only traits that were well-predicted by PCA (i.e. had $Q^2 > 0.5$), the first component distinguishes the traits associated in growth (i.e. N, K and soluble carbon concentrations, and water content) from traits associated with leaf defence and structure (i.e. hemicellulose and Si). The second component is chlorophyll *a*, chlorophyll *b*, carotenoids, anthocyanins and LMA, and mainly separates the traits that were calculated on area basis. The first component distinguishes species relatively well, with less separation of species within the same genus (i.e. A. *campestre* and A. *pseudoplatanus*).

3.2 Spectroscopy of leaf traits

The ability to predict leaf traits from hyperspectral reflectance spectra varied greatly among the 24 traits (Table 2). The R^2 values of validation data varied from 0.92 to 0.16, with traits ranked by goodness of fit as follows (highest first): LMA, leaf water content, Si, phenolics, carotenoids, K, B, efficiency of PSII, N, chlorophyll *a* and chlorophyll *b*. Some minerals, such as P, Zn and Mn, as well as $\delta^{13}C$ and $\delta^{15}N$ showed low R^2. There was virtually no difference in the average reflectance curves of leaves of trees growing on chalk and alluvial soils (Fig. 3a), but the coefficient of variation among plants was greater on the chalk soil (Fig. 3b). Pigments were most accurately modelled using reflectance data from the visible region of the spectra, whilst other traits were most accurately modelled using spectral data in the 1100 - 2500 nm range (Fig. 3). Efficiency of PSII and Fe were the only foliar traits for which the strength of relationship was greatest when all wavelengths between 400 and 2500 nm were used in the model.

Some leaf traits which appeared to be predicted accurately by PLSR do not have absorbance features in the 400-2500 nm range, and were instead predicted because of their close association with leaf traits that do have absorbance features in that range (see correlations in Fig. 4). For instance, Si and B do not have absorption features in the 400-2500 nm range, but their concentrations are highly correlated to hemicellulose, cellulose and lignin concentrations, and these organic polymers do have strong absorbance features in the SWIR region. Likewise, K do not have absorption features in the 400-2500 nm range, but K concentration is highly correlated to leaf water content, soluble carbon, lignin, hemicellulose and cellulose, all of which have absorbance features in the region. The importance of these “constellation effects” (*sensu* Chadwick and Asner 2016) becomes apparent when we examine the partitioning of variance of PLSR-predicted trait values: several rock-derived nutrients vary significantly with soil type when measured in leaves (Fig. 1) but little of that variation is successfully modelled by PLSR (Fig. 5). The explanation for this failure to model soil-related variation correctly is that concentrations of their associated traits remain invariant of soil type (Table 1). The use of PLSR also considerably under-predicted the importance of soil (~ 37 %) on the $\delta^{15}N$ variation, presumably for similar reasons. Some species-soil interaction effects were detected by PLSR modelling, except for traits that showed strong interaction (Mn, P and $\delta^{13}C$). PLSR models were better able to detect intra-specific variation in foliar N concentrations, because much of the nitrogen is contained in proteins, which have strong absorbance features.

4 Discussion

4.1 Patterns of variation in leaf traits

Compared with trees growing on deep alluvium, trees on thin chalk soils had low concentrations of N, P and K macronutrients in their leaves, but high concentrations of several micronutrients. Similar findings have been reported for herbaceous species growing on chalk (Hillier et al. 1990). Phosphorus and several micronutrients form low-solubility compounds in alkaline soils and become less available for plant uptake (Marschner 1995; Misra & Tyler 2000; Tyler 2002; Sardans & Peñuelas 2004), while the low N concentrations may reflect stoichiometric constraints (Niklas et al. 2005). The lower efficiency of PSII in the chalk soil is likely to be a consequence of phosphorus deficiency (Santos et al. 2006). Importantly for our later discussion on indirect estimation of traits by spectroscopy, species did not vary between soil types in their structural and defensive traits (i.e. LMA, lignin, phenolics) despite these differences in rock-derived nutrients. A similar lack of intraspecific change has been found in New Zealand rainforest trees growing on alluvium versus phosphorus-depleted marine terraces (Wright et al. 2010) and in several other studies (Koricheva et al. 1998; Boege & Dirzo 2004; Fine et al. 2006).

Species had a greater influence on trait values than soils for all traits except P, and PCA analyses demonstrated that species with traits associated with fast growth had low concentration of traits associated with defence and structure (see Coley 1983; 1987; Fine et al. 2006). Traits favouring high photosynthetic rate and growth are usually considered advantageous in rich-resource soil environments, while traits favouring resource conservation are considered advantageous in low-resource environments (Aerts & Chapin 1999; Westoby et al. 2002), but in this study the species were generalists growing on both soil types. The traits most influenced by species (in descending order) were Si, leaf water content, 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 between species reflect differences in ion channel activity in roots (Ma & 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 increase B tolerance of plants (Gunes et al. 2007). High Zn organization at the species level corroborates earlier analyses that showed more than 70% of Zn variation occured within family and substantial differences existed between and within species (Broadley et al. 2007).

The patterns revealed by our variance partitioning analysis of six temperate species (Fig. 1) bear similarities to those emerging from an analysis of 3246 species from nine tropical regions (Fig. 5 of Asner & Martin 2016a). The tropical analyses included a “site” term which captured variation due to soil and geology, among other factors. They, like us, found that taxonomic identity explained far more variation than site for most traits. Additionally they found foliar concentrations of P and other rock-derived minerals varied strongly with site, while nitrogen concentrations varied little; found that soluble carbon, structural and defensive traits hardly varied between sites; and observed that pigments (in their case just chlorophyll) was the least predictable of traits, probably because photosynthesis is rapidly up- and down-regulated in response to light environment among other factors (Asner & Martin 2011). Similarly, $\delta^{13}C$ is known to vary strongly with light condition and with relative humidity (Buchmann et al. 1997; Yan et al. 2012) which may explain why species and soil explained little of its variance in our study. These parallels between tropical and temperate systems suggest broad similarities in plant responses to soil across different regions that differ greatly in temperature.

4.2 Measuring interspecific variation in leaf traits with field spectroscopy

The spectral regions selected by our PLSR models match the locations of known spectral absorption features related to proteins, starch, lignin, cellulose, hemicellulose and leaf water content (Knipling 1970; Curran 1989; Elvidge 1990; Fourty & Baret 1998; 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 contributed to the predictions of the efficiency of PSII and Fe 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. According to Kumar et al. (2002), three main protein absorption features reported as important for N estimation are located around 1680 nm, 2050 nm and 2170 nm. In this study, pigments were found to influence the visible region of the spectrum while PSII-efficiency was predicted from features across the VSWIR range. The spectra of chlorophylls are distinct from those of proteins because C-H bonds in their phytols tails create a strong absorption feature not found in proteins (Katz et al. 1966). However, pigments are tightly bound by proteins to form photosynthetic antenna complexes that capture light energy and transfer it to the PSI and PSII reaction centres (Liu et al., 2004). The vibration of the bonds in the pigment–protein complex adds additional absorption features to the spectra of pigments and may help explain why so many bands were involved in PSII-efficiency prediction (Porcar-Castell et al., 2014). The 1500-1900 nm region was important for phenolic compounds prediction, which includes the 1660 nm feature across a variety of species and phenolic compounds (Windham et al. 1988; Kokaly & Skidmore 2015). The primary and secondary effects of water content on leaf reflectance are greatest in spectral bands centred at 1450, 1940, and 2500 nm (Carter & Porter 1991), but has also been predicted using bands between 1100-1230 nm absorption features (Ustin et al. 1998; Asner et al. 2004). With respect to the other rock-derived nutrients, Galvez-Sola et al. (2015) also 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 0.98 for Ca, whereas B showed less accurate results with the use of spectroscopy. The regions of importance for prediction described in those studies were relatively similar to all the mineral nutrients analysed in our study, except for B that had the band between 1500 and 1900 as the best predictive region.

Some of most accurately predicted traits have no absorption features in the visible-to-near-infrared, but were instead estimated indirectly via constellation effects. Leaf mass per unit area (LMA) is consistently among the more accurately predicted traits using spectroscopy (Asner & Martin 2008; Serbin et al. 2014; Chavana-Bryant et al. 2016), but is measured indirectly via its close coupling with water content and leaf structural traits (Asner et al. 2011b). Silicon (Si) concentrations were well-predicted by field spectroscopy, as recently reported by Smis et al. (2014). Silicon is absorbed by plants from the soil solution in the form of silicic acid (H_4SiO_4), being translocated to the aerial parts through xylem, and then deposited as phytoliths (Tripathi et al. 2011). Si is closely associated with phenol- or lignin-carbohydrate complexes (Inanaga et al. 1995), cellulose (Law & Exley 2011), and polysaccharide and peptidoglycans (Schwarz 1973). It seems that spectroscopy is able to predict Si concentrations reliably because it integrates information on several of these foliar traits to make the predictions. Similarly, the relative high precisions for K, Fe and B predictions may be as strong as they are because information on several foliar traits are integrated. Unfortunately, foliar P concentrations are not closely predicted by spectroscopy. RNA and DNA absorb in the ultraviolet (e.g. Tataurov et al. 2008) and phosphates in the longwave infrared, but there are no pronounced absorption features in the VSWIR region (Homolová et al. 2013) and covariance with other traits is weak, making constellation effects unreliable. Whilst a few spectroscopy studies have modelled P with some success, the spectral bands chosen differs among studies (Homolová et al. 2013) suggesting that constellation effects cannot be relied upon.

4.2 Difficulties in measuring intraspecific variation by field spectroscopy and its implications for mapping functional traits

Rock-derived nutrients lack absorption features in visible to shortwave-infrared region of the electromagnetic spectrum so cannot be measured directly by spectroscopy. They can, nevertheless, be estimated indirectly by virtue of the fact that element concentrations co-vary with organic molecules that do have strong absorption features ("constellation effects", see above). This paper identifies a problem with this approach: there were strong differences in rock-derived mineral nutrients between soil types, but we could not measure these because the concentrations of defence and structural traits were barely affected by soil type. We have shown many similarities between our study and those in tropical forests, demonstrating that this problem is likely to be widespread.

There are likely to be implications of the constellation-effect problem for mapping functional traits using imaging spectroscopy. Ever larger areas of earth are being mapped with airborne spectrometers (e.g. Asner et al. 2017) and the anticipated launch of satellite-borne sensors (e.g. EnMAP; DLR 2015; Guanter et al. 2015) will soon enable vegetation and ecosystem function to be characterised at a global scale. The effectiveness of indirect prediction of traits using constellation-effect approaches will depend critically on whether soils act as a strong filter on tree species within a particular region. In the Amazonian lowlands, Asner et al. (2015) found that variation in soil P was mirrored by changes in species composition, and that P variation among species was correlated with changes in structural and defence compounds: in this instance, indirect estimation should be effective (e.g. Dana Chadwick & Asner 2016). However, in low-diversity temperate forests, a single tree species is often found to span many different soil types and show substantial phenotypic plasticity in some traits (Oleksyn et al. 2002; Turnbull et al. 2016). The six species growing on both chalk and alluvial soils in this study are a case in point. In these low diversity systems, it will be much more difficult to map variation using constellation effects, for the reasons explained above. Our study confirms the power of spectroscopy for predicting biochemical and structural plant traits, but we urge caution in interpreting results when species range across contrasting soil types.

5 Conclusions

Trees on thin chalk soils had low concentrations of N, P and K macronutrients in their leaves than trees growing on deep alluvium, but had high concentrations of several micronutrients. Phosphorus is sequestered in insoluble forms in alkaline soils. This shortage of plant available phosphorus was associated in this study with low concentrations of foliar N and low efficiency of PSII, but had no effect on structural and defensive traits. Trait differences were far greater among species than between soil types, for all traits except foliar P. Foliar traits predicted from VSWIR reflectance spectra matched the locations of known spectral absorption features related to proteins, starch, lignin, cellulose, hemicellulose and leaf water content. Some of the most accurately predicted traits have no absorption features in the VSWIR range, and were estimated indirectly through their covariance with structural traits that do have absorption features in that spectral region ("constellation effects") including cell wall constituents. Since these structural traits did not vary with soil type, our models were unable to reliably predict intraspecific variation in rock-derived nutrients via constellation effects. Similarities between our results and those of large-scale tropical studies suggest this problem is likely to be widespread. This study demonstrates the value of spectroscopy for rapid, non-destructive estimation of foliar traits across species, but highlights the difficulties that can arise in detecting within-species changes along environmental gradients.

Authors' Contributions

MHN participated in the chemical analyses, analysed the data and wrote the manuscript; MPD led the chemical analysis and contributed to the writing of the manuscript; DAC conceived of the ideas, designed the methods, supervised the collection of field data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication

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Competing interests

The authors declare that they have no conflict of interest.

References

Adler, P.B. et al., 2014. Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences of the United States of America*, 111(2), pp.740–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24379395.

Aerts, R. & Chapin, F.S., 1999. The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. *Advances in Ecological Research*, 30(C), pp.1–67.

Albert, C.H. et al., 2011. When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics*, 13(3), pp.217–225.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26(1), pp.32–46. Available at: http://libproxy.udayton.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=5472169&site=eds-live.

Asner, G.P., 2014. A chemical-evolutionary basis for remote sensing of tropical forest diversity. In *Forests and Global Change*. Cambridge: Cambridge University Press, p. 462.

Asner, G.P. et al., 2017. Airborne laser-guided imaging spectroscopy to map forest trait diversity and guide conservation. *Science*, 355(6323), pp.385–389.

Asner, G.P. et al., 2004. Drought stress and carbon uptake in an Amazon forest measured with spaceborne imaging spectroscopy. *Proc Natl Acad Sci U S A*, 101(16), pp.6039–6044. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/15071182.

Asner, G.P. et al., 2015. Landscape biogeochemistry reflected in shifting distributions of chemical traits in the Amazon forest canopy. *Nature Geoscience*, 8(May), pp.567–573. Available at: http://www.nature.com/doifinder/10.1038/ngeo2443.

Asner, G. P., Martin, R. E., Carranza-Jiménez, L., Sinca, F., Tupayachi, R., Anderson, C. B. & Martinez, P. (2014), Functional and biological diversity of foliar spectra in tree canopies throughout the Andes to Amazon region. New Phytologist, 204, 127–139. http://doi:10.1111/nph.12895

Asner, G.P., Martin, R.E., Knapp, D.E., et al., 2011. Spectroscopy of canopy chemicals in humid tropical forests. *Remote Sensing of Environment*, 115(12), pp.3587–3598.

Asner, G.P., Martin, R.E., Tupayachi, R., et al., 2011. Taxonomy and remote sensing of leaf mass per area (LMA) in humid tropical forests. *Ecological Applications*, 21(1), pp.85–98.

Asner, G.P. & Martin, R.E., 2011. Canopy phylogenetic, chemical and spectral assembly in a lowland Amazonian forest. *New Phytologist*, 189(4), pp.999–1012.

Asner, G.P. & Martin, R.E., 2016a. Convergent elevation trends in canopy chemical traits of tropical forests. *Global Change Biology*, 22(6), pp.2216–2227.

Asner, G.P. & Martin, R.E., 2008. Spectral and chemical analysis of tropical forests: Scaling from leaf to canopy levels. *Remote Sensing of Environment*, 112(10), pp.3958–3970.

Asner, G.P. & Martin, R.E., 2016b. Spectranomics: Emerging science and conservation opportunities at the interface of biodiversity and remote sensing. *Global Ecology and Conservation*, 8, pp.212–219. Available at: http://dx.doi.org/10.1016/j.gecco.2016.09.010.

Aubin, I. et al., 2009. Comparison of two plant functional approaches to evaluate natural restoration along an old-field - deciduous forest chronosequence. *Journal of Vegetation Science*, 20(2), pp.185–198.

van den Berg, R. a et al., 2006. Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC genomics*, 7, p.142. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16762068.

Boege, K. & Dirzo, R., 2004. Intraspecific variation in growth, defense and herbivory in. *Plant Ecology*, pp.59–69.

Bolster, K., Martin, M. & Aber, J., 1996. Determination of carbon fraction and nitrogen concentration in tree foliage by near infrared reflectances: a comparison of statistical methods. *Canadian Journal of Forest …*, 26(4), pp.590–600. Available at: http://www.nrcresearchpress.com/doi/abs/10.1139/x26-068.

Broadley, M.R. et al., 2007. Zinc in plants: Tansley review. *New Phytologist*, 173(4), pp.677–702.

Buchmann, N., Kao, W.Y. & Ehleringer, J., 1997. Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. *Oecologia*, 110(1), pp.109–119.

Carter, P.W. & Porter, J.D., 1991. Probing of π conjugation in trans-polyacetylene using near-infrared photoluminescence spectroscopy. *Physical Review B*, 43(18), p.14478.

Chavana-Bryant, C. et al., 2016. Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements. *New Phytologist*.

Chen, S. et al., 2004. Sparse Modeling Using Orthogonal Forward Regression With PRESS Statistic and Regularization. *IEEE Transactions on Systems, Man, and Cybernetics, Part B: Cybernetics*, 34(2), pp.898–911.

Coley, P.D., 1983. Herbivory and Defensive Characteristics of Tree Species in a Lowland Tropical Forest. *Ecological Monographs*, 53(2), p.209. Available at: http://www.esajournals.org/doi/abs/10.2307/1942495.

Coley, P.D., 1987. Interspecific Variation in Plant Anti???Herbivore Properties: the Role of Habitat Quality and Rate of Disturbance. *New Phytologist*, 106, pp.251–263.

Couture, J.J., Serbin, S.P. & Townsend, P.A., 2013. Spectroscopic sensitivity of real-time, rapidly induced phytochemical change in response to damage. *New Phytologist*, 198(1), pp.311–319.

Curran, P.J., 1989. Remote sensing of foliar chemistry. *Remote Sensing of Environment*, 30(3), pp.271–278.

Dana Chadwick, K. & Asner, G.P., 2016. Organismic-scale remote sensing of canopy foliar traits in lowland tropical forests. *Remote Sensing*, 8(2).

Davey, M.P. et al., 2007. Species-specific effects of elevated CO2 on resource allocation in Plantago maritima and Armeria maritima. *Biochemical Systematics and Ecology*, 35(3), pp.121–129.

Davey, M.P., Ian Woodward, F. & Paul Quick, W., 2009. Intraspecfic variation in cold-temperature metabolic phenotypes of Arabidopsis lyrata ssp. petraea. *Metabolomics*, 5(1), pp.138–149.

Donohue, K. et al., 2005. ENVIRONMENTAL AND GENETIC INFLUENCES ON THE GERMINATION OF ARABIDOPSIS THALLANA IN THE FIELD. *Evolution*, 59(4), pp.740–757. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.0014-3820.2005.tb01750.x/abstract.

Doughty, C.E., Asner, G.P. & Martin, R.E., 2011. Predicting tropical plant physiology from leaf and canopy spectroscopy. *Oecologia*, 165(2), pp.289–299.

Elvidge, C.D., 1990. Reflectance characteristics of dry plant materials. *International Journal of Remote Sensing*, 11(20), pp.1775–1795.

Feilhauer, H. et al., 2010. Brightness-normalized Partial Least Squares Regression for hyperspectral data. *Journal of Quantitative Spectroscopy and Radiative Transfer*, 111(12–13), pp.1947–1957.

Fine, P.V.A. et al., 2006. The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, 87(7 SUPPL.).

Fourty, T. & Baret, F., 1998. On spectral estimates of fresh leaf biochemistry. *International Journal of Remote Sensing*, 19(7), pp.1283–1297.

Fry, E.L., Power, S.A. & Manning, P., 2014. Trait-based classification and manipulation of plant functional groups for biodiversity-ecosystem function experiments. *Journal of Vegetation Science*, 25(1), pp.248–261.

Funk, J. et al., 2016. Revisiting the Holy Grail: Using plant functional traits to predict ecological processes. *Biological Reviews*.

Galvez-Sola, L. et al., 2015. Rapid estimation of nutritional elements on citrus leaves by near infrared reflectance spectroscopy. *Frontiers in plant science*, 6.

Genty, B., Briantais, J.-M. & Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 990(1), pp.87–92. Available at: http://dx.doi.org/10.1016/S0304-4165(89)80016-9.

Gerke, J., 1992. Orthophosphate and organic phosphate in the soil solution of four sandy soils in relation to pH- evidence for humic- FE- (AL-) phosphate complexes. *Communications in Soil Science and Plant Analysis*, 23(5–6), pp.601–612. Available at: http://www.tandfonline.com/doi/abs/10.1080/00103629209368612?journalCode=lcss20#.VXfhq8hU6Ew.

Gillon, D., Houssard, C. & Joffre, R., 1999. Using near-infrared reflectance spectroscopy to predict carbon, nitrogen and phosphorus content in heterogeneous plant material. *Oecologia*, 118(2), pp.173–182.

Giusti, M.M., Rodríguez-Saona, L.E. & Wrolstad, R.E., 1999. Molar absorptivity and color characteristics of acylated and non- acylated pelargonidin-based anthocyanins. *Journal of Agricultural and Food Chemistry*, 47(11), pp.4631–4637.

Green, J.L., Bohannan, B.J.M. & Whitaker, R.J., 2008. Microbial biogeography: from taxonomy to traits. *Science*, 320, pp.1039–1043.

Grime, J.P. et al., 2000. The response of two contrasting limestone grasslands to simulated climate change. *Science (New York, N.Y.)*, 289(August), pp.762–765.

Grime, J.P.P. et al., 1997. Integrated screening validates primary axes of specialisation in plants. *Oikos*, 79, pp.259–281.

Guanter, L. et al., 2015. The EnMAP spaceborne imaging spectroscopy mission for earth observation. *Remote Sensing*, 7(7), pp.8830–8857.

Gunes, A. et al., 2007. Silicon increases boron tolerance and reduces oxidative damage of wheat grown in soil with excess boron. *Biologia Plantarum*, 51(3), pp.571–574.

Haines-Young, R. et al., 2003. Changing landscapes, habitats and vegetation diversity across Great Britain. *Journal of Environmental Management*, 67(3), pp.267–281.

Hikosaka, K., 2004. Interspecific difference in the photosynthesis-nitrogen relationship: Patterns, physiological causes, and ecological importance. *Journal of Plant Research*, 117(6), pp.481–494.

Hillier, S.H., Walton, D.W.H. & Wells, D.A., 1990. *Calcareous grasslands: ecology and management* Bluntisham Books, ed., Huntingdon.

Hodson, M.J. et al., 2005. Phylogenetic variation in the silicon composition of plants. *Annals of Botany*, 96(6), pp.1027–1046.

Homolová, L. et al., 2013. Review of optical-based remote sensing for plant trait mapping. *Ecological Complexity*, 15, pp.1–16.

Inanaga, S., Okasaka, A. & Tanaka, S., 1995. Does silicon exist in association with organic compounds in rice plant? *Soil Science & Plant Nutrition*, 41(1), pp.111–117.

Jetz, W. et al., 2016. Monitoring plant functional diversity from space. *Nature Plants*, 2(3), p.16024. Available at: http://www.nature.com/articles/nplants201624.

Katz, J.J., Dougherty, R.C. & Boucher, L.J., 1966. *Infrared and nuclear magnetic resonance spectroscopy of chlorophyll* A. Press, ed., New York.

Kleinebecker, T. et al., 2009. Prediction of δ13C and δ15N in plant tissues with near-infrared reflectance spectroscopy. *New Phytologist*, 184(3), pp.732–739. Available at: http://dx.doi.org/10.1111/j.1469-8137.2009.02995.x.

Knipling, E.B., 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. *Remote Sensing of Environment*, 1(3), pp.155–159.

Kokaly, R.F. et al., 2009. Characterizing canopy biochemistry from imaging spectroscopy and its application to ecosystem studies. *Remote Sensing of Environment*, 113(SUPPL. 1).

Kokaly, R.F. & Skidmore, A.K., 2015. Plant phenolics and absorption features in vegetation reflectance spectra near 1.66 ??m. *International Journal of Applied Earth Observation and Geoinformation*, 43, pp.55–83.

Koricheva, J. et al., 1998. Regulation plant secondary metabolism by resource availability : hypothesis testing by means of meta-analysis. *Oikos*, 83(2), pp.212–226.

Kumar, L. et al., 2002. Imaging Spectrometry and Vegetation Science. In *Imaging spectrometry*. pp. 111–156.

Kunin, W.E. et al., 2009. Variation at range margins across multiple spatial scales: environmental temperature, population genetics and metabolomic phenotype. *Proceedings. Biological sciences / The Royal Society*, 276(1661), pp.1495–1506.

Lambers, H. & Poorter, H., 1992. Inherent Variation in Growth Rate Between Higher Plants: A Search for Physiological Causes and Ecological Consequences. *Advances in Ecological Research*, 34, pp.283–362.

Law, C. & Exley, C., 2011. New insight into silica deposition in horsetail (Equisetum arvense). *BMC plant biology*, 11(1), p.112. Available at: http://www.biomedcentral.com/1471-2229/11/112.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148(C), pp.350–382.

Liu, Z., Yan, H., Wang, K., Kuang, T., Zhang, J., Gui, L., An, X. and Chang, W., 2004. Crystal structure of spinach major light-harvesting complex at 2.72 Å resolution. *Nature*, 428(6980), pp.287-292.

Ma, J.F. & Yamaji, N., 2006. Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, 11(8), pp.392–397.

MacGillivray, C.W., Grime, J.P. & The Integrated Screening Programme (Isp) Team, 1995. Testing predictions of the resistance and resilience of vegetation subjected to extreme events. *Functional Ecology*, 9(4), pp.640–649. Available at: http://www.jstor.org/stable/2390156.

Marschner, H., 1995. Functions of Mineral Nutrients: Macronutrients. In *Mineral Nutrition of Higher Plants*. pp. 229–312.

Marschner, M., 2012. *Mineral Nutrition of Higher Plants*, Available at: http://books.google.com/books?id=_a-hKcXXQuAC&pgis=1.

Marten, G.C., Halgerson, J.L. & Cherney, J.H., 1983. Quality prediction of small grain forages by near infrared reflectance spectroscopy. *Crop Science*, 23(1), pp.94–96.

McGill, B.J. et al., 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, 21(4), pp.178–185.

Milton, K. & Dintzis, F.R., 1981. Nitrogen-to-Protein Conversion Factors for Tropical Plant-Samples. *Biotropica*, 13(3), pp.177–181.

Misra, A. & Tyler, G., 2000. Effects of soil moisture on soil solution chemistry , biomass production , and shoot nutrients in two native grasses on a calcareous soil. *Communications in Soil Science and Plant Analysis*, 31(October 2013), pp.37–41.

Mithöfer, A. & Boland, W., 2012. Plant Defense Against Herbivores: Chemical Aspects. *Annual Review of Plant Biology*, 63, pp.431–450.

Niklas, K.J. et al., 2005. Nitrogen/phosphorus leaf stoichiometry and the scaling of plant growth. *Ecology Letters*, 8(6), pp.636–642.

Oleksyn, J. et al., 2002. Needle nutrients in geographically diverse pinus sylvestris L. populations. *Ann. For. Sci.*, 59(4), pp.1–18.

Petisco, C. et al., 2006. Near-infrared reflectance spectroscopy as a fast and non-destructive tool to predict foliar organic constituents of several woody species. *Analytical and Bioanalytical Chemistry*, 386(6), pp.1823–1833.

Pillar, V.D., Sosinski, E.E. & Lepš, J., 2003. An improved method for searching plant functional types by numerical analysis. Journal of Vegetation S*cience*, 14(3), pp.323–332. Available at:

http://dx.doi.org/10.1658/1100-9233(2003)014[0323:AIMFSP]2.0.CO;2.

Porcar-Castell, A., Tyystjärvi, E., Atherton, J., van der Tol, C., Flexas, J., Pfündel, E.E., Moreno, J., Frankenberg, C. and Berry, J.A., 2014. Linking chlorophyll a fluorescence to photosynthesis for remote sensing applications: mechanisms and challenges. *Journal of experimental botany,* 65 (15), 4065-4095.

Richardson, A.D. & Reeves III, J.B., 2005. Quantitative reflectance spectroscopy as an alternative to traditional wet lab analysis of foliar chemistry: near-infrared and mid-infrared calibrations compared. *Canadian Journal of Forest Research*, 35(5), pp.1122–1130.

dos Santos, U.M., de Carvalho Gonçalves, J.F. & Feldpausch, T.R., 2006. Growth, leaf nutrient concentration and photosynthetic nutrient use efficiency in tropical tree species planted in degraded areas in central Amazonia. *Forest Ecology and Management*, 226(1–3), pp.299–309.

Sardans, J. & Peñuelas, J., 2004. Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. *Plant and Soil*, 267(1–2), pp.367–377.

Schachtman, D.P., Reid, R.J. & Ayling, S.M., 1998. Phosphorus Uptake by Plants : From Soil to Cell. *Plant Physiology*, 116, pp.447–453.

Schwarz, K., 1973. A bound form of silicon in glycosaminoglycans and polyuronides. *Proceedings of the National Academy of Sciences of the United States of America*, 70(5), pp.1608–1612.

Serbin, S.P. et al., 2014. Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications*, 24(7), pp.1651–1669.

Sims, D.A. & Gamon, J.A., 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, 81(2–3), pp.337–354.

Smart, S.M. et al., 2003. National-scale vegetation change across Britain; an analysis of sample-based surveillance data from the Countryside Surveys of 1990 and 1998. *Journal of Environmental Management*, 67(3), pp.239–254.

Smis, A. et al., 2014. Determination of plant silicon content with near infrared reflectance spectroscopy. *Frontiers in plant science*, 5, pp.1–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4174135&tool=pmcentrez&rendertype=abstract.

Sultan, S.E., 2001. Phenotypic plasticity for fitness components in Polygonum species of contrasting ecological breadth. *Ecology*, 82(2), pp.328–343.

Tataurov, A. V., You, Y. & Owczarzy, R., 2008. Predicting ultraviolet spectrum of single stranded and double stranded deoxyribonucleic acids. *Biophysical Chemistry*, 133(1–3), pp.66–70.

Team, R.C., 2014. R core team (2014). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www. R-project. org*, p.ISBN 3-900051-07-0, URL http://www.R-project.org/. Available at: http://www.mendeley.com/research/r-language-environment-statistical-computing-96/%5Cnpapers2://publication/uuid/A1207DAB-22D3-4A04-82FB-D4DD5AD57C28.

Tripathi, D.K. et al., 2011. Laser-induced breakdown spectroscopy for the study of the pattern of silicon deposition in leaves of Saccharum species. *Instrumentation Science & Technology*, 39(6), pp.510–521.

Turnbull, M.H. et al., 2016. Separating species and environmental determinants of leaf functional traits in temperate rainforest plants along a soil-development chronosequence. *Functional Plant Biology*, 43(8), pp.751–765.

Tyler, G., 2002. Phosphorus fractions in grassland soils. *Chemosphere*, 48, pp.343–349.

Ustin, S.L. et al., 1998. Estimating canopy water content of chaparral shrubs using optical methods. *Remote Sensing of Environment*, 65(3), pp.280–291.

Ustin, S.L. et al., 2009. Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. *Remote Sensing of Environment*, 113(SUPPL. 1).

Vellend, M., 2010. Conceptual synthesis in community ecology. *The Quarterly review of biology*, 85(2), pp.183–206.

Violle, C. et al., 2012. The return of the variance: Intraspecific variability in community ecology. *Trends in Ecology and Evolution*, 27(4), pp.244–252.

Weiner, J., 2004. Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 6(4), pp.207–215. Available at: http://www.sciencedirect.com/science/article/pii/S1433831904700773.

Wessman, C.A. et al., 1988. Foliar analysis using near infrared reflectance spectroscopy. *Canadian Journal of Forest Research*, 18(1), pp.6–11.

Westoby, M. et al., 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, 33(1), pp.125–159.

Windham, W.R., Fales, S.L. & Hoveland, C.S., 1988. Analysis for tannin concentration in sericea lespedeza by near infrared reflectance spectroscopy. *Crop Science*, 28(4), pp.705–708.

Wold, S., Sjöström, M. & Eriksson, L., 2001. PLS-regression: A basic tool of chemometrics. In *Chemometrics and Intelligent Laboratory Systems*. pp. 109–130.

Wright, D.M. et al., 2010. Do leaves of plants on phosphorus-impoverished soils contain high concentrations of phenolic defence compounds? *Functional Ecology*, 24(1), pp.52–61.

Wright, I.J. et al., 2004. The worldwide leaf economics spectrum. *Nature*, 428(6985), pp.821–827.

Wu, J. et al., 2016. Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests. *New Phytologist*.

Yan, C.F. et al., 2012. Needle-age related variability in nitrogen, mobile carbohydrates, and ?? 13c within pinus koraiensis tree crowns. *PLoS ONE*, 7(4).

Zhao, N. et al., 2015. Optimization of Parameter Selection for Partial Least Squares Model Development. *Scientific Reports*, 5, p.11647. Available at: http://www.nature.com/doifinder/10.1038/srep11647.

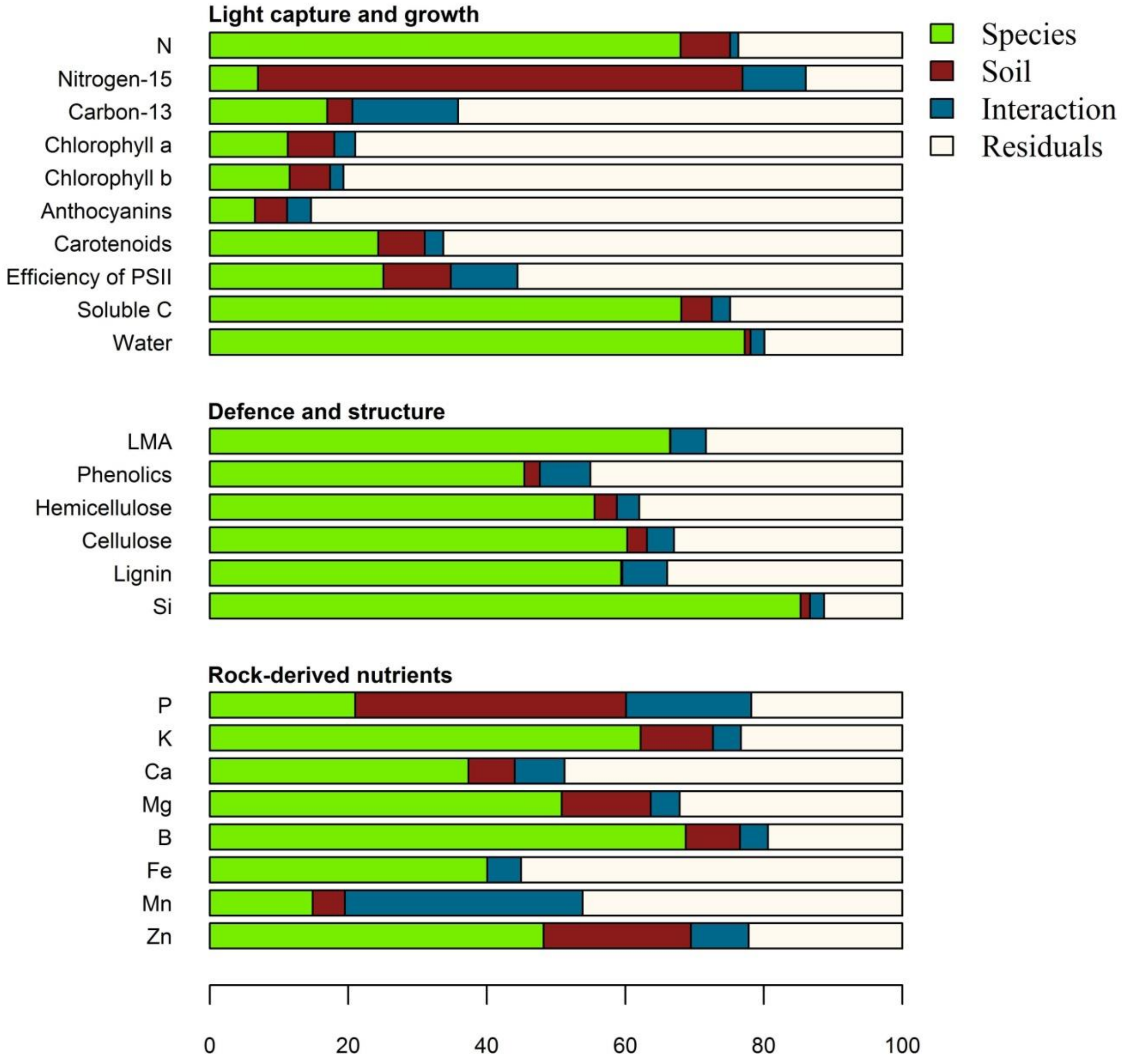

Figure 1. Partitioning of variance of foliar traits between species, soil, species-soil interaction and residual components for six generalist species found on both chalk and alluvial soils. Residual variation arises from within-site intraspecific variation, micro-site variability, canopy selection and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

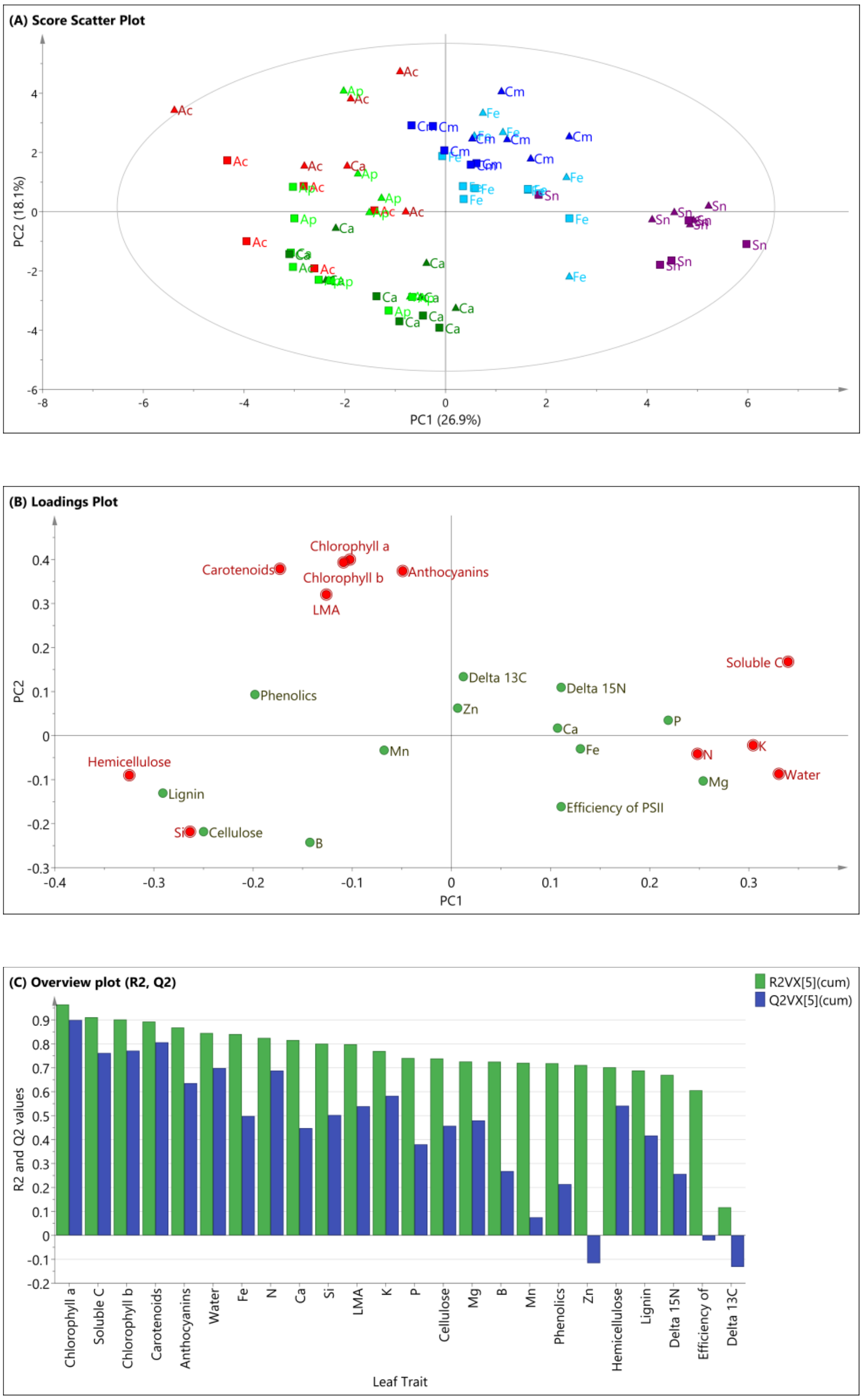

Figure 2. Principal component analysis of all leaf traits (unit variance scaled) measured across all species and sites. (A) Score scatter plot showing first and second principal components using all six species for which data exist for all 24 traits on two contrasting soil types. Colours represent species identity: Fe = *Fraxinus excelsior*; Sn = *Sambucus nigra*; Ac = *Acer campestre*; Cm = *Crataegus monogyna*; Ca = *Corylus avellana*; Ap = *Acer pseudoplatanus*. Samples from chalk sites are denoted by squares symbols and alluvium sites are denoted by triangles. (B) Loadings plot showing position and correlation of all leaf traits. Traits highlighted in red denote are those with $Q^2 > 0.5$; (C) cumulated R^2 of PCA axes 1-5 (Green bars denote how well a trait can be explained

in the model) and Q^2 (Blue bars denote how well a trait can be predicted) values for each trait. The traits are in descending R^2 order of how well they correlate with the other traits in the data set.

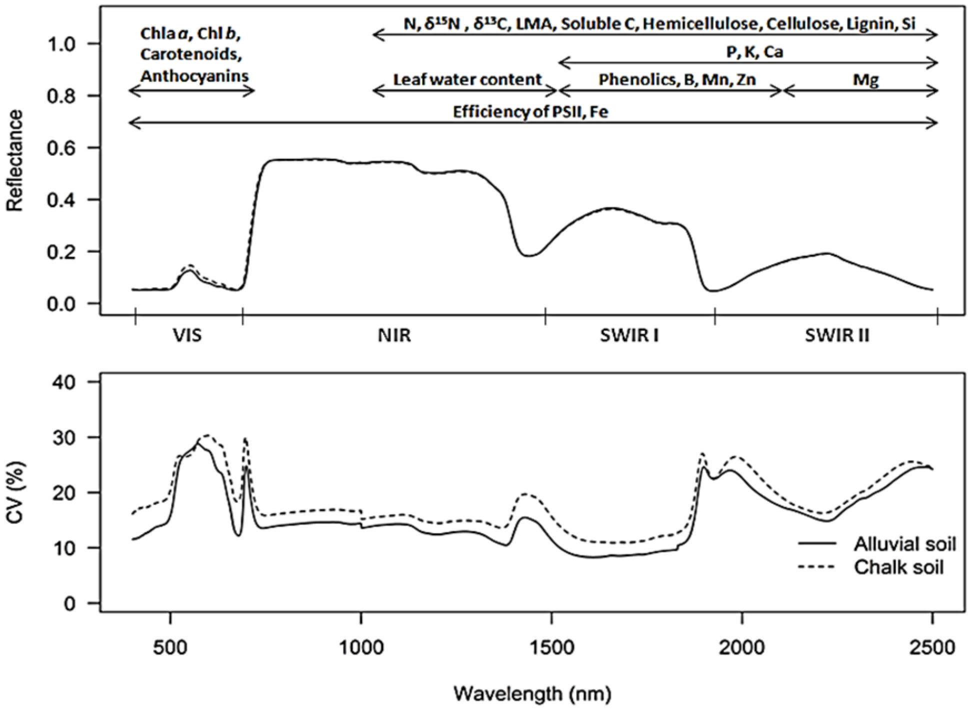

Figure 3. Spectral reflectance and percentage coefficient of variation (CV) of reflectance of six generalists species for alluvial and chalk soils. The spectral regions for each trait were selected based on the model that minimised RMSE.

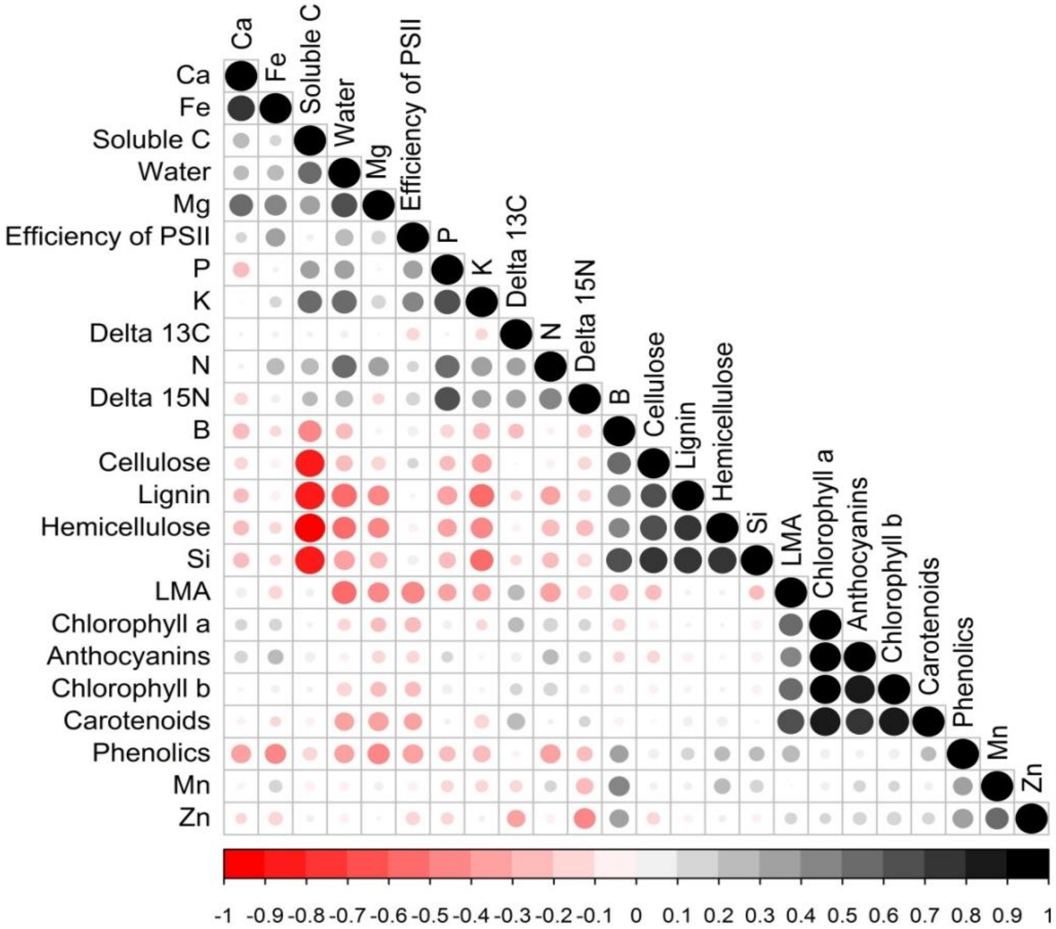

Figure 4. Spearman correlation rank test among leaf traits of 6 species growing on both soil types. Red and black circles mean, respectively, negative and positive correlations. Foliar traits were organised using cluster analysis.

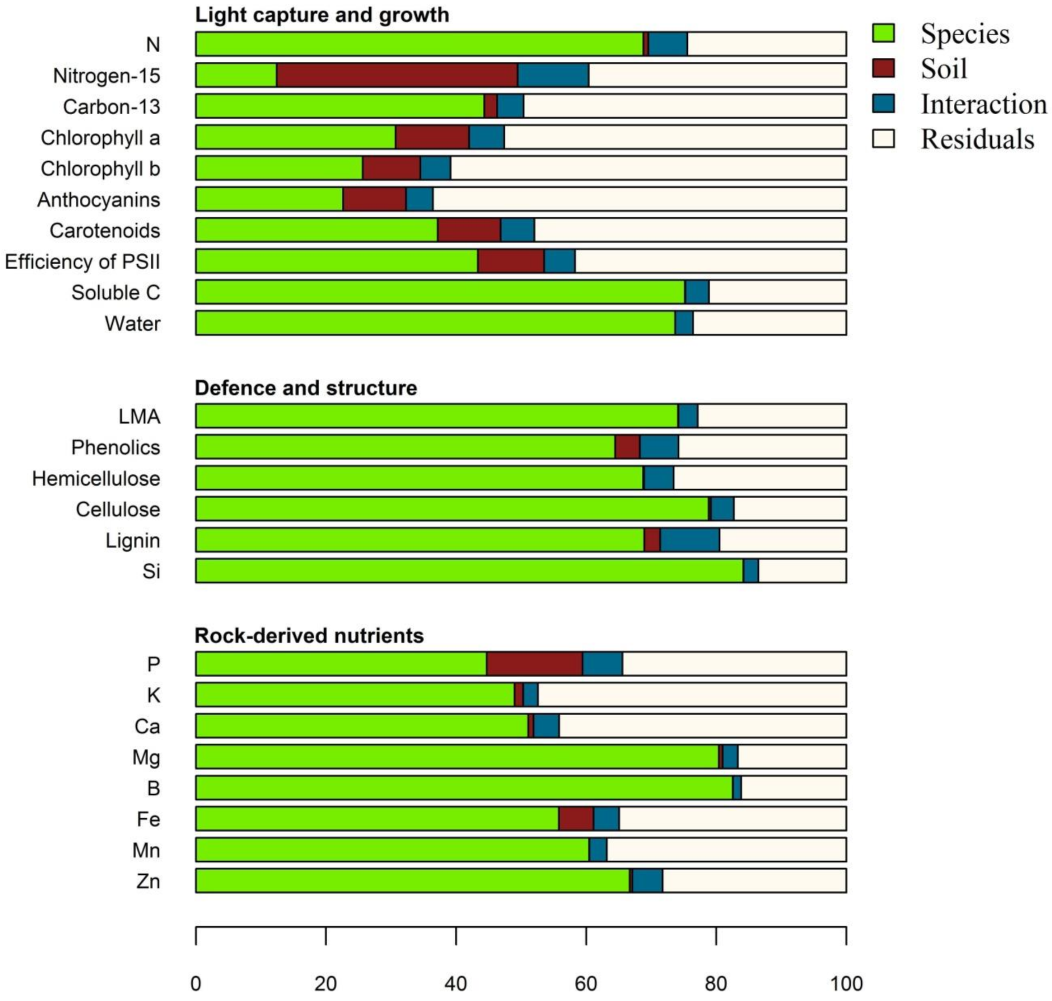

Figure 5. Partitioning of variance of foliar traits between species, soil, species-soil interaction and residual components for six generalist species found on both chalk and alluvial soils from predicted data. Residual variation arises from within-site intraspecific variation, micro-site variability, canopy selection but not measurement error variance, and is therefore smaller than for field measurements (Fig. 1). Predicted data were obtained from partial least square regression (PLSR).

Table 1. Average, standard deviation (SD) and coefficient of variation (CV) in percentage for leaf traits of six generalist species growing on alluvial and chalk soils. Foliar trait was statistically different between soil types with *P*-value < 0.05 *, < 0.01 ** and < 0.001 ***. Note that water content and the concentrations of defence and structure compounds are invariant of soil type, as this is key to understanding why variation in elemental concentrations between soil types cannot be predicted indirectly by “constellation effects”.

Traits	**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
δ^{15}N (‰) ***	3.43 ± 2.65	77.3	-3.83 ± 2.01	52.3
δ^{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
Leaf water content (%)	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

+log transformed prior to ANOVA.

Table2. Partial Least Squares Regression (PLSR) on spectral data and leave-one-out cross-validation for 24 leaf traits of 6 species occurring on both alluvial and chalk soils. The model calibration (indicated with subscript cal) and validation (indicated as subscript val) performance was evaluated for each leaf trait by calculating the coefficient of determination (R^2), root mean square error (RMSE) and the percentage root mean square error (%) based on the given number of latent variables (nL) for each PLS model.

Leaf trait	**Spectral 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
δ^{15}N (‰)	1100 – 2500	9	0.41	0.16	3.28	4.01	23.5	28.7
δ^{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
Leaf water content (%)	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

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