



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 functional plant 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 interspecific 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 concentrations 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 to 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), while all other traits were best modelled using reflectance data from the shortwave infrared region (1100–2500 nm). 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 covarying 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.

1 Introduction

There is currently great interest in using plant traits to understand the influence of environmental filtering and species identity on the functioning of plant communities and to model community responses to the 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 interspecific and intersite variation may reflect both genetic variation and phenotypic plasticity in response to environment (Davey et al., 2009; Sultan, 2001; Donohue et al., 2005). Despite sub-

stantial 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 trade-offs among these traits are often interpreted in terms of the life histories 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, 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 and Dintzis, 1981), soluble C compounds and leaf water content, C isotope discrimination ($\delta^{13}\text{C}$), N isotope discrimination ($\delta^{15}\text{N}$); (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 and Boland, 2012), and LMA, a primary axis of specialisation 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); 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 focused 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 hyperspectroscopy 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 and Martin, 2016b; Jetz et al., 2016; Asner et al., 2017). Plans to put hyperspectral sensors into space (e.g. the German Aerospace Center, DLR, plans to launch the Environmental Mapping and Analysis Program, 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* us-

ing 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 and 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 and Gamon, 2002; Wu et al., 2016). The ability of spectroscopy to measure intraspecific variation in multiple 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- and 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 as follows: (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 2 h. For each branch, 10 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. 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 fibre analyser (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, 649 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 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 (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–Ciocalteu 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 were 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 to 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 midpoint between the main vein and the leaf edge, approximately halfway between the petiole and leaf tip, with the abaxial surface pointing towards the probe. The readings were calibrated against a Spectralon white reference every five 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). 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 influence of species identity and soil type on each of the 24 leaf traits. Species, soil and soil \times 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 and between soil types, the interaction between soil and species and the unexplained variance (resid-

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

^a log-transformed prior to ANOVA.

ual variance). The residual variance comprises analytical error and various types of intraspecific variation including microsite 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 soil–species interaction 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 (10 000 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 data set. 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 denotes how well a trait can be predicted from the data set. 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 and 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 infrared (NIR, 700–1500 nm) and shortwave infrared I (SWIR I, 1500–1900 nm), shortwave infrared II (SWIR II, 1900–2500 nm) regions, as well as combinations of the regions (700–1100, 700–1900, 700–2500, 1100–1500, 1100–1900, 1100–2500, 1500–2500 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 the methods section).

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 than on chalk soils (Table 1). Nitrogen isotope discrimination ($\delta^{15}\text{N}$) 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 op-

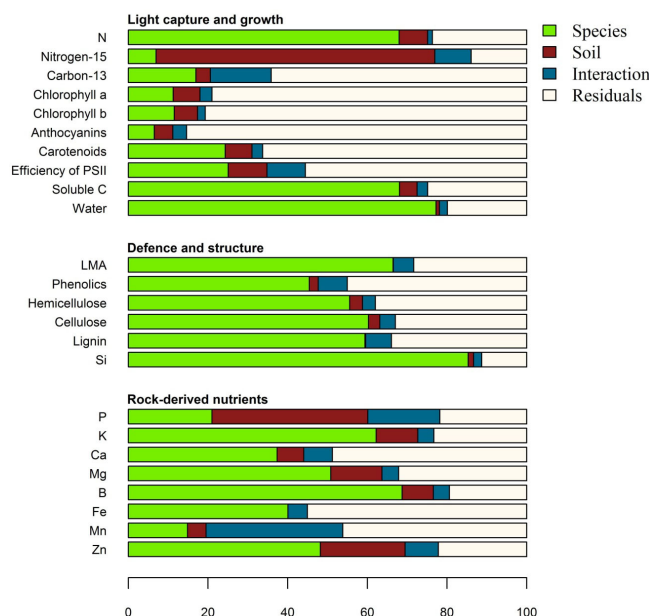


Figure 1. Partitioning of variance of foliar traits between species, soil, species–soil interaction and residual components for six generalist species found on both chalk and alluvial soils. Residual variation arises from within-site intraspecific variation, microsite variability, canopy selection and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

posite 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 ($\delta^{13}\text{C}$ 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, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, P, Ca or Mn concentrations. The interactions between species and soil (blue bars, Fig. 1) explained little variation and were significant for $\delta^{15}\text{N}$, P, Mn and Zn, but for no other traits. The pigments, efficiency of PSII and $\delta^{13}\text{C}$ had the largest unexplained variance. PERMANOVA analyses showed that, overall, species identity accounted for 25 % of the variation in leaf traits and soil type accounted for 5 %, while the interaction between

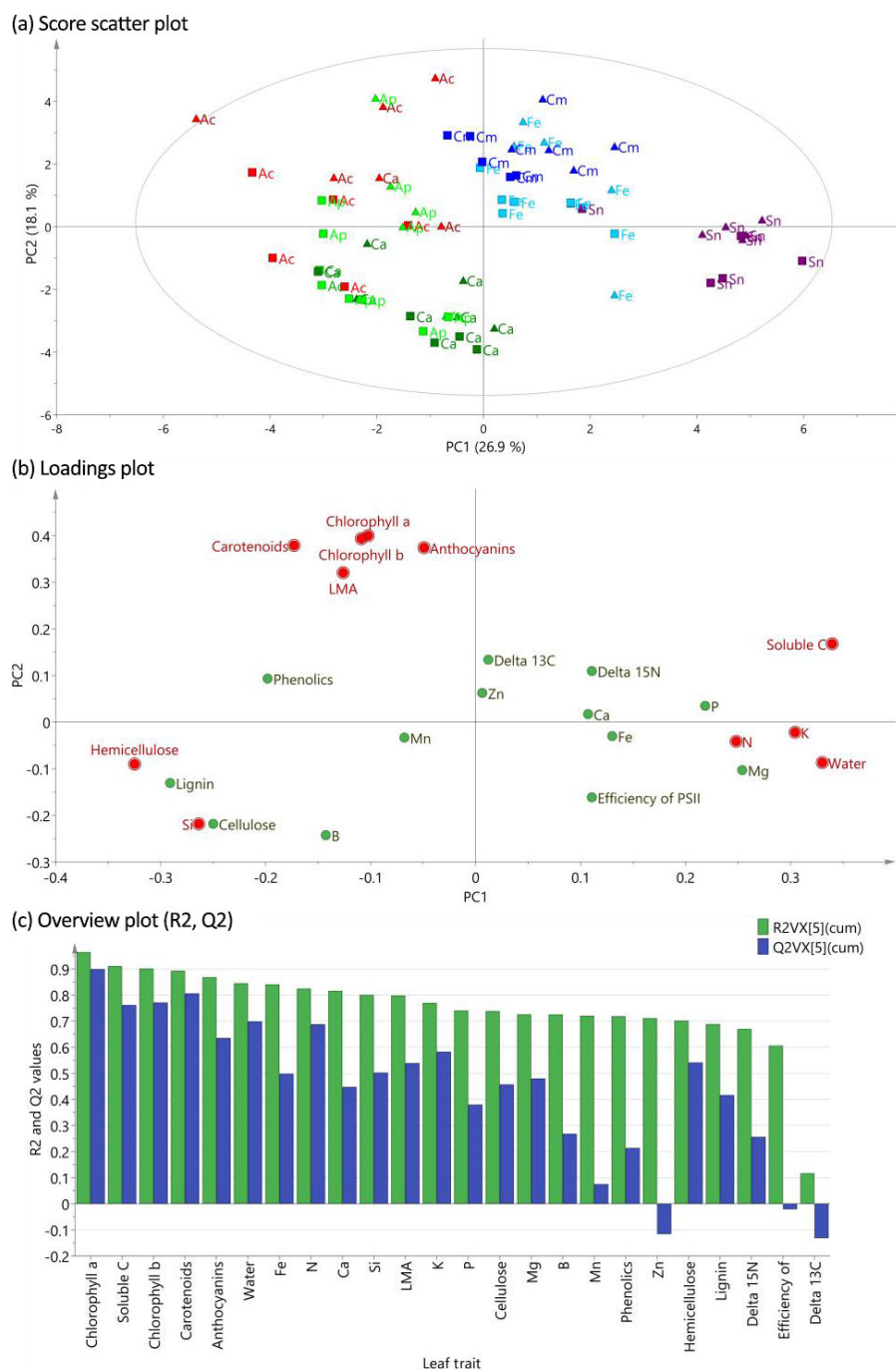


Figure 2. Principal component analysis of all leaf traits (unit variance scaled) measured across all species and sites. **(a)** Score scatter plot showing first and second principal components using all six species for which data exist for all 24 traits on two contrasting soil types. Colours represent species identity: Fe is *Fraxinus excelsior*, Sn is *Sambucus nigra*, Ac = *Acer campestre*, Cm is *Crataegus monogyna*, Ca is *Corylus avellana*, Ap is *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.

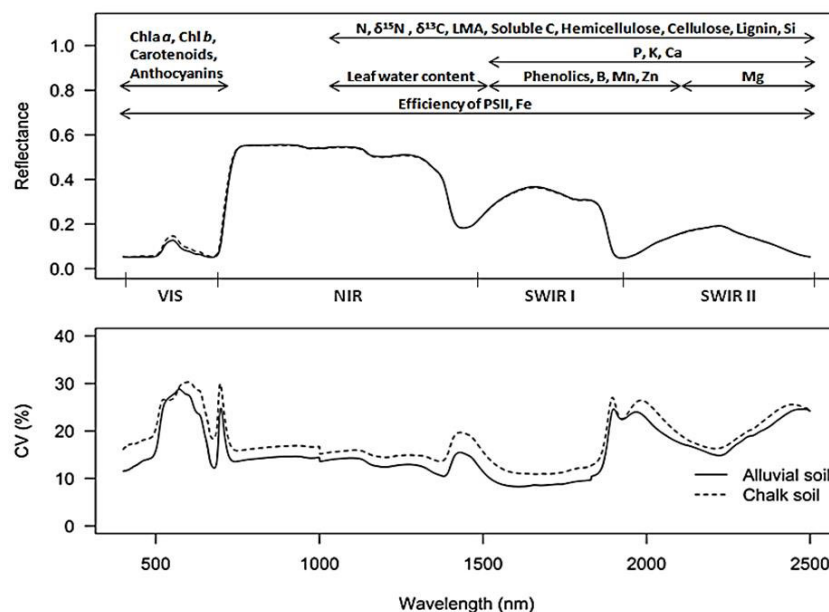


Figure 3. Spectral reflectance and percentage coefficient of variation (CV) of reflectance of six generalists species for alluvial and chalk soils. The spectral regions for each trait were selected based on the model that minimised RMSE.

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 components 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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, while 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 does 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 Dana 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

Table 2. Partial least squares regression (PLSR) on spectral data and leave-one-out cross-validation for 24 leaf traits of six 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	R^2		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
$\delta^{15}\text{N}$ (‰)	1100–2500	9	0.41	0.16	3.28	4.01	23.5	28.7
$\delta^{13}\text{C}$ (‰)	1100–2500	6	0.46	0.30	0.85	0.96	16.1	18.2
*Chlorophyll <i>a</i> (mg m ^{−2})	400–700	7	0.65	0.53	60.05	69.62	13.5	15.7
Chlorophyll <i>b</i> (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.

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 underpredicted the importance of soil ($\sim 37\%$) on the $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$). PLSR models were better able to detect intraspecific 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 and Tyler, 2000; Tyler, 2002; Sardans and 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

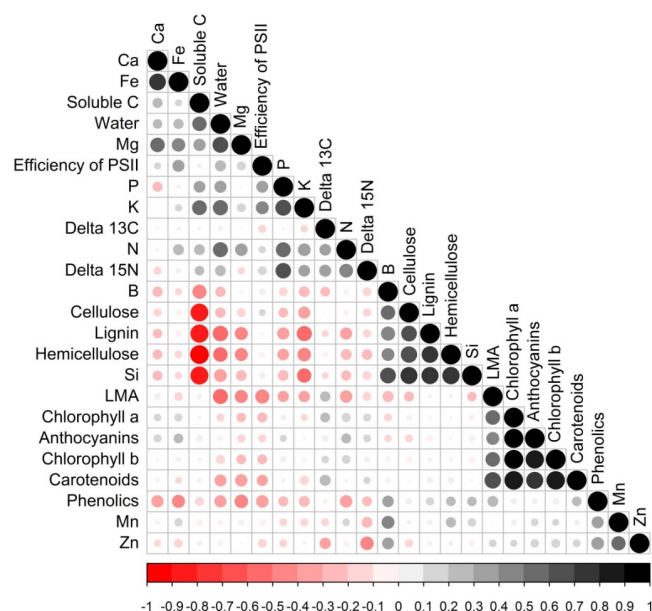


Figure 4. Spearman correlation rank test among leaf traits of six species growing on both soil types. Red and black circles mean, respectively, negative and positive correlations. Foliar traits were organised using cluster analysis.

to be a consequence of phosphorus deficiency (dos 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 or phosphorus-depleted marine terraces (Wright et al., 2010) and in several other studies (Koricheva et al., 1998; Boege and 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 concentrations 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 resource-rich soil environments, while traits favouring resource conservation are considered advantageous in low-resource environments (Aerts and 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 and Yamaji, 2006). Previous studies have also shown Si to be under strong phylogenetic control, and

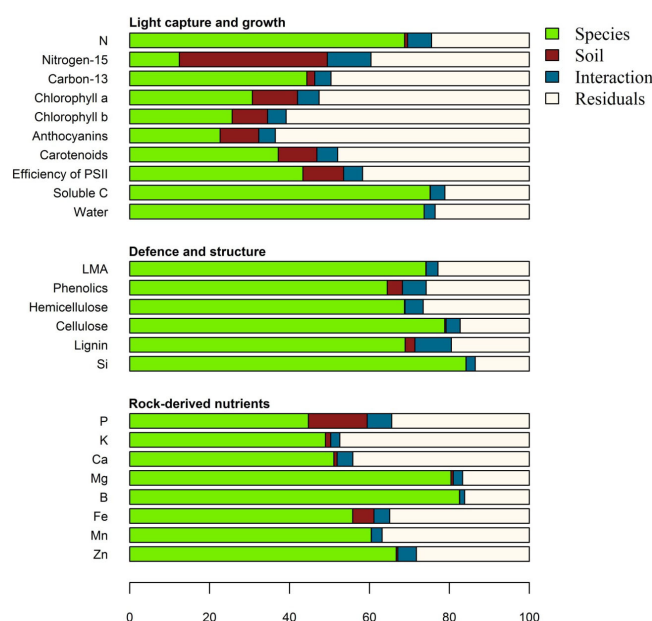


Figure 5. Partitioning of variance of foliar traits between species, soil, species–soil interaction and residual components for six generalist species found on both chalk and alluvial soils from predicted data. Residual variation arises from within-site intraspecific variation, microsite variability, 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).

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 organisation at the species level corroborates earlier analyses that showed more than 70 % of Zn variation occurred within a 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 and 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 that foliar concentrations of P and other rock-derived minerals varied strongly with site, while nitrogen concentrations varied little; soluble carbon, structural and defensive traits hardly varied between sites; and pigment (in their case just chlorophyll) was the least predictable of traits, probably because photosynthesis is rapidly up- and downregulated in response to light in the environment among other factors (Asner and Martin, 2011). Similarly, $\delta^{13}\text{C}$ is known to vary strongly with light and relative humidity (Buchmann et al., 1997; Yan et al., 2012),

which may be 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 (Knippling, 1970; Curran, 1989; Elvidge, 1990; Fourty and 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 pigment concentrations and contributed to the predictions of the efficiency of PSII and Fe only, whereas the infrared region was associated with the most traits. The region of importance with correlated wavelengths with nitrogen varies between 1192 nm in deciduous forest (Bolster et al., 1996) and 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 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 phytol 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 and 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 and Porter, 1991), but have also been predicted using bands between 1100 and 1230 nm (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 different citrus leaves with the 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, which 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. LMA is consistently among the more accurately predicted traits using spectroscopy (Asner and Martin, 2008; Serbin et al., 2014; Chavana-Bryant et al., 2016), but is measured indirectly via its close coupling with water content and structural traits of leaves (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), having been 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 and 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 (Homolova et al., 2013) and covariance with other traits is weak, making constellation effects unreliable. While a few spectroscopy studies have modelled P with some success, the spectral bands chosen differ among studies (Homolova et al., 2013), suggesting that constellation effects cannot be relied upon.

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

Rock-derived nutrients lack absorption features in the 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 covary with organic molecules that do have strong absorption features (constellation effects). 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 was approved by the DLR, with launch planned for mid-2018; Guanter et al., 2015) will soon enable vegetation and ecosystem function to be characterised at the 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 and 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 lower 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.

Data availability. The data has become publicly available through deposit to NERC's Environmental Information Data Centre (EIDC; Coomes et al., 2017).

Author 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 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.

Competing interests. The authors declare that they have no conflict of interest.

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