Dear Dr Bahn,

Thank you for your email regarding manuscript *Leaf trait variation and field spectroscopy of generalist tree species on contrasting soil types.* We are grateful to reviewers for their very thorough review. We were pleased that referee #1 regarded the manuscript as “a well written, interesting paper” based on “a solid analysis” which “asks a relevant question of interest to the readers of this journal”. However, the review proceeded to identify many typographical errors and points that needs clarification. Referee #2 explained that they were unable to understand two of our analyses and the associated figures.

We have revised the text carefully following the reviewers’ suggestions. Both referees criticised our choice to focus the discussion on the spectroscopy of foliar silicon; we now present a much broader perspective on the uses and limitations of field spectroscopy for detecting multiple traits. We have also sought to emphasize the key points of the paper by replacing two figures that the referees found difficult to understand with much simpler figures that convey the same message. As requested by reviewer #2, we have included a figure that shows the reflectance spectra along with an indication of the regions relevant to estimation of different leaf traits (Figure 1) and have removed the sections on functional groupings. Also, as requested by reviewer #2, P supply limitation was better discussed, as well as the soil and species effects on traits.

We now focus the paper around one issue: the challenges of measuring intraspecific variation in some leaf traits using field spectroscopy. 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 because element concentrations co-vary with organic molecules that do have strong absorption features (“constellation effects”). Our 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 (used to indirectly estimate the mineral nutrient concentrations) 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.

You requested major revision of the manuscript. These revisions have resulted in many changes to the text, as you will note in the track-changed document following the responses to the reviewers on the same document. However, the underlying analyses are unchanged. We thank the referees again for their insightful comments, and hope they find our revisions satisfactory.

Yours sincerely

Matheus Henrique Nunes and David Coomes.

Response to Anonymous Referee #1’s comments

Received and published: 07 December 2016

General Comments :

Referee comment: This is a well written, interesting paper that attempts to use leaf spectroscopy to predict leaf traits in two contrasting soil types. They found that traits tended to be specific to species and that soil type had much less of an influence. They used the PLSR methodology to predict traits with spectroscopy and found reasonably good relationships which reflect previous studies. Overall, this is a solid analysis and asks a relevant question of interest to the readers of this journal.

Author response: We thank the referee for these positive comments.

Referee comment: Below I suggest a few areas where the paper could be strengthened and a number of minor points. **Specific comments:**

Referee comment: 1) The Material and Methods ‘Statistical Analyses’ section needs to be much expanded and clarified. Especially in regards to Figures 3 and 4. Without knowing how the data for those sections were acquired, it is difficult to evaluate the claims made in the results and discussion section.

Author response: We have removed the former figure 4 and replaced it with a new figure that can be more easily interpreted (current Figure 5). We also have expanded the text in the methods section to clarify how we acquired and analysed the data. In particular, we have added the following sentence to clarify the methods used to construct the correlation matrix graphic: “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.”

We have provided a clearer explanation for Figure 5: “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).”

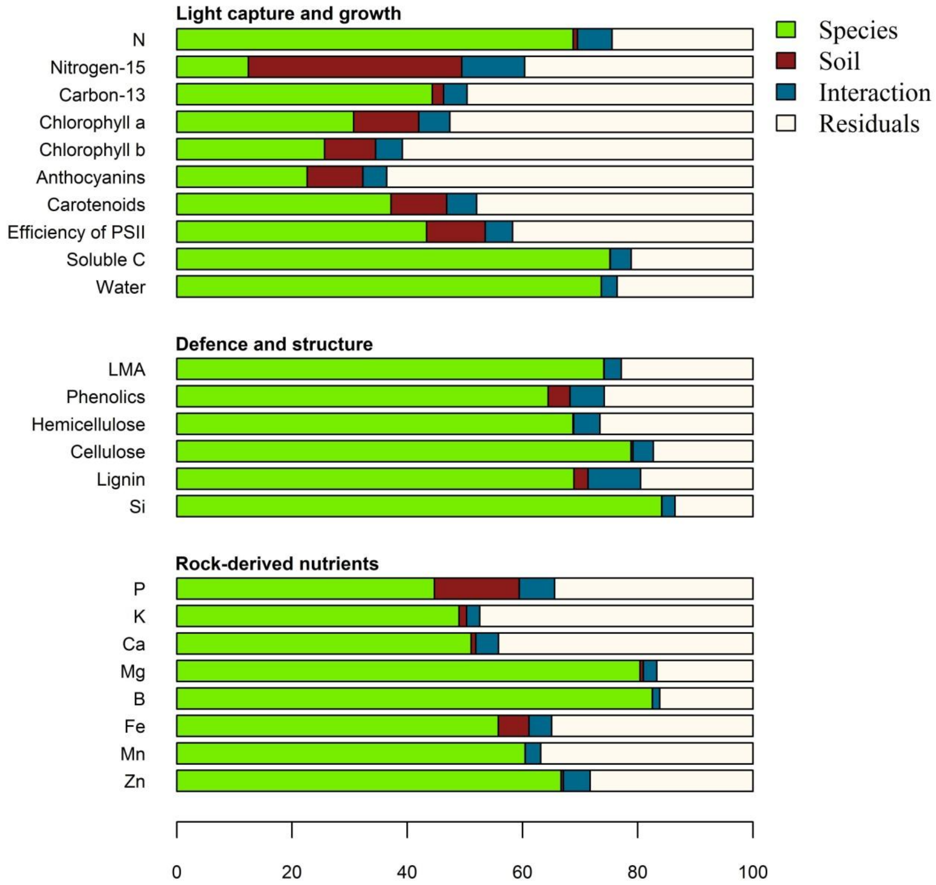

Figure 5. Partitioning of variance of foliar traits between species, soil, species-soil interaction and residual components for six generalist species found on both chalk and alluvial soils from predicted data. Residual variation arises from within-site intraspecific variation, micro-site variability,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

Referee comment: 2) Some of the findings discussed in the abstract need to be made clearer.

Author response: We have added more references in the discussion that support our findings. Particularly, we have substantially improved the “Variation in traits between chalkland vs alluvial soils” section, by discussing how P deficiency could be associated with variation in leaf traits. Furthermore, we have improved the “Inter-specific and residual variation” section in the discussion.

Referee comment: 3) Some of the claims/statements made in the abstract and intro either need to be changed or better supported with relevant literature.

Author response: We removed two questions that were initially part of the paper. We have decided that rather than force our traits into the three functional groups, we should run a single PCA to discover how the traits were related to each other, and to both species identity and soil type. Furthermore, we agree that the fourth question was similar to question 3, and have merged both questions into a single one: “is field spectroscopy effective at predicting phenotypic variation in leaf traits between soil types, as well as interspecific differences?”. We changed some claims and statements in both introduction and discussion as highlighted by the referees.

Referee comment: 4) Many frequently used terms throughout the paper need to be changed/clarified (see below).

Author response: Many thanks for the suggestions on terminologies. It was done throughout the document, see comments below in minor amendments.

Referee comment: 5) Why there is such an emphasis on being able to predict Si using PLSR throughout the paper needs to be clarified!

Author response: We have reduced the discussion on Si and broadened the review out to include other traits.

Referee comment: 6) Discuss more clearly the relevance of the findings in terms of future high resolution aircraft campaigns. Based on these results, what sort of aircraft data could be produced for temperate forests.

Author response: We have added as the last section of the discussion the “Difficulties in measuring phenotypic variation by field spectroscopy and its implications for mapping functional traits”. We have included some considerations on the relevance of our findings in terms of high resolution aircraft campaigns.

Referee comment: Technical Corrections Terminology.

Change uses of “among species’ to “between species” (if that is what is meant).

Change uses “species x soil interaction” to “species-soil interaction” or something similar.

Change uses of “goodness-of-fit” to “strength of relationships” or something similar. Change uses of “leaf property” to “leaf trait”.

Line 58 – typo. Change “include phosphorous” to “including phosphorus”.

Line 84 – “leaf property”. Replace with “leaf trait”?

Line 108: “Leaf areas were measured”. Suggested “Leaf area was measured?”

Line 169: “strong co-llinearity”. Typo.

Line 326: “relative”. Typo (relatively)

Line 357: “A review in the literature”. “A review of the literature”

Author response: Thank you for pointing out these issues. We have made the corrections requested.

Referee comment: Abstract/summary Line 10 – change “traits variation” to “trait variation”

Author response: We now use the terms “trait variation” and “variation in traits”.

Referee comment: Line 12 – “Hyperspectroscopy is a recently developed technology for estimating the traits of fresh leaves” – disagree (the technique dates back to the 90s – e.g. Curran, 1989)

Author response: The claim was wrong indeed. We have replaced it with a sentence highlighting the importance of hyperspectroscopy for vegetation science. “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 (G. P. Asner et al., 2017; Gregory P. Asner & Martin, 2016b; Jetz et al., 2016; Ustin et al., 2009). 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.”.

Referee comment: Line 13 – “Few studies have evaluated its potential for assessing inter- and intraspecific trait variability in community ecology” – This is a contentious claim given a lot of Asner’s work (e.g. Asner and Martin, 2011). This statement is not supported in the introduction.

Author response: We agree that the Asner’s team have published several papers on this topic for temperate forests. But we argue on the text that analyses involving this large suite of traits provide optimism to develop general, predictive rules in community ecology as we refine our understanding of which traits are varying in a given environment. There is also a need for broader testing of the mechanisms underlying interspecific variation in phenotypic plasticity across traits and environmental variables (e.g. Weiner, 2004; Funk et al., 2016) and how this variation ultimately informs species and community responses to environmental change (Funk et al., 2016).

Referee comment: Line 14 – “Working with 24 leaf traits”. Contradicted by line 151 which mentions 26 leaf traits.

Author response: The number is 24 and we have altered the text accordingly.

Referee comment: Line 19 - “(iv) Can leaf spectra be used to detect inter-soil as well as inter-specific variation in traits?” – I don’t understand how this question differs from iii (“what traits can be estimated precisely using field spectroscopy?”). If you can precisely estimate a trait using field spectroscopy, then surely it will be able to detect inter-soil and inter-specific variations. Unless the estimation only works on one species type on one particular soil type. Maybe rephrase?

Author response: We agree with you. We have rephrased and merged both questions into a single one: “is field spectroscopy effective at predicting phenotypic variation in leaf traits between soil types, as well as interspecific differences?”.

Referee comment: Line 20 – “The contribution of species and soil-type effects to variation in traits were evaluated using statistical analyses” – maybe state a few of the main statistical analyses used?

Author response: Thanks for the comment. We agree that it should have been better explained in the abstract. We changed it to “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 Ltd, 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, Hoefsloot, Westerhuis, Smilde, & van der Werf, 2006) to avoid the effects of variables with high variance. The PCA was used to obtain score scatter, loadings, as well as R2 and Q2 overview plots to evaluate whether traits clustered into functional groups. R2 values denote how well a trait can be explained in the model and Q2 denote how well a trait can be predicted from the dataset.”

Referee comment: Line 21 – “Foliar traits were predicted from spectral reflectance using partial least square regression, and so inter- and intra-specific variation.” – Presumed typo – rewrite.

Author response: We have changed the text to: Foliar traits were predicted from spectral reflectance data using partial least square regression.

Referee comment: Line 22 – “Most leaf traits varied greatly among species” – a) replace ‘among species’ with either within or between species (presumed between?) b) Also this sentence is confusing – suggests that there was simply a wide variation in leaf trait measurements - slightly random to mention in abstract. Actual meaning I think is something along these lines “Inter-specific variation was the largest contributor to trait variation”.

Author response: We have altered the sentence to “. Analysis of variance showed that inter-specific differences in traits were generally much stronger than phenotypic differences related to soil type, accounting for 25% versus 5% of total trait variation, respectively.” in the abstract and further explained in the results.

Referee comment: Line 23 – “Macronutrient concentrations were greater on alluvial than chalk soils while micronutrient concentration showed the opposite trend” – Foliar macronutrient concentrations or soil macronutrient concentrations? (presumed the former?). Also, slightly odd sentence – what’s the significance? Maybe meant to say something along these lines? - “However, foliar macro- and micronutrient concentrations were found to be more strongly influenced by soil type”.

Author response: We have changed the text to: “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.”

Referee comment: Line 24 – “Si predictions using spectroscopy appear to be promising” – what’s so special about Si predictions?! Why do they get singled out?

Author response: It was the first time Si was reported as a trait able to be predicted using spectroscopy in forests. But we agree that it should not be singled out as Si is not the main focus for the paper. We have rephrased the sentences that were mentioning Si as a very important finding and we have reduced the amount of text on Si in the discussion.

Referee comment: Line 28 – “However, it [field spectroscopy] was unable to detect subtle within species variation of traits associated with soil type” – repetition of line 25? (“Field spectroscopy. . ..was less effective at detecting subtle variation of rock-derived nutrients between soil types”). Combine sentences to keep abstract concise?

Author response: We have rephrased it and considerably expanded the text on the lack of detection of subtle variation of rock-derived nutrients due to soil variation as follows: “Some of most accurately predicted traits have no absorption features in the visible-to-near-infrared, but were instead estimated indirectly via constellation effects. 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.”

Introduction

Referee comment: Line 64 – “along environmental change”. Typo. Suggested “along environmental gradients”?

Author response: We have changed the text to “In response to environmental change”.

Referee comment: Line 71 – “However, spectral and chemical properties may be uncoupled if intraspecific variation in foliar traits is high and/or phenotypic plasticity exceeds phylogenetic patterns among leaf properties”. Disagree. Spectral and chemical relationships would still hold, it would just be harder to identify species type based on their reflectance signatures.

Author response: We agree with you. We found references supporting your statement: “Wu et al., (2016) found that leaf traits and spectra vary systematically and predictably with leaf age between forest sites on contrasting soil types thousands of kilometers apart.” Madritch et al. (2014) also demonstrated the high correlation between spectral properties, traits and soil very well.

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 phenotypic variation in multiples traits between soil types, particularly when some of those traits are indirectly determined through constellation effects, has not been critically evaluated.

Madritch, M.D., Kingdon, C.C., Singh, A., Mock, K.E., Lindroth, R.L. and Townsend, P.A., 2014. Imaging spectroscopy links aspen genotype with below-ground processes at landscape scales. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *369*(1643), p.20130194.

Wu, J., Chavana-Bryant, C., Prohaska, N., Serbin, S.P., Guan, K., Albert, L.P., Yang, X., Leeuwen, W.J., Garnello, A.J., Martins, G. and Malhi, Y., 2016. Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests. *New Phytologist*.

Referee comment: Line 73 – “Martin and Aber (1996) demonstrated that equations for estimating leaf properties from one site were unable to predict leaf properties for other sites, due to variability in the magnitudes of foliar traits levels between data sets and environmental influences”. Very old reference and what about all the evidence to the contrary (e.g. all of Asner’s work) ???

Author response: As per previous comment, we have deleted that reference. We also agree that we can find high correlation between spectral properties, traits and soil (see previous comment).

Referee comment: Line 75 – "To our knowledge, the link between foliar traits and spectral properties of trees has not been broadly demonstrated for temperate forests" – query this statement. The remote sensing of foliar traits began in temperate forests.

Author response: We are no longer making this statement, which can be proved wrong indeed. "There is currently great interest in hyperspectroscopy in vegetation science, particularly because improved airborne sensors and faster computing make it possible to map functional traits from the air (Asner and Martin, 2016; Jetz et al., 2016; Asner et al., 2017)". We have slightly changed the focus of the paper. "The ability of spectroscopy to measure phenotypic variation in multiples traits between soil types, particularly when some of those traits are indirectly determined through constellation effects, has not been critically evaluated."

Referee comment: Line 86 – "what is the relative contribution of soil type and species to leaf trait variation?". Missed word? "what is the relative contribution of soil type and species type to leaf trait variation".

Author response: We have included the word species identity.

Referee comment: Line 88 – "does the importance of the three functional groups change due to soil or more due to species variation?" – awkward phrasing. Rephrase.

Author response: We have rephrased all the sentences involving functional groups.

Material and Methods

Referee comment: Line 102: "Leaves of 66 trees of six species were collected from the two contrasting soil types. The six species were in common to both sites". Suggested "Across both sites, leaves were collected from 66 trees, representing six species. The six species common to both sites were:"

Author response: Many thanks. We have made this amendment.

Referee comment: Line 103: "Acer campestre L. (Field Maple)" – what does the L stand for?

Author response: L. is the authority - the person who named the species formally. In this case Linnaeus, who back in the 1700s invented the Latin binomial system for naming species that is still used today. Some biology journals insist on including these. We have removed them from this paper

Referee comment: Line 105: “Two fully sunlit branches were selected, were cut and placed on ice in a cool box, and transported to a lab for processing within 2 hours (and often within 30 minutes)”.

Author response: We have changed the text to: Two fully sunlit branches were selected, cut and placed in a cool box, and subsequently transported to a laboratory for processing within two hours.

Referee comment: Line 149: “2.4 Statistical analyses”. Needs to be split up into each statistical analysis performed and titled accordingly.

Author response: This has been carried out as requested.

Referee comment: Line 156: “Where necessary, variables were log transformed to meet assumptions of ANOVA”.

Author response: Table 1 has additional information concerning which variables were log transformed and how they can be found.

Referee comment: Line 168: PLSR section – no mention of using 70% to calibrate and 30% to test but Cal and Val appear on Table 3. No mention of how the data for Figure 3 and 4 is acquired!!!

Author response: We added the following sentences to the text to make it clearer: “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.”

Regarding Figure 3: We have rephrased the text to: “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.”

Regarding Figure 4: Picture 4 no longer exists as previously explained.

Results

Referee comment: Line 204 – “Species exerted little or no influence on pigment concentrations” – Refer to species in this context (and throughout paper) as ‘species type”?

Author response: Yes, it does. We have changed this throughout the document to species identity.

Referee comment: Line 241: “Ability to predict leaf traits from hyperspectral reflectance varied greatly among the 24 traits fitted using the 6 species (Table 3)”. “fitted using the 6 species” - confusing. Rephrase.

Author response: We have removed the “fitted using the 6 species” as it did not make any sense.

Referee comment: Line 243: “PLSR modelling for LMA, water, Si, phenolics, carotenoids, K, B, efficiency of PSII, N, chlorophyll a and chlorophyll b were in descending order the best performing in terms of”

Author response: Thank you. We have corrected the text.

Referee comment: Line 248- “higher goodness-of-fit” – use a different term? E.g. stronger relationships/correlations etc.

Author response: OK – we have changed the text to higher strength of relationship.

Referee comment: Line 256: “There were strong correlations among some of the leaf properties (Fig. 3) that can be potentially leveraging the estimation of other leaf traits from the use of PLSR”. Interesting. Explain further?

Author response: We added the following sentences in the results: “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 δ15N 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 δ13C). 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.”

Referee comment: Line 257: “The correlation graphic also shows the similarity among variables through cluster analysis”. Explain. Cluster analysis was not been mentioned in the Materials and Methods. Explain how this was achieved, why it was done and expand on results.

Author response: We have made it clearer in the Material and methods as follows: “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.”

Discussion

Referee comment: Line 271: “Some leaf traits were strongly influenced by both species and soil type, while others were hardly affected by soil and only varied with species”. Vague. Make more specific.

Author response: We added many references on the P supply implications in chalk soils as follows: “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, Walton, & Wells, 1990).” And “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).”

Referee comment: Line 305: “water” – change to ‘leaf water content’.

Author response: Thank you. We have done all the corrections ad changed the term “water” to “leaf water content” throughout the text.

Referee comment: Line 321: “but their study sampled only from fully sunlit leaves”. Suggested - “Similarly, their study sampled only from fully sunlit leaves”.

Author response: Thank you. Alteration made.

Referee comment: Line 325: “The investment in light capture had high intra-specific variation, and neither species nor soil accounted for variation in [these] foliar properties”. Missing word.

Author response: We have restructured the sentence.

Referee comment: Line 327: “separating out some species”. Confusing. Rephrase?

Author response: We have improved the discussion and included the following sentence that had the same meaning: “The investment in structure and defence-related traits were little influenced by soil type and was mainly determined by species identity.” “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.”

Referee comment: Line 327: “Investment in traits related to defence and leaf structure is species-mediated, and may be separated into two defensive strategies”. State the two defensive strategies?

Author response: We improved the discussion regarding the functional grouping as follows: “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, Falster, Moles, Vesk, & Wright, 2002), but in this study the species were generalists growing on both soil types. “

Referee comment: Line 342: “Doing so revealed that. . .”. Awkward. Rephrase.

Author response: This sentence does not exist anymore after the changes throughout the discussion.

Referee comment: Line 351: “Although chlorophylls also contain nitrogen, the spectra of chlorophylls differ greatly from proteins because of their dissimilar chemical structures, showing strong absorption due to C-H bonds in the phytol tail of the molecule (Katz et al., 1966), also confirmed in this work when visualizing the regions of importance for predictions.” Require a full stop after (Katz et al. 1996) and develop last sentence (“also confirmed in this work when visualizing the regions of importance for predictions”).

Author response: We have refined the text to “Although chlorophylls also contain nitrogen, the spectra of chlorophylls differ greatly from proteins because of their dissimilar chemical structures, showing strong absorption due to C-H bonds in the phytol tail of the molecule

(Katz, Dougherty, & Boucher, 1966). That can be confirmed in this work as the visible region of the spectrum showed the best predictions of pigments.”

Referee comment: Line 360: “On the other hand, the use of spectroscopy on fresh leaves is particularly better for LMA predictions”

Author response: We have edited the paragraph, which included that specific sentence to “Leaf mass per unit area (LMA) is consistently among the more accurately predicted traits using spectroscopy (ASNER & Martin, 2008; Chavana-Bryant et al., 2016; Serbin, Singh, McNeil, Kingdon, & Townsend, 2014), but is measured indirectly via its close coupling with water content and leaf structural traits (Asner et al. 2011b).”

Referee comment: Line 365: “The use of spectroscopy for Si predictions on fresh leaves appears to be promising considering our accurate results”. Maybe, but why are Si predictions so important? What ecological function does Si perform?!

Author response: We have reduced the text on Si to avoid singling it out, as it is not a specific question on this paper.

Referee comment: Line 339: 4.4 Predictions of foliar traits using spectroscopy – this section maybe a bit long? Could condense? Says some interesting things but I’m not sure they’re all relevant to the paper.

Author response: We have condensed and restructured the entire section named “Measuring interspecific variation in leaf traits with field spectroscopy”. We have also discussed the Difficulties in measuring phenotypic variation by field spectroscopy and its implications for mapping functional traits.

Referee comment: Line 384: Consideration on the use of spectroscopy to quantify patterns of foliar traits. Typo - Consideration of the use of spectroscopy to quantify patterns of foliar traits.

Author response: Thanks. The correction was made.

Referee comment: Line 385. “The range of variation within species for most predicted traits tend to be smaller with the use of PLSR on reflectance”. Very confusing. Rephrase.

Referee comment: Line 399: “This study particularly provides findings for a large range of traits that indicate that the use of spectroscopy may be useful to quantify structural traits but can be misleading to measure the environmental filtering on traits that are indirectly predicted, such as macro- and micronutrients”. I might agree if I understood Figure 4 but, as I don’t, I query this statement.

Author response: The updated Figure 4 is Figure 5. However, we have changed to picture to one that can be easily interpreted. We have restructured the text to make the Figure 4 clearer.

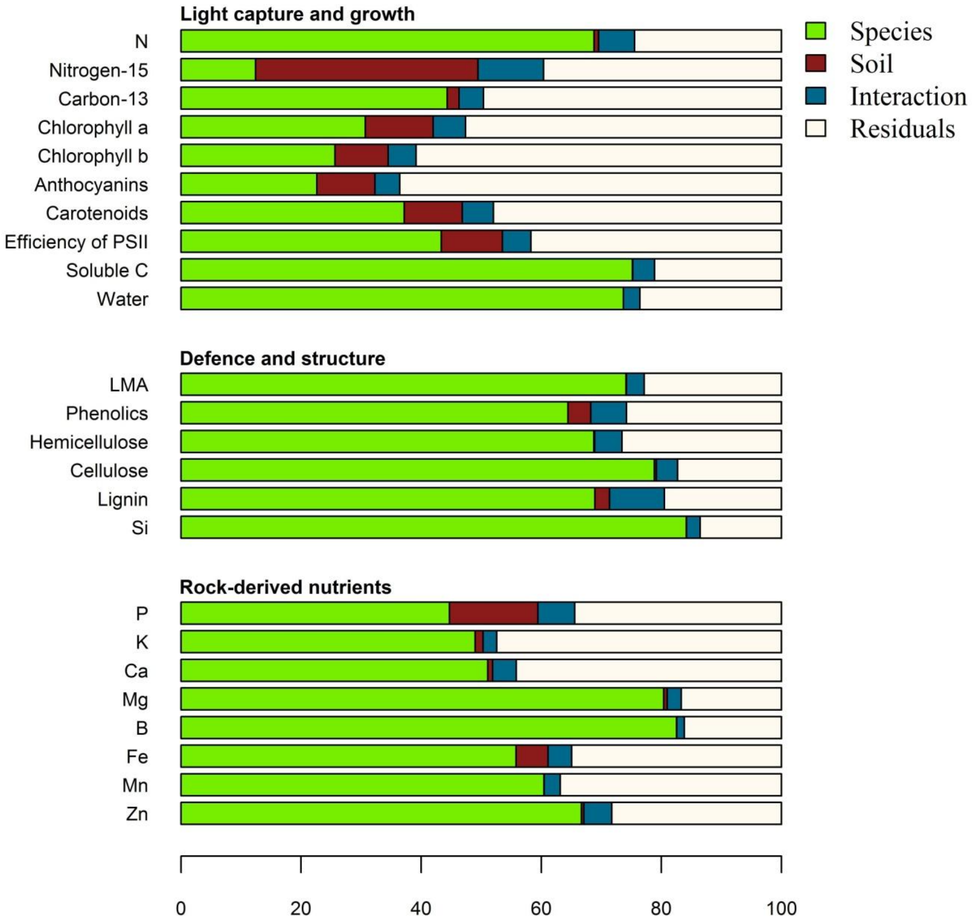

Figure 5. Partitioning of variance of foliar traits between species, soil, species-soil interaction and residual components for six generalist species found on both chalk and alluvial soils from predicted data. Residual variation arises from within-site intraspecific variation, micro-

site variability, canopy selection and measurement error variance. Predicted data were obtained from partial least square regression (PLSR).

Referee comment: Line 401: “While remote sensing is not a direct replacement of field sampling, the ability of remote sensing platforms to assess biological phenomena at large spatial scales is unparalleled”. Slightly random – doesn’t follow from previous statement/results section.

Author response: We agree that it completely disagree with previous statements and results. We are no longer including this statement on the paper.

Conclusion

Referee comment: Line 407: “rock-derived nutrients are strongly influenced by the soil characteristics”. Need to tone down or change previous sentence, otherwise statements are contradictory.

Referee comment: Line 409: “This study also demonstrates the potential for estimating foliar traits by field spectroscopy and its promising use to predict Si”. a) “demonstrates the potential” –this has already been done many times. Maybe something more along the lines of “agrees with the existing literature in demonstrating the potential. . .” b) “its promising use to predict Si”. Once again – what is so important about Si?!?!?!

Author response: We no longer have the conclusion but we decided to expand the discussion on the Difficulties in measuring phenotypic variation by field spectroscopy and its implications for mapping functional traits

Figures

Referee comment: Line 661: “Red and black circles mean negative and positive correlations”. Which way round?

Referee comment: Line 668: “The greyness and size of each dot reflects the goodness-of-fit of the PLSR for each foliar trait, with darker and bigger points representing the most accurate PLSR predictions. goodness-of-fit". Give statistical boundaries for how dots were sorted into each size/shape category.

Author response: We have the changed the figure to:

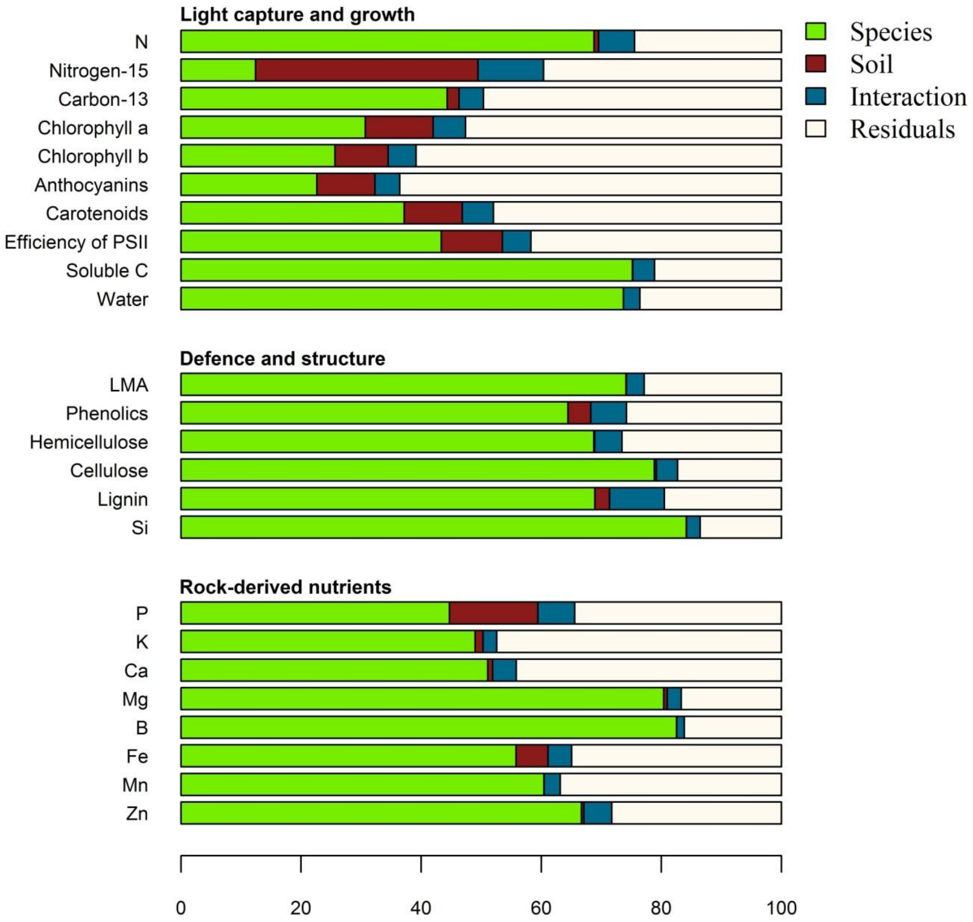

Figure 5. Partitioning of variance of foliar traits between species, soil, species-soil interaction and residual components for six generalist species found on both chalk and alluvial soils from predicted data. Residual variation arises from within-site intraspecific variation, micro-site variability, canopy selection and measurement error variance. Predicted data were obtained from partial least square regression (PLSR).

Referee comment: Perhaps add the word "…respectively" at the end to clarify which is which?

Author response: Thank you. The correction was made.

Referee comment: Line 675: Table 1. CV needs to be represented as %CV, as stated in the heading.

Author response: Thank you. It is corrected now.

Response to Anonymous Referee #2's comments

Received and published: 16 December 2016

Referee comment: The article "Leaf trait variation and field spectroscopy of generalist tree species on contrasting soil types" by Nunes and co-authors analyzed field spectroscopy data collected on different European tree species on contrasting soil types. The authors worked with 24 leaf traits and explored the following questions: What contribution do soil type and species identity make to trait variation? When traits are clustered into three functional groups (light capture and growth, leaf structure and defence, as well as rock-derived nutrients), are some groups more affected by soil than others? What traits can be estimated precisely using field spectroscopy? Can leaf spectra be used to detect inter-soil as well as inter-specific variation in traits? The authors found that most leaf traits varied greatly among species. The effects of soil type were generally weak by comparison.

Specific Comments:

Referee comment: Line 28 variation in foliar traits and Si predictions using spectroscopy appear to be promising. Not clear what Si means at this stage, it becomes clear later. But in general all the discussion on Si is poor

Author response: Firstly, we spelled out Si and all the nutrients that were presented on the paper as an acronym. We previously singled out the performance of Si as a promising result but its performance should not be the main focus of the manuscript. We have reduced the discussion on Si and broadened the review out to include other traits.

Referee comment: Line 162 We recognize that grouping leaf properties into functional classes can be controversial, given that a single leaf property can contribute to. This is particularly true for P, this assumption has to be justified as foliar P can be easily considered a trait associated to growth.

Author response: 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; Aubin, Ouellette, Legendre, Messier, & Bouchard, 2009; Fry, Power, & Manning, 2014; Pillar, Sosinski, & Lepš, 2003). In this study we measured 24 traits which we organise into three functional groups (Gregory P. Asner et al., 2015; Gregory Pa Asner, 2014). 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). Leaf traits were grouped using principal component analysis (PCA) using Simca-P (2016) software (Umetrics Ltd, 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, loadings, as well as R2 and Q2 overview plots to evaluate whether traits clustered into functional groups. R2 values denote how well a trait can be explained in the model and Q2 denote how well a trait can be predicted from the dataset.

Results Section Spectroscopy of leaf properties

Referee comment: The results of PLSR are on one hand encouraging because the portion of spectra selected for specific traits are in line with what expected from the literature. Some examples from the article: 1) higher goodness-of-fit were obtained for K, Ca and P in the SWIR regions. 2) Pigments were the only traits that predictions were more accurate when using the visible region (400 – 700 nm)

Author response: Many thanks. We thought this encouraging too.

Referee comment: I think would be useful to have more discussion on what is known and what is new compared for instance to the review from Homolova et al., which discuss many of the traits mentioned by the authors and how these traits can be predicted from remote sensing data. What do we learn from these results? I think the authors should make an effort to improve this aspect because can be quite relevant considering the great dataset they have. For example a figure with a reflectance spectra with an indication of the regions relevant to estimate other the traits indicated might be useful for the reader.

Author response: I agree that it would be interesting to have a figure with an average reflectance spectrum indicating the relevant regions for each trait, as per Figure 1. We included the Coefficient of variation (%) and the average reflectance with the regions partitioning indicating which part of the spectrum is more suitable for each trait. There are amendments in the Material and Methods, as well as Results sections on the graphic.

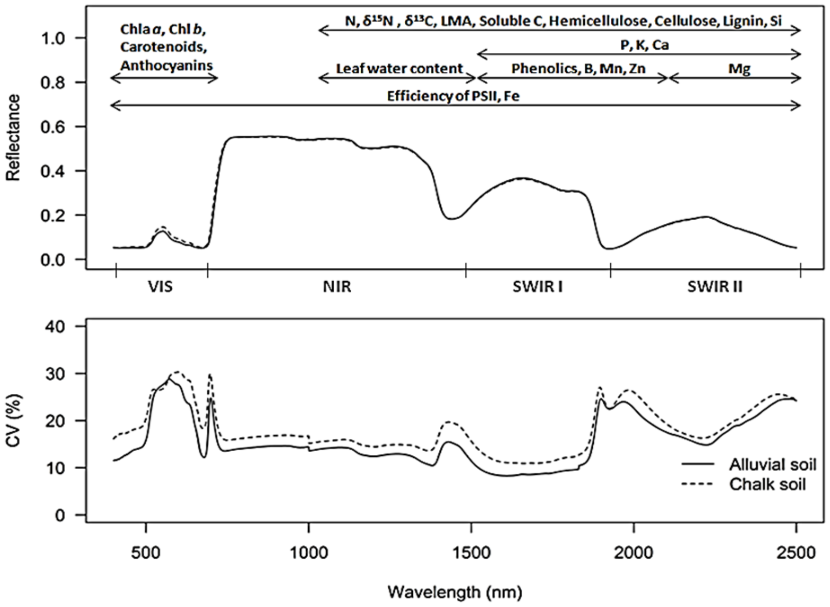

Figure 3. Spectral reflectance and coefficient of variation (% CV) of reflectance of six generalists species for alluvial and chalk soils. The spectral regions for each trait were selected based on the model that minimised RMSE.

Referee comment: Line 267 The species x soil interaction effects were detected by PLSR modelling, except for traits that showed strong interaction (Mn, P and δ 13C). This should be better discussed

Author response: We have improved considerably the discussion on the ability of field spectroscopy to predict trait variation. The following paragraph was added to the results: “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 δ15N 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 δ13C). 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. ”.

And this to the discussion: “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.

What are the 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 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). On the other hand, 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, Reich, Zytkowiak, Karolewski, & Tjoelker, 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 urges caution in interpreting results when species range across contrasting soil types. ”

Referee comment: Line 279 Our findings that trees growing on the chalk soils had relatively low concentrations of N, P and K in their leaves, and relatively high concentrations of Ca, Mg, B, Mn, Si and Zn, is consistent with previous analyses of mineral nutrition in calcareous soils. Please add a reference here

Author response: 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).

Referee comment: The discovery that structural and defensive traits do not vary with soil is consistent with a previous study in New Zealand’s lowland temperate rain forests (Wright et al., 2010). That study compared traits of trees growing on phosphorus rich alluvium versus phosphorus-depleted marine terraces. Foliar phosphorus concentrations of species were halved on the marine terraces, but there was no detectable variation in structural traits, phenolic or tannin concentrations. I would add more discussion at line 298. At the moment is more a description of results. Please specify at the beginning which traits are you talking about and why they do not change between poor and rich soils:

Author response: We have added more references and made the sentences clearer: “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 phenotypic 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 (Boege & Dirzo, 2004; Fine et al., 2006; Koricheva, Larsson, Haukioja, & Keinanen, 1998).”

Referee comment: "Water“ was defined as trait. Please define exactly what do you mean with water and how this was computed also here

Author response: We included the following sentence on the paper: “Leaf water content was computed as the ratio between the quantity of water (fresh weight – dry weight) and the fresh weight.” We also used the term leaf water content throughout the paper.

Referee comment: Line 304: Species had a greater influence on trait values than soils for all traits, except P. This makes completely sense to me because the content of P in leaves should be more related to the P available in the soil for the plants and not too much to the species. But again I found the discussion poor. There is a lot of literature about the leaf stoichiometry and P stoichiometry and the relationship with physical and chemical properties of the soil.

Author response: We agree that some discussion on P was missing out and we expanded the soil and species effects on traits considering that P supply limitation: “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; Sardans & Peñuelas, 2004; Tyler, 2002), while the low N concentrations may reflect stoichiometric constraints (Niklas, Owens, Reich, & Cobb, 2005). The lower efficiency of PSII in the chalk soil is likely to be 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 phenotypic 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 (Boege & Dirzo, 2004; Fine et al., 2006; Koricheva et al., 1998).

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, White, Mead, & Broadley, 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, Inal, Bagci, Coban, & Sahin, 2007). High Zn organization at the species level corroborates earlier analysis that show more than 70% of Zn variation occurs within family and substantial differences exist between and within species (Broadley, White, Hammond, Zelko, & Lux, 2007).

The patterns revealed by our variance partitioning analysis of six temperate species (Fig. 1) bear surprising 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. They, like us, found foliar concentrations of P and other rock-derived minerals varied strongly with site, while nitrogen concentrations varied little. They, like us, found that soluble carbon, structural and defensive traits hardly varied between sites. And they, like us, 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 (Gregory P Asner & Martin, 2011). Similarly, δ13C is known to vary strongly with light condition and with relative humidity (Buchmann, Kao, & Ehleringer, 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. ”

Referee comment: Also with the database the authors have they can also explore how the reflectance is related to ratio such as C/P N/P or C/N ratios.

Author response: Unfortunately, P is not well predicted; the few studies spectroscopy studies available differ in the spectral bands they chose to model P (Homolová, Malenovský, Clevers, García-Santos, & Schaepman, 2013). 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 so constellation effects are unreliable. 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”). Because of the confounding factors revolving around rock-derived nutrients predictions, we decided not to include ratios that would not be directly detected.

Referee comment: Line 350 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.

Please also cite other recent papers showing similar results with spectrometers similar to the one used in this study (e.g. Homolova et al., 2013).

Author response: Thank you for the suggestion. We have included it: “According to Kumar et al. (2001), three main protein absorption features report as important for N estimation are located around 1680 nm, 2050 nm and 2170 nm.”

Referee comment: Line 353 Although chlorophylls also contain nitrogen, the spectra of chlorophylls differ greatly from proteins because of their dissimilar chemical structures, showing strong absorption due to C-H bonds in the phytol tail of the molecule (Katz et al., 1966), Here if I understand correctly the authors they want to make the point that Chl and N are estimated with different regions of the spectrum despite N is one component of Chl and should covary. If my interpretation is correct I suggest another line of argumentation: Nitrogen Chl are contained in the green vegetation and N content and Chl are correlated (see Houborg et al., 2013). However, in dry leaves there is only N and not Chl. And therefore we cannot expect that the PLSR select similar regions for Chl and N.

Author response: We have added the following sentences to the text: “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. (2001), three main protein absorption features reported as important for N estimation are located around 1680 nm, 2050 nm and 2170 nm. Although chlorophylls also contain nitrogen, the spectra of chlorophylls differ greatly from proteins because of their dissimilar chemical structures, showing strong absorption due to C-H bonds in the phytol tail of the molecule (Katz et al., 1966). That can be confirmed in this work as the visible region of the spectrum showed the best predictions of pigments.”

Chl and N were not correlated in our study and the spectral measurements were done on fresh leaves. The main reason for PLSR to select different regions was that N is correlated to the proteins and Chl (even though they contain nitrogen) to the phytoil tails.

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

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Summary

Understanding the causes of variation in plant functional traits is a central issue in ecology, particularly in the context of global change. Spectroscopy is increasingly used for rapid and non-destructive estimation of foliar traits, but few studies have evaluated its accuracy when assessing intraspecific 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

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Deleted: Analyses of the drivers of traits variation based on thousands of tree species are starting to unravel patterns of variation at the global scale, but these studies tend to focus on interspecific variation, and the contribution of intraspecific changes remains less well understood. Hyperspectroscopy

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species, but highlights problems with predicting intraspecific variation indirectly. We discuss the implications of these findings for mapping functional traits by airborne and space-borne 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 intraspecific variation 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

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Deleted: (Grime et al., 1997; Lambers and Poorter, 1992), 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

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Deleted: (Marschner, 2012). Analyses involving this large suite of traits are so far restricted to comparisons of tropical forests, and emphasize cross-site and cross-specie differences with little consideration or within-species variation (Asner et al., 2011; Asner et al., 2015). Placing traits into functional groups, and analysing

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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

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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).

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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.

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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

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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

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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 ≥ 60% of the variation of eight traits (in descending order Si, water content, B, soluble C, N, LMA, K and cellulose concentrations), and ≥ 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).

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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 δ^{13}C and δ^{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

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

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The majority of leaf properties showed higher goodness-of-fit using the regions of the spectrum betwee

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Deleted: . The soil importance was precise for the majority of leaf properties, but PLSR did not detect precisely the variation of rock-derive 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 δ^{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 δ^{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

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

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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, δ^{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. Although chlorophylls also contain nitrogen, the spectra of chlorophylls differ greatly from proteins because of their dissimilar chemical structures, showing strong absorption due to C-H bonds in the phytol tail of the molecule (Katz et al. 1966). That can be confirmed in this work as the visible region of the spectrum showed the best predictions of pigments. The 1500-1900 nm region was also important for phenolic compounds prediction, which includes the 1660 nm feature across a variety of species and phenolic compounds (Windham et al. 1988; Kokaly & 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;

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¶
4.3 Functional groups on contrasting soils ¶
We investigated how traits in generalist species are responding to different soil conditions and the factors most contributing to changing leaf properties. The investment in light capture had high intra-specific variation, and neither species nor soil accounted for variation in foliar properties. The investment in growth showed relative high inter-specific variation separating out some species. Investment in traits related to defence and leaf structure is species-mediated and may be separated into two ...

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A review in the literature suggests that the use of dry leaves may ...

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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). However, it seems likely that spectroscopy is able to predict Si concentrations reliably because it integrates information on several of these foliar traits to make the predictions. Similar to Si, the relative high precisions for K, Fe and B predictions is likely to be stronger due to the integrating information on several foliar traits simultaneously. Unfortunately, P is not well predicted; the few studies spectroscopy studies available differ in the spectral bands they chose to model P (Homolová et al. 2013). 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 so constellation effects are unreliable.

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.

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The use of spectroscopy for Si predictions on fresh leaves appears to be promising considering our accurate results. The data available in the literature show that the ecological functions of Si have generally been poorly studied, and that there are almost no data about the role of Si structures in the reflection and transmission spectra of short-wave or photosynthetically active radiation in plants. Silicon is absorbed by plants from the soil solution in the form of silicic acid (H4SiO4) being translocated to the aerial parts of the vegetal through xylema, and then deposited along

Deleted: plant as phytoliths (silicified bodies) (Tripathi, 2011). Smis et al. (2014) showed for the first time the potential use of NIR spectroscopy to predict Si concentration. Si shows strong interactions with plant biomolecules such as phenol- or lignin-carbohydrate complexes (Inanaga et al.,), cellulose (Law and Exley,), and proteins (Perry and Keeling-Tucker,). Predictions of Si concentrations, and other traits, from leaf spectra reflectance can be stronger than expected likely because leaf spectra integrate information on several foliar traits simultaneously. ¶
Galvez-Sola et al. (2015)

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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 intraspecific variation 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.

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.

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4.5 Consideration on the use of spectroscopy to quantify patterns of foliar traits¶
The range of variation within species for most predicted traits tend to be smaller with the use of PLSR on reflectance, resulting in consistent slight overpredictions of the inter-specific variance. The interrelationships between foliar chemical and spectral properties for each species help to explain the successful results reported in developing species-level variation from leaf spectral data (Asner et al., 2009). In general, the residuals variation was lower for most leaf trait with the use of spectroscopy, possibly because the use of spectroscopy affects the ability to quantify measurement error, one of the residual variation components.¶
The variation caused by soil on mineral nutrients and δ^{15}N allocated to the leaves remained unchanged with the use of spectroscopy, possibly because structural leaf traits, such as LMA, cellulose, water, as well as pigments, contribute more to leaf reflectance. As these structural traits remained unchanged between soil types for the six species, it possibly explains why the analyses were not able to detect the mineral nutrients and δ^{15}N effects on reflectance, considering that spectroscopy sensitivity to these properties are an artefact of traits correlation rather than a real feature. The same occurs when accounting for variation related to the interaction between soil and species. The soil component in the interaction tends to be underestimated for rock-derived and δ^{13}C. ¶
This study particularly provides findings for a large range of traits that indicate that the use of spectroscopy may be useful to quantify structural traits but can be misleading to measure the environmental filtering

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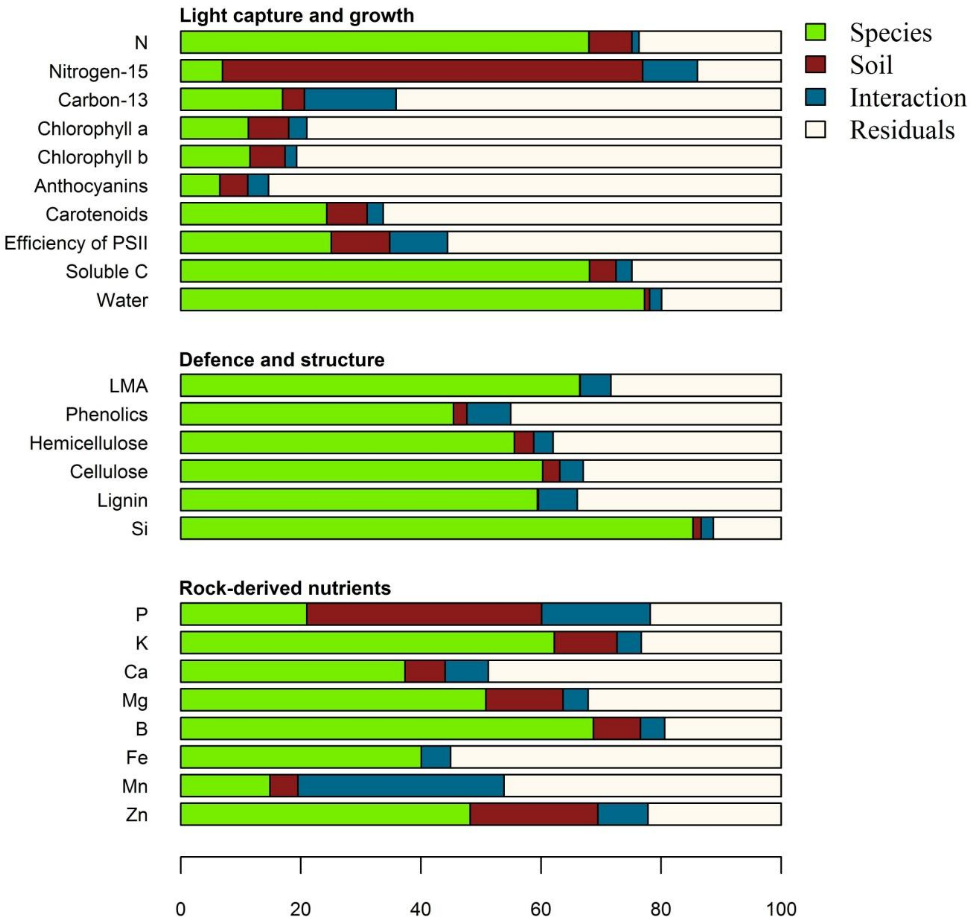

Figure 1. Partitioning of variance of foliar traits between species, soil, species-soil interaction and residual components for six generalist species found on both chalk and alluvial soils. Residual variation arises from within-site intraspecific variation, micro-site variability, canopy selection and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

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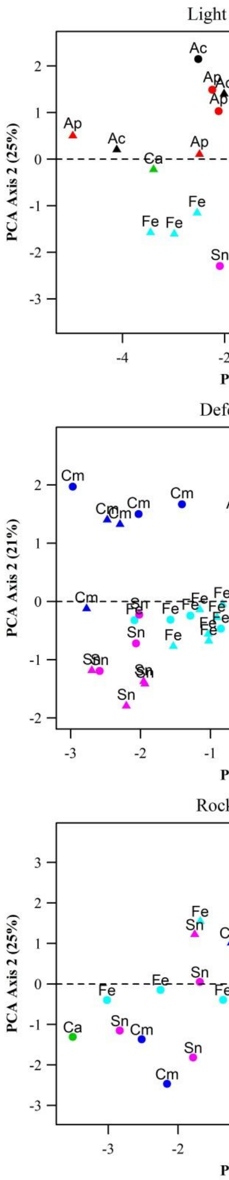

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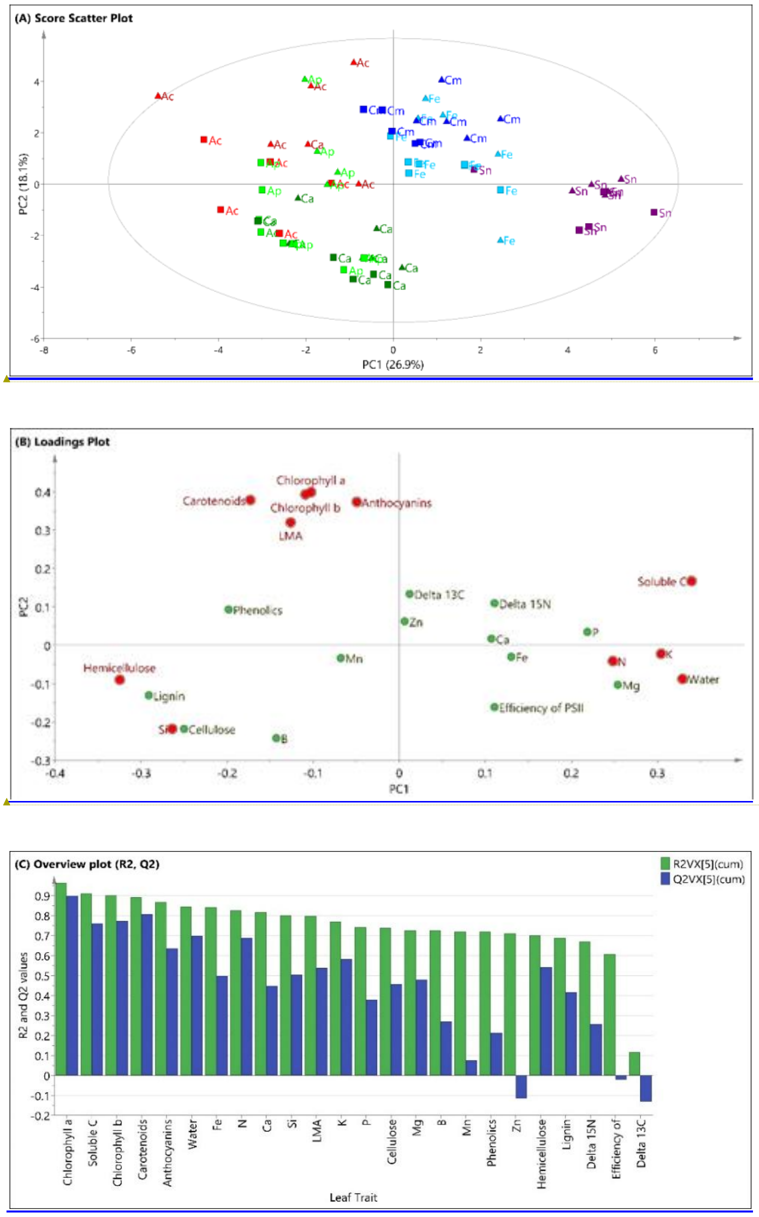

Figure 2. Principal component analysis of all leaf traits (unit variance scaled) measured across all species and sites. (A) Score scatter plot showing first and second principal components using all six species for which data exist for all 24 traits on two contrasting soil types. Colours represent species 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

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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.

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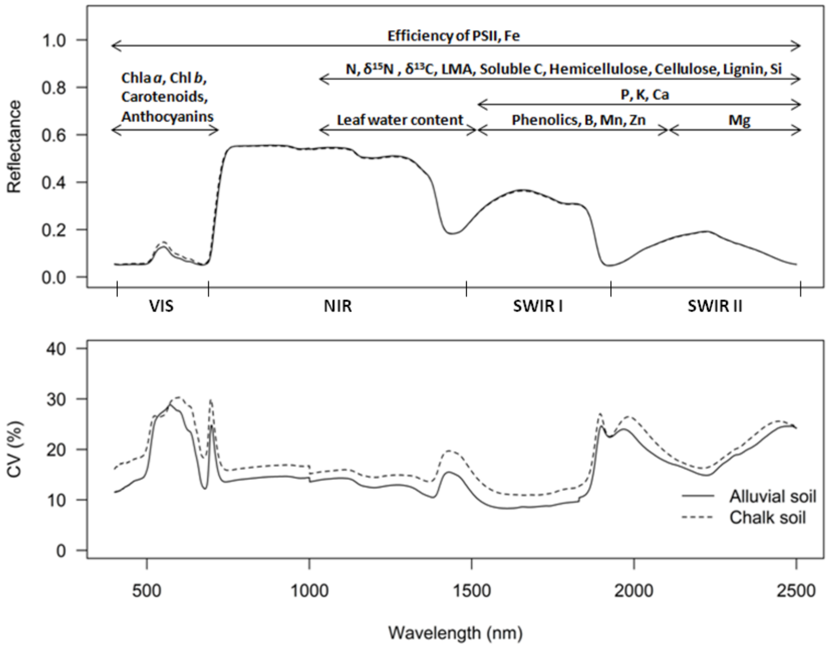

Figure 3. Spectral reflectance and percentage coefficient of variation (CV) of reflectance of six generalists species for alluvial and chalk soils. The spectral regions for each trait were selected based on the model that minimised RMSE.

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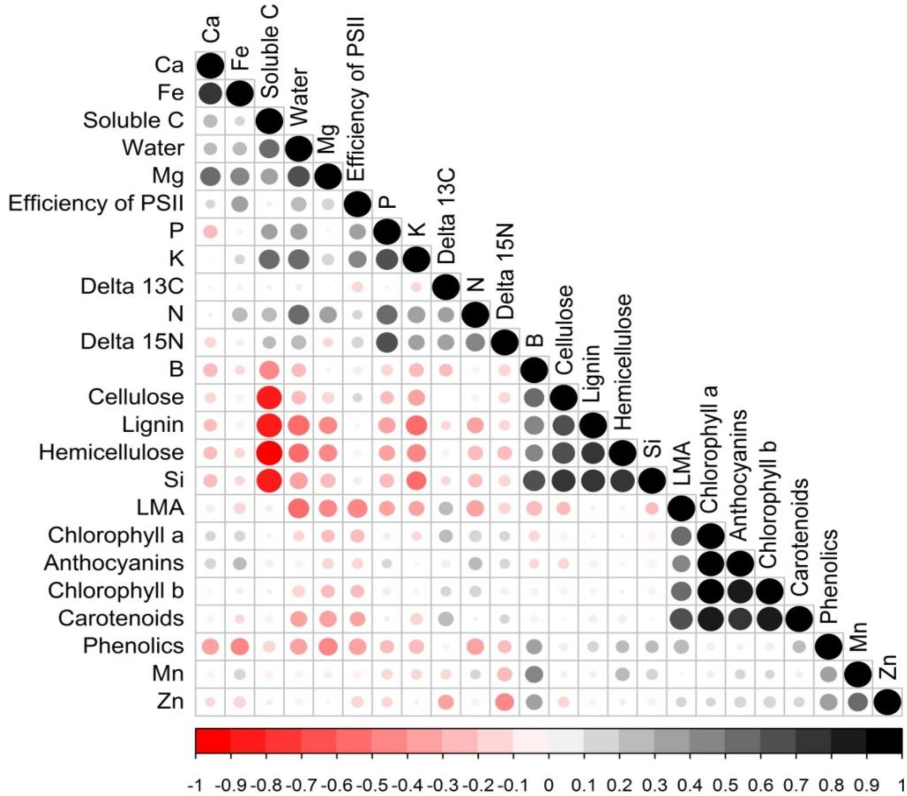

Figure 4. Spearman correlation rank test among leaf traits of 6 species growing on both soil types. Red and black circles mean, respectively, negative and positive correlations. Foliar traits were organised using cluster analysis.

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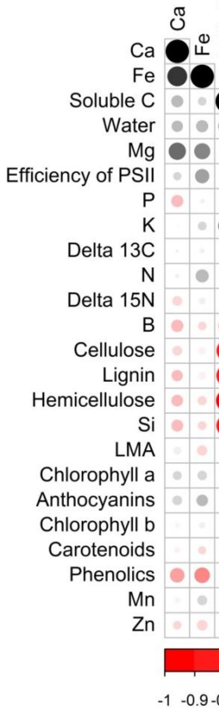

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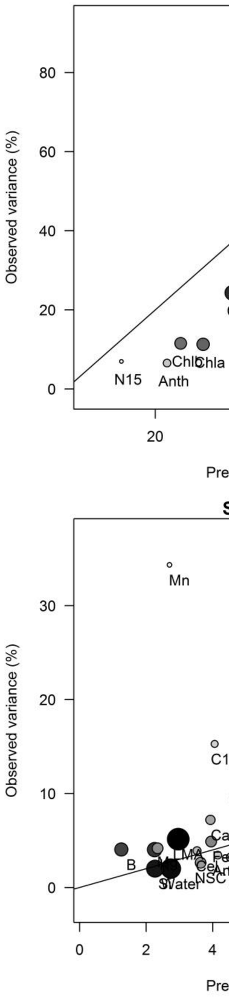

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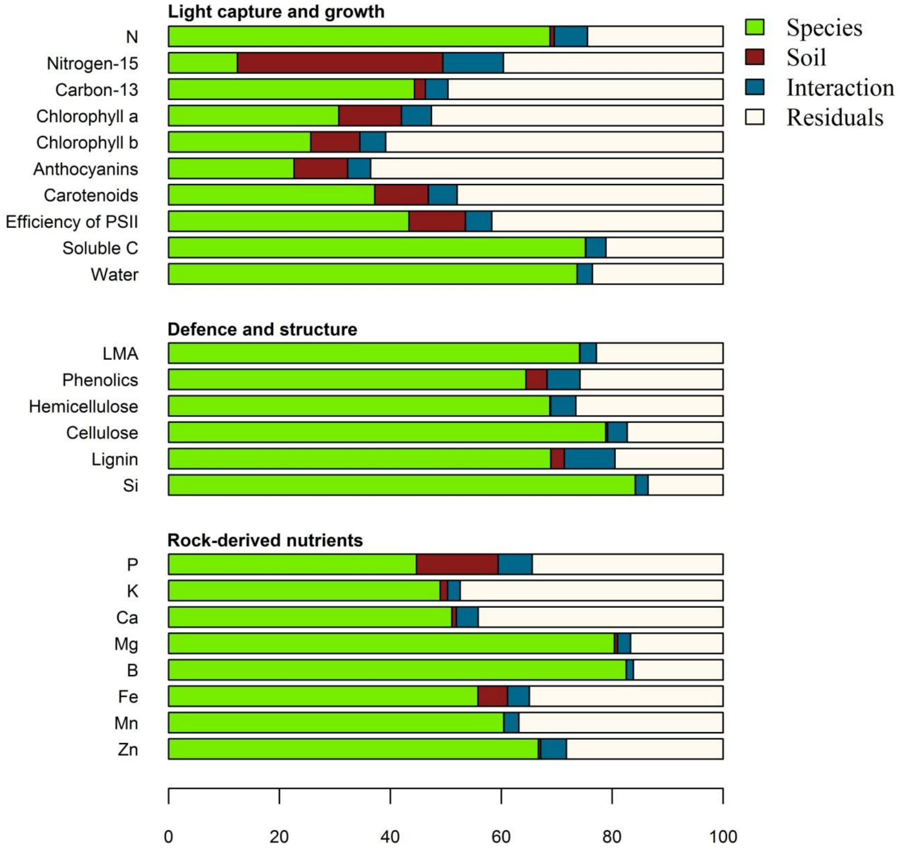

Figure 5. Partitioning of variance of foliar traits between species, soil, species-soil interaction and residual components for six generalist species found on both chalk and alluvial soils from predicted data. Residual variation arises from within-site intraspecific variation, micro-site variability,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

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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

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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

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