

Dear Dr. Michael Bahn,

Thank you for your email regarding manuscript ***Leaf trait variation and field spectroscopy of generalist tree species on contrasting soil types***. We were pleased that Referee #1 made positive comments. We are grateful that the reviewer went through the text carefully and spotted numerous minor issues, all of which we have resolved. We have improved the Material and Methods section to make it clearer, have supported with relevant literature the claims and statements as suggested by the reviewer and clarified both major and minor points. We have reduced the discussion on Si and broadened the review out to include other traits.

Yours sincerely

Matheus Henrique Nunes

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 expanded the text in the methods section to clarify how we acquired data and statistical analysis. Particularly, we added the sentence to clarify the Fig.3**

"To evaluate the correlation among traits, Spearman correlation coefficient was calculated between all trait pairs and the variables were organised in the graphic using hierarchical clustering order to arrange traits into groups."

The necessity of the fourth question was questioned (see below) given its similarity to the third question. In response, we have merged them into only: "What traits can be accurately and precisely estimated using spectroscopy in temperate woodlands?" We decided that Figure 4 was no longer necessary as it didn't not provide additional insights over the results provided elsewhere in the paper.

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 “Phenotypic variation associated with soil” 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 removed it. This gives us more opportunity to discuss the literature focusing on the two questions we have remaining. 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: **These points are clarified, changed or added to the manuscript. See below for more details.**

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-linearity”. 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 numerous issues. We have made the corrections throughout the paper.**

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

Author response: **We now use the term “trait variation” and, in some cases, “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 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)”.

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 are not assessing directly the use of spectroscopy to predict inter- and inter-specific variance anymore because this question was overlapping with the third question. However, we agree that there are numerous works that evaluate the variation of a large range of traits in tropical forests (e.g. Asner’s studies), however few studies have done in temperate forests for a large suite of traits.

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 that this question do not differ from question iii. We decided to leave it out to make the paper more concise and focus on the question iii.

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 be better clarified in the abstract. We changed it to “The contribution of species identity and soil-type effects to variation in traits were evaluated using analysis of variance (ANOVA). Foliar traits were predicted from spectral reflectance using partial least square regression.”

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 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: “Foliar traits were predicted from spectral reflectance using partial least square regression. inter-specific variation was the largest contributor accounting for 25% of the total variation in all leaf traits”

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: Overall, inter-specific variation was the largest contributor accounting for 25% of the total variation in all leaf traits and soil type accounted for 5%, and the interaction between species and soil accounted for virtually no variation. In general, plants investing in traits related to growth had less investment in defence or foliar structure, whereas soil played minor important role in these traits. Nonetheless, foliar macro- and micronutrient

concentrations were found to be strongly influenced by soil type, and foliar phosphorus had the largest variation among all traits due to soil variation.

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 are not assessing directly the ability of spectroscopy to detect variation of traits associated with soil type, so we have removed the sentences related to it.

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.

We have changed the text and added the reference: “...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 (Sims and Gamon, 2002).”

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 rephrased that statement showing that temperate forests have been studied to : To our knowledge, the link between foliar traits and spectral properties of trees has not been broadly demonstrated for a large range of traits on contrasting soil types in temperate forests. 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)

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 recognise that grouping functional classes is controversial. We decided to eliminate this question as it was in fact confusing and somewhat misleading.

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 was 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 ?? what?? , as in Feilhauer et al., 2010. RMSE and R^2 were acquired during both model calibration (indicated with subscript cal) and after model validation (indicated as subscript val).”

Regarding Figure 3: To evaluate the correlation among traits, Spearman correlation coefficient was calculated between all trait pairs and the variables were organised in the graphic with hierarchical clustering order.

Regarding Figure 4: We deleted question iv and, subsequently, Figure 4.

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. 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 removed the “fitted using the 6 species” as it did not make 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: There were strong correlations among some of the leaf traits that may have leveraged the estimation of other leaf traits from the use of PLSR. Si and B were highly correlated to hemicellulose, cellulose and lignin, for instance, but spectroscopy sensitivity to those traits is an artefact of traits correlation rather than a real feature. Likewise, K was highly correlated to leaf water content, soluble carbons, lignin, hemicellulose and cellulose, and its strong relationship with the spectral information can also represent the artefact of correlation with other traits directly predicted using spectroscopy.

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 correlation coefficient was calculated between all trait pairs and the variables were organised in the graphic with hierarchical clustering order.

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 have been more specific and have changed the text to: Some leaf traits were greatly influenced by species identity with influences of soil type, such as soluble carbons, N, K, Mg, B and Zn, whereas other traits were hardly affected by soil and only varied with species, such as cellulose, hemicelluloses, lignin, foliar water content, Si and phenolics. Soil had a strong influence on concentrations of mineral nutrients in the leaves. Other foliar traits – mostly those involved in structure, defence and growth - varied between species but soil had little detectable effect.

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.”

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 identity is driving investment in defence and foliar structure, as well as investment in traits related to growth. In general, our study indicates that plants investing in compounds related to defensive strategies (e.g. either high phenolics or high carbon fractions concentrations) are associated with plants investing less in traits related to growth (e.g. P, N, water content, soluble carbons), although traits are not necessarily individually correlated to each other.”

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 et al., 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 : “The use of spectroscopy on fresh leaves is particularly positive for LMA predictions.”

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 the whole section 4.4 as requested

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: **As we deleted the question iv off, this sentence is no longer on the paper.**

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 have changed to: "This study demonstrates the potential for rapid and accurate estimation of foliar traits. Soil fertility is not constraining the use of spectroscopy to a particular soil type, indicated by the strong relationship between foliar traits and spectral information across both soils. Spectroscopy provides the opportunity to characterize important sources of variation in foliar traits related to chemistry without having to measure the entire range of foliar constituents. Our results provide the basis for additional studies to use spectroscopy to identify additional foliar constituents that may vary among temperate forests."**

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.

Author response: We have changed to: “Species identity had a much stronger influence on most traits than the substrate upon which the trees grew. In particular, most traits associated with light capture, growth, cell wall structure and defence were uninfluenced by substrate. The exception to this rule, was that foliar concentrations of rock-derived nutrients were strongly affected by the soil type. Plants investing into defence or foliar structure are investing less in growth, patterns greatly influenced by species identity and, with much less influence, by soil type.”

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 have relatively changed the discussion to make it clearer and subsequently the conclusion. That sentence is now as follows: “Some traits, for instance Si, B and K, are likely to be accurately predicted due to the integrating information on several foliar traits simultaneously. This study demonstrates the potential for rapid and accurate estimation of foliar traits of forest canopies with contrasting soil types. Soil fertility is not constraining the use of spectroscopy to a particular soil type, indicated by the strong relationship between foliar traits and spectral information across both soils.”

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: The figure is no longer on the paper as question iv was deleted.

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.