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# *Interactive comment on* "Leaf trait variation and field spectroscopy of generalist tree species on contrasting soil types" *by* Matheus Henrique Nunes et al.

### Anonymous Referee #1

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### **General Comments**

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. Below I suggest a few areas where the paper could be strengthened and a number of minor points.

Specific comments 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. 2) Some of the findings discussed in the abstract need to be made clearer. 3) Some of the claims/statements made in the abstract and intro either need to be changed or better supported with relevant literature. 4) Many frequently used terms throughout the paper need to be changed/clarified (see below). 5) Why there is such an emphasis on being able to predict Si using PLSR throughout the paper needs to be clarified! 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.

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".

Abstract/summary

Line 10 - change "traits variation" to "trait variation"

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)

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.

Line 14 - "Working with 24 leaf traits". Contradicted by line 151 which mentions 26 leaf

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traits.

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?

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?

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

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".

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".

Line 24 – "Si predictions using spectroscopy appear to be promising" – what's so special about Si predictions?! Why do they get singled out?

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Line 28 – "However, it [field spectroscopy] was unable to detect subtle withinspecies 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?

Introduction

Line 58 – typo. Change "include phosphorous" to "including phosphorus".

Line 64 – "along environmental change". Typo. Suggested "along environmental gradients"?

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.

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) ???

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.

Line 84 - "leaf property". Replace with "leaf trait"?

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". BGD

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

Material and Methods

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:"

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

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)".

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

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

Line 156: "Where necessary, variables were log transformed to meet assumptions of ANOVA". Reference Table 1, where info concerning which variables were log transformed can be found.

Line 169: "strong co-llinearity". Typo.

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!!!

Results

Line 204 – "Species exerted little or no influence on pigment concentrations" – Refer to species in this context (and throughout paper) as 'species type"?

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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.

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

Line 248- "higher goodness-of-fit" – use a different term? E.g. stronger relation-ships/correlations etc.

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?

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.

Discussion

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.

Line 305: "water" – change to 'leaf water content'.

Line 321: "but their study sampled only from fully sunlit leaves". Suggested - "Similarly, their study sampled only from fully sunlit leaves".

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.

Line 326: "relative". Typo (relatively)

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Line 327: "separating out some species". Confusing. Rephrase?

Line 327: "Investment in traits related to defence and leaf structure is speciesmediated, and may be separated into two defensive strategies". State the two defensive strategies?

Line 342: "Doing so revealed that...". Awkward. Rephrase.

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").

Line 357: "A review in the literature". "A review of the literature"

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

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?!

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.

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.

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.

Line 399: "This study particularly provides findings for a large range of traits that indi-

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cate 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.

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.

#### Conclusion

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

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?!?!?!

#### Figures

Line 661: "Red and black circles mean negative and positive correlations". Which way round?

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.

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

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