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 #2 saw merit in our work. We are grateful that the reviewer went through the text carefully and gave us positive ideas on variation in traits, all of which we have resolved. We have substantially improved the discussion, have included a figure that includes the reflectance spectra along with an indication of the regions relevant to estimate each leaf trait (Figure 1) and have dealt with functional grouping from a different perspective. We also have reduced the discussion on Si and broadened the review out to include other traits.

Yours sincerely

Matheus Henrique Nunes

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: We recognise that grouping leaf traits into functional classes can be controversial, given that a single leaf trait 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). We based our leaf traits grouping on previous studies that attempted to investigate this chemical portfolio that expresses multiple strategies undertaken by plants to maximize fitness over the lifetime of the individual or species (Asner and Martin, 2012; Asner et al., 2015). Furthermore, we have deleted the second question where we attempt to model variation within each pre-determined group, but we use principal component analysis to group all traits based on the data instead to see whether our traits follow those groupings.

Asner, G.P. and Martin, R.E., 2012. Contrasting leaf chemical traits in tropical lianas and trees: implications for future forest composition. *Ecology Letters*, *15*(9), pp.1001-1007.

Asner, G.P., Anderson, C.B., Martin, R.E., Tupayachi, R., Knapp, D.E. and Sinca, F., 2015. Landscape biogeochemistry reflected in shifting distributions of chemical traits in the Amazon forest canopy. *Nature Geoscience*, *8*(7), pp.567-573.

Results Section Spectroscopy of leaf properties

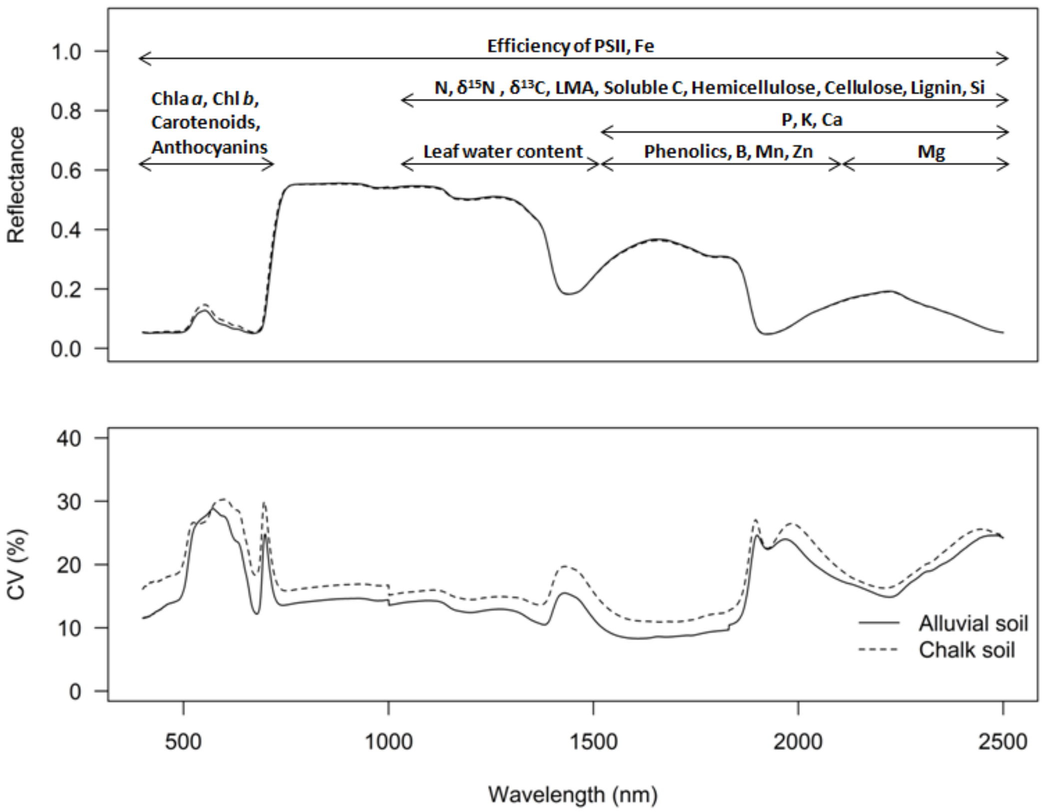
T Referee comment: he results of PLSR are on one hand encouraging because the portion of spectra selected for specific traits are in line with what expected from the literature. Some examples from the article: 1) higher goodness-of-fit were obtained for K, Ca and P in the SWIR regions. 2) Pigments were 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 1. 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 thought that the fourth question could be leading to question iii, and was irrelevant to bring more information into the paper. Thus, the abovementioned sentenced is no longer on the manuscript.

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: 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 analyses of foliar nutrients in chalk grasslands species by Hillier et al. (1990). Thin chalk soils contain small quantities of macronutrients needed by plants, and are unproductive for growing crops unless heavily fertilized; however, cation exchange sites in the soil contain high concentrations of calcium and magnesium (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: "The investment in structure and defence-related traits were little influenced by soil type and was mainly determined by species identity. The discovery that structural and defensive traits (i.e. lignin, phenolics) do not vary with soil is consistent with a previous study in New Zealand's lowland temperate rain forests (Wright et al., 2010). The authors compared traits of trees growing on phosphorus-rich alluvium versus phosphorus-depleted marine terraces, and found that concentrations of these compounds were invariant (see also Koricheva et al. 1998; Long et al. 2016). LMA does not vary with soil type and did not correlate with nutrients in the leaves. High LMA, however, is associated with higher pigments in the leaves and, therefore, pigments play a role in modulating LMA variability. The effect of low nutrient availability on leaf anatomy is much smaller than the effect of light (Shields, 1950) and, consequently, the overall effect of nutrients on LMA is moderate, and (on average) only appears when plants are severely limited in growth (Poorter et al., 2009). In general, the concentration of rock-derived nutrients in leaves is highly dependent on soil type as environmental filter. Traits favouring high photosynthetic rate and growth are considered to be advantageous in rich-resource soil environments, whereas expressions of traits favouring resource conservation are considered advantageous in low-resource environments (Aerts and Chapin, 1999, Westoby et al., 2002).

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. We have included some sentences on P and the relationship with other variables along the discussion: Leaf P is related to soil P, which not necessarily affects foliar N (Ordoñez et al., 2009), however the effect of soil P on leaf N seems determined by a tight coupling of leaf N and leaf P (Niklas et al., 2005).

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: We did not obtain a strong relationship between P and spectral data, which can be attributed to the low P concentration in the leaves (Homolova et al., 2013). According to these authors, there is a limited number of studies that estimated P using spectroscopy revealing inconsistent spectral bands among the reviewed literature. P is poorly predicted with field spectroscopy, and so did tested ratios including P, and for this reason we decided against evaluating stoichiometry in this paper, interesting though it is. As P predictions using spectroscopy might be an artefact of correlation with other traits, 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: 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 report 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.

Homolova, L., Malenovsky, Z., Clevers, J.G.P.W., García-Santos, G., Schaepman, M.E. Review of optical-based remote sensing for plant trait mapping (2013) Ecological Complexity, 15, pp. 1-16.

Houborg, R., Cescatti, A., Migliavacca, M., Kustas, W.P. Satellite retrievals of leaf chlorophyll and photosynthetic capacity for improved modeling of GPP (2013) Agricultural and Forest Meteorology, 117 (1), pp. 10-23.