

Interactive comment on “Spatial trends in leaf size of Amazonian rainforest trees” by A. C. M. Malhado et al.

A. C. M. Malhado et al.

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Here, we immensely thank the referee J. Lloyd and the anonymous referee for all their constructive comments on our work, and hope we have satisfactorily addressed all comments, as detailed below.

Section 2 - Comments by Referee 2 Anonymous

General Comments This manuscript presents an investigation into leaf structural traits and their correlation with environmental as well as physiological gradients within the Amazon Basin. The manuscript presents the research topic with much clarity and an in depth background, with an array of ideas that represent our current state of knowledge on leaf economics and a strong foundation for the subsequent analysis.

1. Referee 2 -Anonymous With such a complex dataset, containing multiple covariates,

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a complete statistical analysis is challenging. The statistics presented here may not fully capture some of the leaf-environment relationships that exist (i.e. cut-off p-value of 0.05 is quite conservative) but may also over estimate the relationship due to the application of multiple ANOVA tests and regrouping of leaf area data.

Malhado et al. (response) Yes this is true. We have tried to statistically control as much as possible for other effects but, to our knowledge (and I have asked around), there is no ideal analyses. In this paper, due to the complexity of many of the relationships, we intentionally tried to be conservative in our analysis. Our main challenge was to deal with autocorrelation, pseudo-replication and phylogeny at the same time. The grouping and regrouping of data was far from ideal but allowed us to partition the analyses exploring different points of view.

2. Referee 2 - Anonymous The collection of leaf characteristics from Herbarium specimens might also affect the analysis because intra-specific plasticity was not considered, presuming that the Herbarium sample were collected from one site, and then applied to all site by species combinations (which might justify the broader categories for leaf area used in the ANOVA).

Malhado et al. (response) Yes this again is true and a limitation to the work. In the current study herbaria and flora specimens were used to designate traits to a given species and these data were extrapolated onto all of the individuals of that species in the permanent plot database. This procedure, from one side, necessarily eliminates potentially interesting variability among sub-populations and, at worst, may critically reduce the strength of environment-trait associations limiting our ability to uncover patterns. I have compiled different herbaria specimens collected at different times and places to address this limitation, but here is not the place for such a complex analysis it deserves a paper on its own. On the positive side, we believe we have used a typical leaf for each species and therefore we would be removing some of the noise from the sampling method.

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Referee 2 Anonymous It would be interesting to compare the spatial patterns of the findings in this study with recent work on the patterns of drought resilience in the Amazon from remote sensing and/or whole-ecosystem experiments. Also useful in the discussion would be a mention of possible site-level feedbacks that may act to minimize water stress (i.e. hydraulic lift and deep soils and roots) that might confound climatic variables and their role in determining leaf structure.

Malhado et al. (response) I agree completely with this comparison and believe that we would be able to see some interesting patterns. All the leaf data are now available to the RAINFOR network researchers and there are plans to integrate these data on this type of analyses. The RAINFOR trait database with the RAINFOR-PLOTS/TREES database. I also think that now it would be interesting to compare the ground leaf data with spectral responses of remote sensing data. We did some preliminary analysis on this and found some interesting correlations.

The discussion has been improved taking the advice of the referee and a paragraph has been added on site-level feedback for minimizing water stress.

5. Referee 2 Anonymous - Specific Comments (SC) 1. An elaboration on the pioneer-climax gradient. This is used in the analysis as a covariate, but it is unclear why.

Malhado et al. (response) We were interested in assessing if leaf size varies with the growth strategy of trees and the pioneer index gave us a useful, if simplified, method of doing this. The functional hypothesis is that if a plant is adapted for quicker growth will it have bigger leaves in order to capture more light and increase the rate of photosynthesis.

6. Referee 2 Anonymous (SC) 2. A better description of herbarium methodology is needed. Were these images scanned and measured digitally?

Malhado et al. (response) The methodology for assigning the leaf-size category was as follows.

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a) The list of RAINFOR tree species was generated.

b) A digital image for each species was located using a number of online herbaria. The major source of data used for this study is the online Neotropical Herbarium Specimens of the Field Museum of the State of Illinois, United States of America. The Field Museum team provides a desktop reference set of high-quality images of dried herbarium specimens for comparison. These will represent a broad range of Neotropical genera and common species. The underlying strategy is to have just a few examples of each species, specimens that are typical or illustrative of that species. Preference is given to specimens that have a good set of leaves as well as flowers or fruit, and to specimens with an authoritative identification.

c) When a range of specimens were available, priority was given to specimens collected in the Amazon region and furthermore, all the available specimens were assessed with the objective of choosing the one that was most representative (typical) of the available images and which was of sufficient quality for measuring and further analyses.

d) After running searches on the first five sources listed in Table 1 (see manuscript), the outstanding species were cross-referenced with herbaria on the GBIF database (Global Biodiversity Information Facility). A list of herbaria containing specimens of the outstanding species was created and each herbarium was contacted with a loan request for the (digital) specimens.

e) The leaf-size category template (Figure A, below in smaller scale) created by the Leaf Architecture Working Group (1999) was used as a guide for assigning leaf shape categories. Specimens without an accompanying measurement scale were discarded.

All the measurements were made manually. We have added a sentence to the methods to clarify this.

Figure A: Physical representation of leaf size categories (taken from the Leaf Architec-

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ture Working Group 1998). (I can not upload it here).

7. Referee 2 Anonymous (SC) 3. Was any data transformation required before analysis? The raw data are not presented (i.e. plots between precipitation and leaf size) and so it is not possible to evaluate this.

Malhado et al. (response) All the frequency data was ArcSine-square root transformed. The compositional data was analysed with both ArcSine-square root transformation and untransformed but this did not influence the results. For this reason we reported the untransformed data.

8. Referee 2 Anonymous (SC) 4. Section 2.3, first paragraph, its unclear from this paragraph why the splitting into groups is necessary, perhaps explain why the comparison to other studies is necessary earlier on.

Malhado et al. (response) The main reason is to allow comparison with previous studies this has been explained in more detail in the text. People working on RAINFOR and others networks have described a series of differences between forests in Amazonia. For instance, previous studies using the RAINFOR database have suggested that forests in western Amazonia have higher wood productivity (Malhi et al. 2004), higher turnover (Phillips et al. 2004), and lower wood density and biomass (Baker et al. 2004) than forests growing on infertile soils further east. Furthermore, Malhado (2008) work on 4 leaf traits in Amazonia also described some patterns, for example, that trees with compound leaves do not show any similarly straightforward regional patterns.

9. Referee 2 Anonymous (SC) 5. It would be helpful to define "pioneer" species and how this concept was posed to the experts for their classification.

Malhado et al. (response) (Information took from Butt et al., 2008). A Pioneer index was used to rank genera according to the extent to which they are perceived to be pioneers (plants that are specialists in forest gaps and other disturbed areas). This index approach, adapted from an ethnobotanical technique (cf. Phillips & Gentry, 1993),

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reflects the degree of consensus among botanists familiar with the taxa, in this case three botanists (O.Phillips, Rodolfo Vasquez, Abel Monteagudo) and collector label comments in herbarium reference material. Each taxon was judged by each botanist on one or more independent occasions, as a non-pioneer; (0) or a pioneer (1), and the average calculated for that taxon expert combination. Each taxon consensus score was the sum of these average values divided by the number of experts consulted, so that the index had a potential range of zero to unity. For our analyses, scores ranged from 0 to 1.

10. Referee 2 Anonymous (SC) 6. A table summarizing the wood density relationship, one of the main points of the paper, is not included.

Malhado et al. (response) The wood density relationship is indeed important. However, we are not convinced that a table would provide a more thorough account of this relationship than is already in the text. Therefore, in this case, we have left it as it is.

11. Referee 2 Anonymous -Technical Corrections (TC) 1. Throughout, be more precise than " humped " distribution, perhaps " unimodal " would be appropriate?

Malhado et al. (response) Yes, many thanks for the correction - this is more appropriate and has been corrected.

12. Referee 2 Anonymous (TC) 2. Was the ANOVA on p2141, line 5-9 for just two groups? A t-test would perhaps have been more appropriate, but would have given the same results. What were the criteria for this data grouping, multiple tests might need to be weighted statistically.

Malhado et al. (response) Yes, the ANOVA was for just two groups. It has been redone using a non-parametric statistic to respond to the other referee. It is true; we found the same general result. There are a few reasons for the grouping of the leaf size categories: we are trying to identify broad patterns for large/small leaves; these groupings have been used in other works (eg. Dolph and Dilcher 1980); the grouping avoids the

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effect of the phylogeny (see Figure 3 on the manuscript).

13. Referee 2 Anonymous (TC) 3. Would be useful to see xy plots

Malhado et al. (response) Plots are listed on the end of these comments.

14. Referee 2 Anonymous (TC) 4. Figures: In general, the axis labels should be made larger

Malhado et al. (response) Figures redone.

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