

Interactive comment on “Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate” by N. M. Fyllas et al.

Anonymous Referee #1

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GENERAL COMMENTS

The manuscript by Fyllas et al. describes the effect of genetics, soils and climate on leaf traits of 1040 individual trees sampled across the Amazon basin. The main findings are 1) that some traits are mainly determined by genetics, and others by the environment, 2) species from more fertile soils tend to have higher leaf nutrient concentrations and lower LMA than species from more infertile soils, 3) soil fertility, and to a lesser extent temperature and rainfall affect intraspecific variation in leaf traits, whereas solar radiation does not. Although the first two findings are not really novel, the third finding is, in my opinion, and has rarely been tested before because most studies did not encompass such a large range of environmental conditions.

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This is an impressive study in terms scale and coverage across the Amazon basin. This strength is at the same time its weakness, as it is a highly unbalanced sampling design, in which 1040 trees were sampled belonging to 508 species, of which 303 were only sampled once! I was therefore not sure what exactly I was looking at. The authors attempted to correct for this by partitioning leaf trait variation into genetic and environmental (plot) components. I found this a very elegant solution, but the authors should consult a statistician whether they are really able to do so, with such a highly unbalanced design and little replicates. I found it also elegant that the authors associated inherent leaf traits with each other, after having corrected for the environment, and that they associated intraspecific variation in leaf traits to 4 important environmental gradients in the Amazon basin. This should get a stronger focus in the paper, because here is where its strength is (lots of environmental variation because of the basin-wide coverage)

This is a massive manuscript, with > 12000 words, and with nearly as many statistical tests as leaves sampled. I got totally lost in the overkill of statistical tests and results. The manuscript lacks a clear structure, partly because the authors try to address too many points at the same time, they try to relate it to too many current topics; and because they do not have clear questions and hypotheses. The discussion lacks headers and structure, meanders back and forth, and jumps from physiological detail to sweeping generalization. I have read the manuscript several times and I did not get away with a clear feeling what it was about. If I wouldn't have been a reviewer, then I would have given up reading it. So I think my main suggestion to the authors is to rethink what their main message is, focus on the most novel points, and structure their manuscript around it. Now this main message is snowed under by the tremendous amount of detail and result, which is a pity, given the nice results and the magnitude of the study. Please find below some major and minor suggestions. I hope they are of help to the authors to improve the manuscript.

SPECIFIC COMMENTS

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Reduce the statistical overkill and amount of data presented. Although I appreciate it that the authors tried to analyze their data statistically as careful as possible, I got totally swamped and lost as a reader by all these nearly-similar-but-slightly-different results. You really should weed thoroughly in this. I suggest to delete half of it. 1. Fig 1. Histogram of raw trait data and how this differs between fertile and infertile soils. DELETE. You present this analysis before the analysis in which you partition variance into genetic and environmental effects, whereas you should present it after it. Moreover, you do later the proper statistical test in which you remove genetic variation and look only to environmental effect, with soil fertility as a continuous instead of a categorical variable

2. Fig 2. Here you partition trait variance into genetic and environmental effects. This is a very nice analysis and the figure provides a very elegant overview of the data. What I missed in the discussion is an interpretation of the results. Why are especially Al, Mg, C and LMA genetically determined, and why are especially P, K, Ca environmentally determined?

3. Fig 3. Family differences in trait values. You spent a lot of text on this in the results, and very little text on it in the discussion. This is nice for people who work in the region, but not of general interest and not crucial for your discussion. I would put it in a digital appendix.

4. Fig 4 & 5. Genus and species variation in leaf traits. Delete. See the point above. This is even less interesting, and you do not need it at all for your discussion (and you did not discuss it at all)

5. Fig 6 and Table 2. Bivariate trait relations between the genetic components. This is one of the core results of the study. However, it is not clear at all why you show especially these relationships? Because they showed the highest correlation? Because you had an a-priori hypothesis about it? It is no clear how you divided species in high and low fertility species, especially because for 303 species you had only a single observa-

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tion (!), so it might erroneously have been classified as a low fertility species whereas in fact it is a generalist or might even show a significant positive association with high fertility, but the single individual that you sampled happened to occur at a low fertility site. I am sorry, but you simply do not have the proper data to do this analysis! You can fit a slope through the whole cloud (please provide N, r, and P in the panels), but it does not make sense to fit different slopes for assumed "low-fertility"; and high-fertility species, and test for differences in slopes and elevations. So please omit these analyses from Table 2. Fig 6, please also plot as tic values the back-transformed values on the axes, so that the reader gets a better feeling what he/she is looking at.

6. Fig 7. Bivariate trait associations between plot values. This is another key-result, and mirrors partly Fig 6 for the species values. So why don't you select the same bivariate panels for Fig 6 and 7, so that the reader can compare them. The authors stress that trait associations may differ when compared within (e.g., by comparing plots) and across species. I missed an analysis of how often they are different (or are they in general just congruent?), and the discussion lacks an explanation of why they would/should be different.

7. Table 3. SMATR analysis of bivariate scaling relationships between fertile and unfertile plots. I got lost here. First why are you doing this? Why do you a-priori expect differences in scaling relationships? And second, why do you divide a continuum in soil fertility arbitrary into two groups? Wouldn't it be a more appropriate test to evaluate how the CONTINUOUS soil fertility PCA axis affects the RATIO between two trait values? Moreover, the statistical results indicate that low and high soil fertility sites for most traits do not differ in slopes and elevations. So if there are no clear a-priori hypotheses, if the test is less than ideal, if the results are inconclusive and non-significant, why doing then this whole exercise after all?

8. Table 4. The PCA with soil traits is useful

9. Table 5. Multiple regression results of plot trait values on environmental axes. This

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is repeated 3 times, once just normal, and twice with different techniques to remove spatial autocorrelation. I would suggest to remove this spatial autocorrelation analysis. It is said in the methods that spatial autocorrelation can be brought about by 1) similarities in species composition or 2) by similarities in the environment. If the genetic species effect is already removed by the variance partitioning analysis, then point 1 is not an issue anymore. That would leave us with point 2 that we want to analyze the relationship between traits and the environment by removing via spatial autocorrelation the environmental effect. This does not make sense to me at all! Maybe it makes sense to a geostatistician, but if the goal is only to make the data points more independent, then I think a statistical canon is used to kill a mosquito (I think this statistical autocorrelation is much less of a problem than the highly unbalanced sampling of species and trees!!!). So please remove this spatial autocorrelation, that will save you two pages in the methods, 3 (!) pages in the results, and many readers that, along with me, totally lost track. Instead, devote the gained space to explain more clearly in your discussion why these trait-environment relationships are the way they are, and why this is important.

10. Table 6& Fig 8,9. Plot-environment relationships. This is a core result of your paper. Very few studies have been able to show this. Here is where the strength is of your basin-wide approach, so take advantage from it!

11. Table 1. Difference between N fixing Fabaceae, non-fixing Fabaceae, and non-Fabaceae. This is an interesting analysis with interesting results, but a total side-track of the paper. Delete.

12. The study presents a highly unbalanced sampling design, in which 1040 trees were sampled belonging to 508 species, of which 303 were only sampled once! I was therefore not sure what exactly I was looking at. The authors attempted to correct for this by partitioning leaf trait variation into genetic and environmental (plot) components. I found this a very elegant solution, but the authors should consult a statistician whether they are really able to do so, with such a highly unbalanced design and little replicates.

And they should more explicitly discuss the limitations of this sampling approach and to what extent it confounds their results

13. The whole paper is not very well-structured, things are discussed on an ad-hoc basis. Make in the intro 3 clear questions: 1) partitioning of trait variance between genetics and environment, 2) bivariate traits associations (genetics and plot level), 3) trait-environment relationships. End the intro with 3 clear corresponding hypotheses (else it remains a fishing expedition). Give the same 3 headers in your discussion, start with your hypo and then what you found, and end each section with a clear conclusion.

14. The authors discuss many of the 36 potential bi-variate trait relationships for their nine traits. It would be very worthwhile to include a PCA for the genetic component of these 9 traits, so that the reader gets a better feeling how they are associated.

TECHNICAL COMMENTS You divided your 63 plots in low and high fertility plots, based on total reserve bases, with 80 as a natural breakpoint. This feels a bit odd, first because the exact value of this breakpoint is arbitrary, second because at the same time you present in Table 4 a soil PCA with a soil fertility axis (the first PCA axis) based on many variables and this should therefore be a better and more complete description of soil fertility, and third why do you want to break up a continuum in classes, thus losing resolution? If you want to break it up (I would not do so), I would use the PCA axis value. This becomes also clear from Fig 8 where both the low and high fertility plots may share the same fertility PCA value!

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