Preprint bg-2025-391

Response to Reviewer 1

I thank the authors for their detailed and relevant look into nutrient cycling in tropical forest and appreciate the hard work that has undoubtedly been done to achieve the results presented. However, I have many remarks throughout the text on phrasing, word choice, nuance, and more importantly, on the quality of the scientific analysis, discussion, and the data analyses. Nonetheless, I believe the expected changes/clarifications are feasible, which is why I would reconsider the manuscript after major revisions. Please find my general remarks below; for the detailed and textual remarks, I refer to the attached annotated pdf (please do not mind what is written on the first page - I noted those comments more clearly in the 'major general comments' below). The reason for providing the annotated pdf is that there were many textual issues that would simply take too much time to write in this reviewer comment.

Response: We thank the reviewer for their timely and detailed analysis and comments for improving our manuscript. We also appreciate the annotated pdf, which we printed and reviewed carefully. Many of our edits were made using the annotated pdf, so some responses to the comments and suggestions noted there may not appear explicitly here in this document. We tried to implement all recommended modifications regarding the text, statistical analyses, and figures. A revised version of the manuscript with tracked changes will be uploaded shortly.

Major general comments:

- The title does not represent the core of the study: at first, it seemed the study was about the difference between specifically sand- or clay soils, whereas the focus was actually on topography.

Response: We changed the title to: Comparative analysis of nutrient concentrations in generalist and specialist tree species across topographic positions in the Central Amazon

- Did you use pith-to-bark samples for basic wood density? You always mentioned the diameter of the wood samples you collected, but not their length (thus also not if they were pith-to-bark cores). Additionally, these samples are usually collected with 2 replicates per stem, to account for asymmetry in the stem's shape. Note that you can correct for not using pith-to-bark sensu https://bsapubs.onlinelibrary.wiley.com/doi/full/10.3732/ajb.0900243).

Response: We thank the reviewer for the observation and shared paper of measuring wood specific gravity. The core sample to determine wood density was obtained by extracting a core using an increment borer (Haglöf Sweden®) with approximately 5.15 mm diameter and variable length (approximately equal to half the stem radius (including both sapwood and heartwood). This variable length was due to the diameter of each individual tree. In the laboratory at Instituto Nacional de Pesquisas da Amazônia (INPA), Brazil, the bark was removed from each core, which was then submerged in water until reaching a constant weight (~ 20 days), to ensure full saturation. The saturated volume was obtained by measuring the core dimensions (three measurements each of diameter and length at the base, middle, and top) using a digital caliper (model Mitutoyo Absolute, mm scale), and calculating the average. The dry mass was determined by drying the cores in an oven at 105 ± 2ºC until a constant mass was achieved, then weighing them on an analytical precision balance (model Toledo Prix Ps 360 R2; 360g x 0.001 g precision).

- Additionally, please explicitly mention how you treated the bark - was it included in measurements or not? This is also relevant for the wood nutrient analyses.

Response: The wood samples collected in the field were stored in liquid nitrogen and sent directly to the laboratory for chemical analysis without any prior processing. As such, the bark was included in the samples and in the subsequent nutrient analyses.

- The methodology of submerging the cores in water for 20 days has certain limitations: you probably over-estimate the fresh volume, so mention that one can also use conversion factors sensu https://bsapubs.onlinelibrary.wiley.com/doi/full/10.1002/ajb2.1175.

Response: We thank Reviewer 1 for this valuable comment. It was surprising to see that Vieilledent et al. showed that Global Wood Density Database have been estimated with an overestimated conversion factor. We compared our direct WD estimates using tree cores with Pastorello et al., 2024 (https://data.ess-dive.lbl.gov/view/doi:10.15486/ngt/1898906) who reported WD at species and genus level in the same site. Based on the reviewer comment, we have revised our WD estimates and used the empirical value of 0.828 for the conversion factor as proposed by Vieilledent et al.

- Add more info in the methods section. The authors often refer to a protocol used by the lab, but the reader would have to see which steps were taken in the sample preparation, extraction methods, which machines were used to analyze the contents, ...

Response: We have added more details of the protocols, methods and machines used in the methods section.

- The statistical analyses were carried out or discussed (or both) to a limited extent. The paragraph in the M&M section discusses the check of normality, but not of homoscedasticity. Additionally, and more importantly, the authors mention ANOVA as the method to test "whether leaf and trunk nutrient- and C concentrations differ between the generalist and specialist species," or Kruskal-Wallis in case the normality assumption was violated. The authors need to acknowledge that they have a categorical variable 'tree functional type' with 3 levels: valley spec., plateau spec., and generalist. ANOVA can only tell you if at least 1 of these levels differs significantly from the others, not if which levels differ from each other. For the latter comparison, you would need a suitable post-hoc test. Nonetheless, e.g. Fig. 4 shows the 3 factor levels with specific indications of significance marked as '*', which would have to mean post-hoc testing has been conducted. If this is the case, please mention in the M&M section. Additionally, Fig. 3 reports t-test results, while the method was not mentioned in the M&M, nor the assumptions to be evaluated. Finally, Fig. 6 shows Spearman correlations with p-values. Which statistical test was carried out? And why was a non-patametrical correlation coefficient used?

Response: We thank the reviewer for the observations. We have revised and expanded the Material and Methods section to provide a more detailed description of statistical analyses. In addition to the normality tests, we also report the homoscedasticity in the reviewed version of the manuscript. Figures 3, 4 and 5 were updated and reorganized into two composite figures (Figures 3 and 4, are shown below). In this new layout, soil, leaf, and wood nutrient concentrations are presented together in order to improve visualization of nutrient patterns and trends across the topographic levels (plateau and valley) and functional types (generalists, valley and plateau specialists). We also updated the text and figures captions to clarify the statistical methods used. we applied ANOVA when both normality and homoscedasticity assumptions were met, and the Kruskal-Wallis test when these assumptions were violated. We reported in the new version of the manuscript post hoc tests (in both methods and results sections) to identify differences between functional types: Tukey's following ANOVA, and Dunn's test following Kruskal-Wallis. The significance levels for both post hoc tests are indicated with asterisks (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$) in the figures. Additionally, we clarified in the methods that *t*-tests were used for soil nutrient concentrations in the analysis described in Figures 3 and 4. For Figure 6, the Spearman's rank correlation was used because most of the data did not meet the assumptions of normality, justifying the use of a non-parametric correlation coefficient.

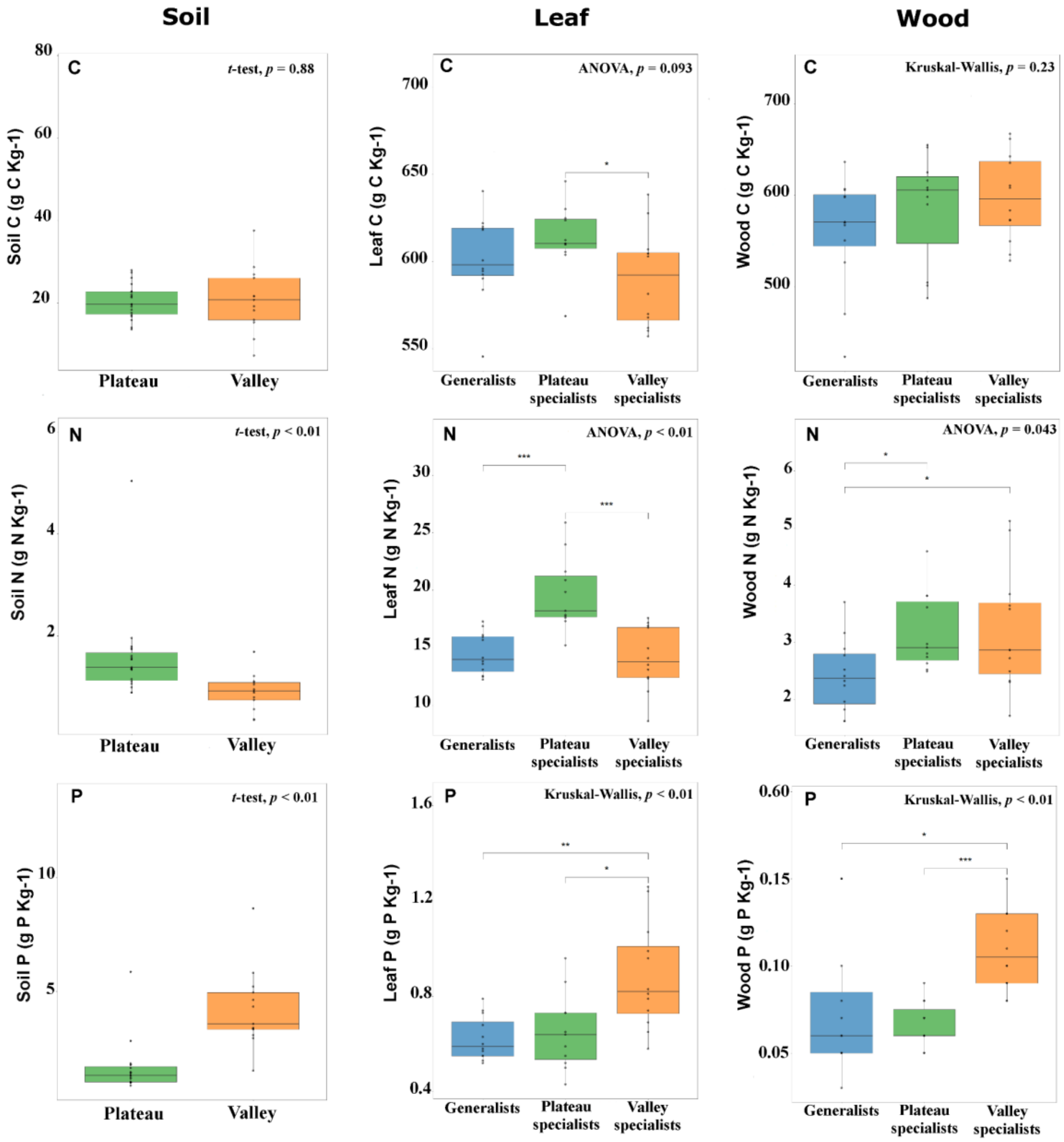


Figure 3 Carbon (C), nitrogen (N), and phosphorus (P) concentrations in soil, leaves, and wood across topographic habitats (plateau vs. valley) and among functional groups (generalists, plateau specialists, and valley specialists). Statistical tests are indicated in each panel (*t*-test, ANOVA, or Kruskal–Wallis), with post hoc significance denoted by asterisks (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

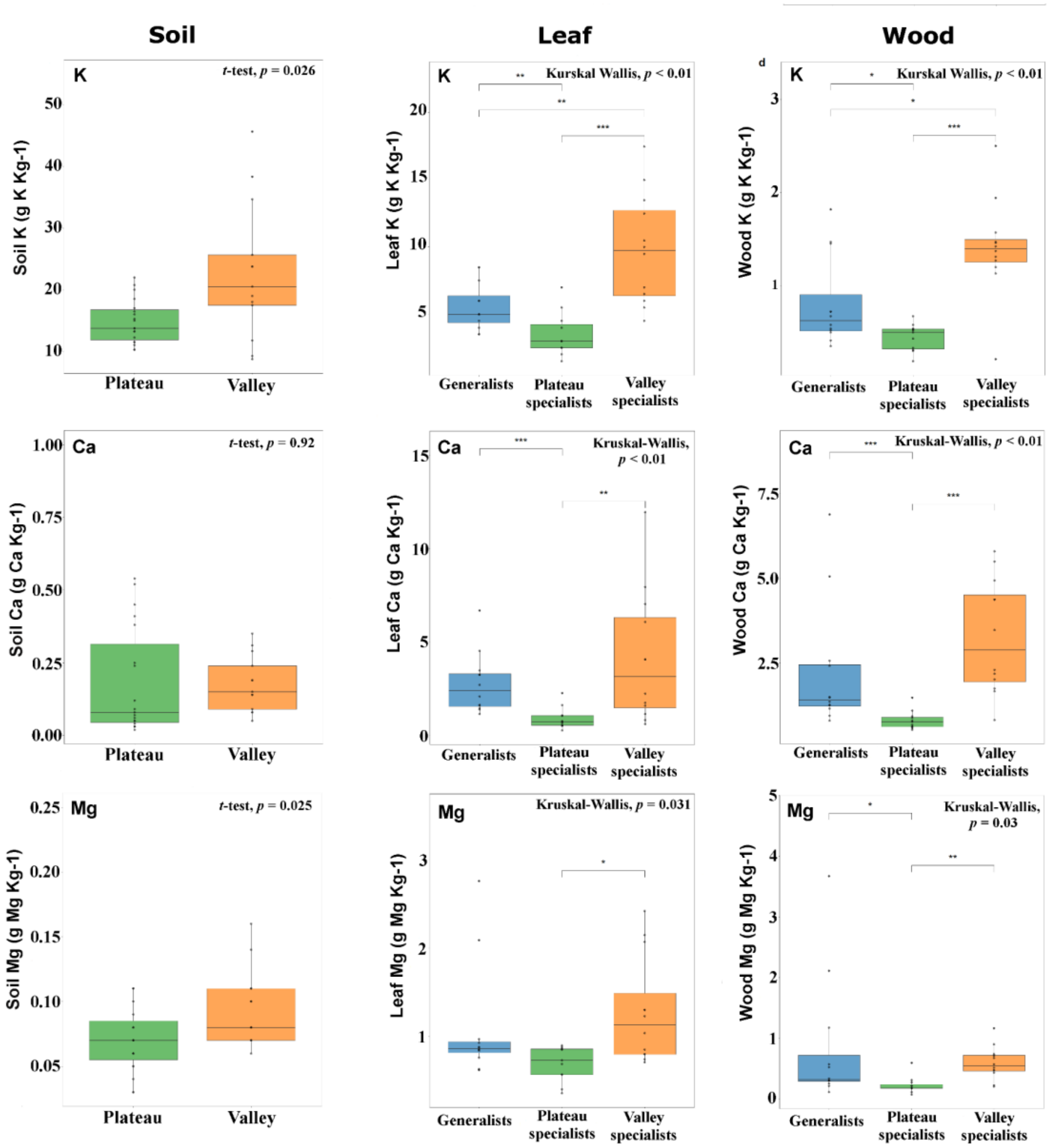


Figure 4 Potassium (K), calcium (Ca), and magnesium (Mg) concentrations in soil, leaves, and wood across topographic habitats (plateau vs. valley) and among functional groups (generalists, plateau specialists, and valley specialists). Statistical tests are indicated in each panel (*t*-test, ANOVA, or Kruskal–Wallis), with post hoc significance denoted by asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$).

- Further, for now, the discussion of the results is quite limited and a bit 'shallow'. I believe the results are valuable, but require better understanding and more consistent and robust analysis. It is, e.g., important to acknowledge that you are not looking into soil nutrient availability, only to total nutrient stocks. Hence, is it surprising you don't find strong correlations with tissue nutrient contents?

Response: We thank the reviewer for this valuable observation. We have now expanded the discussion section to better cover our results especially regarding the new analysis that we did on the revised version of the manuscript. Our study was carried out at a more localized scale, in comparison with, for example, the study of Bauters et al. (2022) who conducted at a much broader, pantropical scale. Bauters et al. found strong correlations between nutrient concentrations in soils and plant tissues, particularly for calcium (Ca), magnesium (Mg), and potassium (K). In our case, variations in nutrient availability along the topographic gradient may be more subtle compared to other pantropical studies, as the underlying bedrock and edaphoclimatic conditions are relatively the same. However, P for example, is highly mobile within plants, facilitating internal redistribution through processes such as foliar nutrient resorption, while it remains relatively immobile in soils (see Vergutz et al., 2012). Therefore, the results obtained for nutrient concentrations from different functional groups in this study, as well as discussions addressing their internal nutrient demands, may be more informative at local scales. Even for other nutrients besides P, such as Ca and Mg, plant tissue concentrations could be better described by investigating different plant functional groups (e.g., generalists and specialists according to topographic position). Nonetheless, adequate considerations are needed, since, for example, Ca is one of the main components of plant cell structure and is a practically immobile element within plants. In this context, although we maintained the Spearman correlation matrix between soil, wood, and foliar nutrient concentrations (which we now recognize may overwhelm the reader with numerous correlations and obscure the main findings), we aimed to highlight the most significant results of this study: the coordination between wood and leaf nutrient concentrations (as shown in the figure below) and their variation across different plant functional groups (Figures 3 and 4), with some relationships to soil to a certain extent. The figure below was generated using the amount of nutrients in plant tissues (expressed on a fresh weight basis in kg), calculated with the biomass equations proposed by Silva (2007) and described in the Materials and Methods section (*Section 2.6: Quantification of carbon and nutrients in leaf and wood* – equations 4 to 6). These calculations were performed for each sampled individual using their respective DBH values to estimate the total amount of each nutrient per compartment (leaf and wood). In this way, DBH variation was reflected in the biomass of each individual and, consequently, in the calculated nutrient amounts. This approach allowed us to account for individual differences in size, effectively incorporating size variation into the nutrient estimates. This point was raised by the reviewer in the annotated pdf.

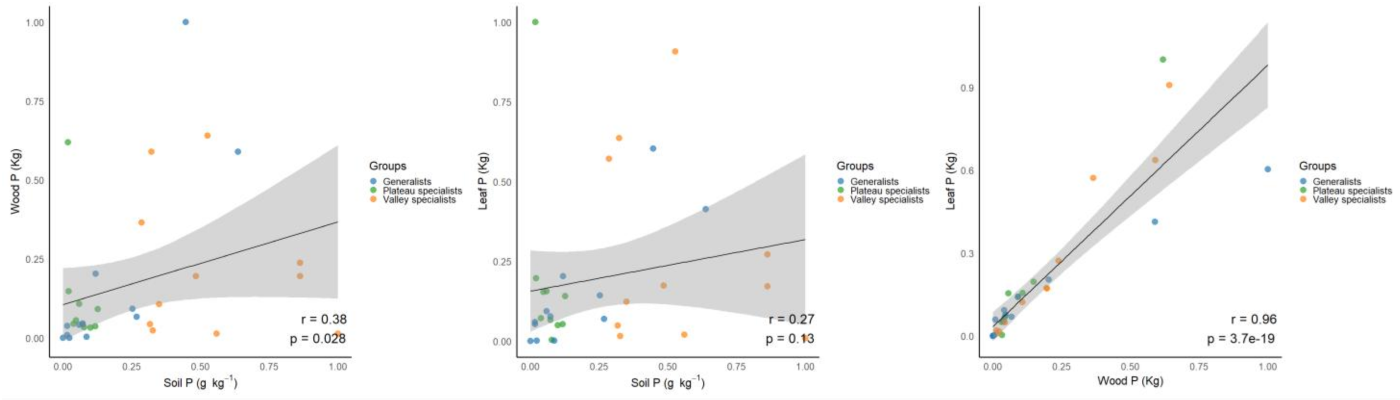
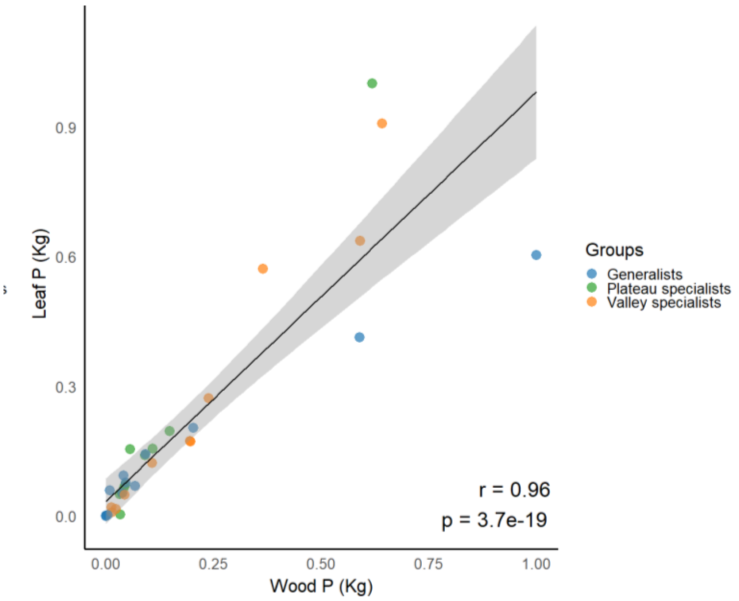
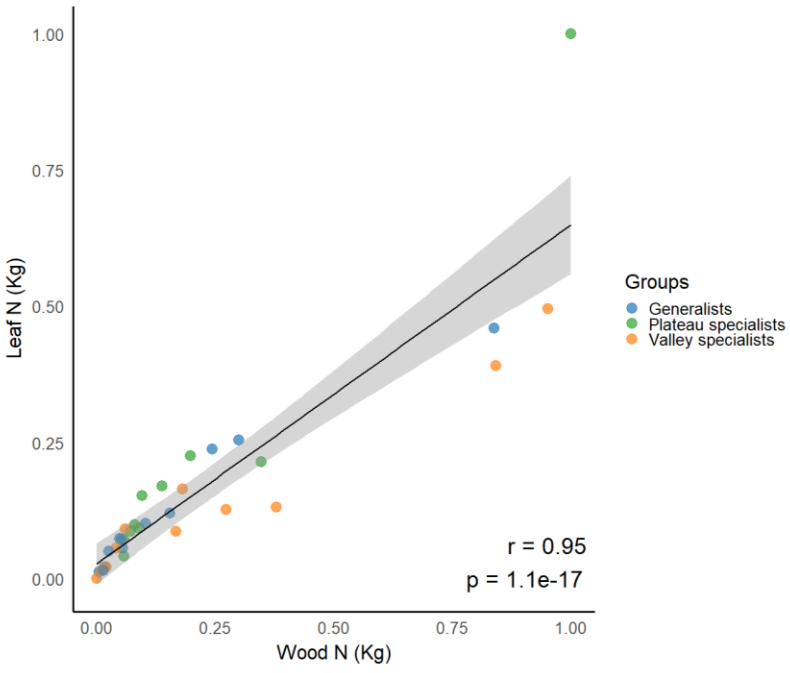


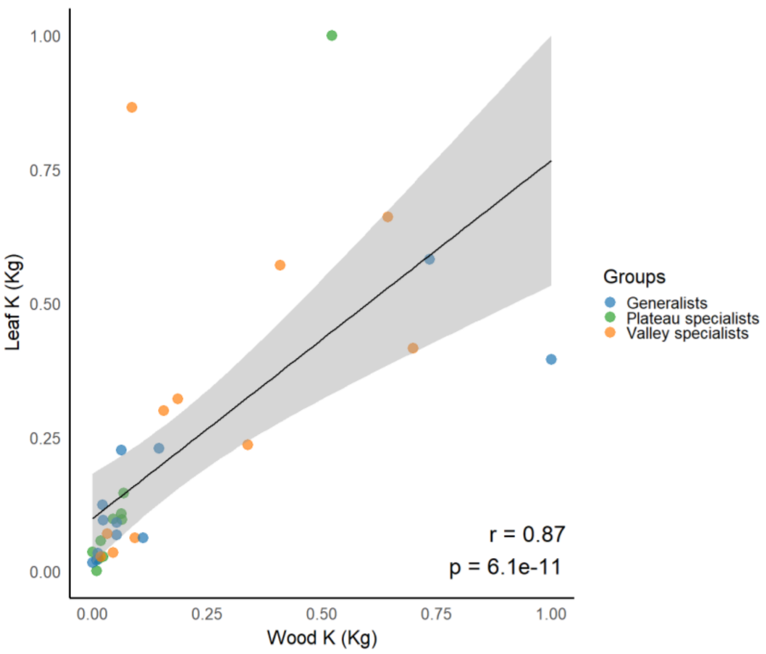
Figure X – Scatterplot showing the relationship between Soil P, Leaf P and Wood P nutrient amount concentrations (based on their fresh weight) for functional groups. This figure will be incorporated in the revised version of the manuscript.

- I'm still a bit unclear to what the actual impactful result is you found. As mentioned before, the discussion contains many general statements that are not necessarily informative. Following is an example from the abstract: "Trunk carbon concentrations did not vary significantly compared to leaves, suggesting that other biological or environmental factors influenced tree nutritional status." --> What do we learn from this? Is it maybe simply to be expected that wood vs. foliar carbon contents vary to a different extent? How is this linked to the 'tree nutritional status'?

Response: In the revised version of the manuscript, we clarified the key findings more explicitly**.** In our view, the most impactful result of the study is the strong correspondence between nutrient stocks in plant tissues (leaf and wood), as shown in the figures above, and, to a certain extent, between plant tissues and soil nutrient concentrations. For instance, plateau soils exhibited significantly higher nitrogen concentrations (*t*-test, $p < 0.01$) than valley soils. This pattern was mirrored in the higher N concentrations in the leaves and wood of plateau specialist species compared to valley specialists and generalists (ANOVA, $p < 0.001$) (Figure 3). Similarly, valley soils, which are sandier, had significantly higher concentrations of phosphorus and potassium, and this was reflected in the leaf and wood tissues of valley specialists, which had the highest P and K concentrations. A similar analysis can be conducted comparing leaf and wood tissues, which showed strong correspondences (please see the new figures below). These patterns between soil and nutrient in tissues suggest that soil nutrient availability along topographic positions plays to some extent a role in plant nutrient status. While we did not explicitly quantify nutrient use efficiency (NUE) or focus on plant nutrient acquisition strategies (e.g., fine roots, AMF associations), our results offer a foundation for future research exploring how species-specific adaptations to nutrient availability influence species distributions and functional traits across heterogeneous environments such as those found in sandy valleys and high clay content plateaus in the Central Amazon. Despite that, we recognize that this relationship between soil properties and species distributions is not entirely new in tropical forests (e.g., John et al., 2007; Russo et al., 2007; Zuleta et al., 2020; Davies et al., 2005). However, regarding the Central parts of the Amazon basin our contribution lies in demonstrating, at a local spatial scale, that the relatively differences in soil nutrient stocks across topographically distinct habitats (plateaus vs. valleys) are partially reflected in nutrient allocation to leaves and wood among generalist and specialist tree species. This highlights the importance of incorporating spatial heterogeneity of soil nutrients in trait-based and ecosystem modeling studies, particularly in the Amazon, where topography is closely linked to nutrient availability, forest structure, productivity, and hydraulic traits (e.g., Cosme et al., 2017; Soong et al., 2020; Sousa et al., 2022; Costa et al., 2023). In summary, our results demonstrate that soil nutrient variability partially reflects plant nutrient patterns across functional groups, and that there is strong coordination of nutrient stocks between wood and leaf. The new figures shown below, which are being developed for the revised version of the manuscript, will illustrate these findings.







- Additionally, in the discussion, always start with your results, and then put them in a context using literature.

Response: We have added a paragraph at the beginning of the discussion section highlighting the major findings of the study, supported by appropriate references.

Additional comments were written on the attached document, close to the corresponding sentence in the MS. Please also formulate a reply to these comments/questions in case of resubmission.

Response: We thank the reviewer for the valuable and careful review included in the attached document. We are carefully addressing each point and will provide detailed responses and corresponding revisions shortly.

Minor general comments:

- Use 'wood' instead of 'trunk' or 'trunk woody material'

Response: We have changed 'trunk' for 'wood' in the entire text.

- Use 'content' instead of 'concentration' for mass-based measures (µg /g etc)

Response: done.

- Add a soil properties description table to the site description or results.

Response: We have added soil properties description as a supplementary table.

- The colors in Figures 3, 4, 5 are not particularly meaningful

Response: We have updated the graph colors to a blue/orange palette to broaden the paper's accessibility and ensure the figures are more colorblind-friendly.

- Make a clear distinction in terminology between plant functional types, often defined in the context of pioneer trees vs. shade-tolerants etc. Just to avoid confusion.

Response: We have standardized the terminology for plant functional types (PFTs) throughout the manuscript.

- Make sure to use abbreviations in full only at first mention: e.g., 'carbon (C)' --> Later only 'C'

Response: Thanks for the observation, we reviewed all the abbreviations throughout the manuscript.

- Fig. 3: plot means with diamond shapes, make y-axis labels bigger and simpler: 'Soil C (g C kg-1)', make sure to add the specific element after 'g' as in this example, why are they called 'average' concentrations? (similar remarks for Fig. 4 and 5)

Response: We updated the y-axis labels to be bigger and more straightforward, such as "Soil C (g C kg^{-1})," as recommended. The layout of figures 3 and 4 (which were derived from the figures 3, 4, and 5 in the previous version) was completely revised and improved.

- Fig. 6: what is the shaded area?

Response: The shaded area represents the 95% confidence interval of the regression.

- Fig. 7: improve names: "Ca_Soil_Mg.ha" --> "Soil_Ca", put units in caption + What is the logic behind the colors?

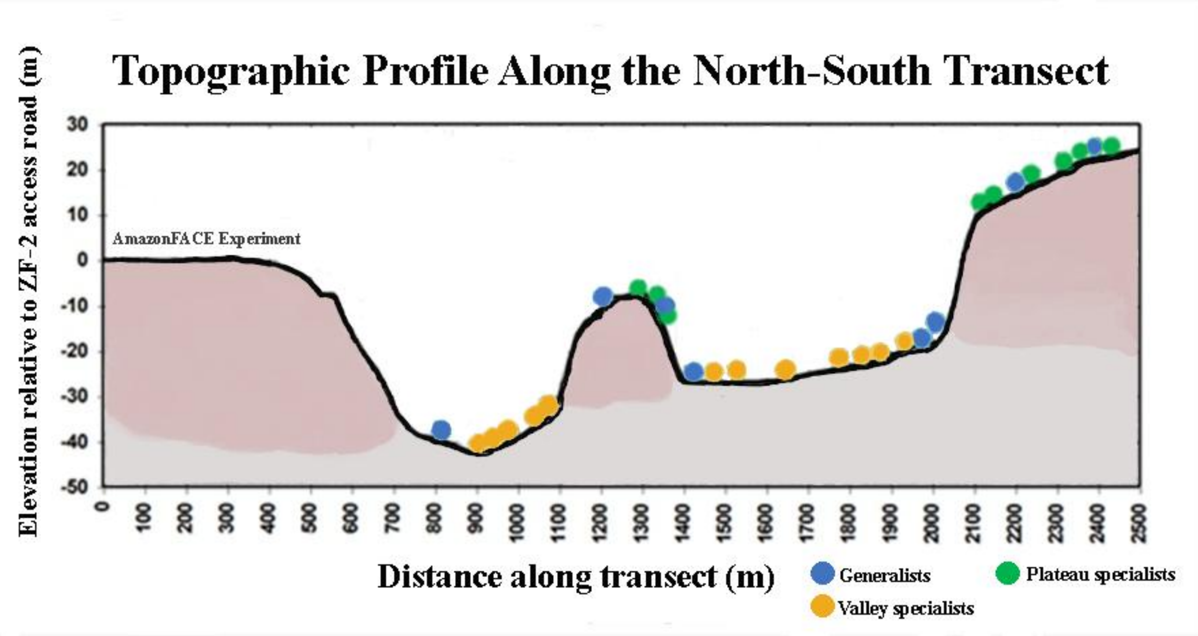
Response: We have updated the names for Soil_Ca; Soil_P; etc. The logic behind the colors is that the "warm"colors (red) represent the tendency to positive correlations and the "cold" colors (blue) represent negative correlations.

- Why do you note down doi links for a few references only?

Response: We have updated the references to include doi links for all of them.

- Fig. S1: use different colors for different tree categories (--> how spatially separated are the classes actually?) + add classification what you define as valley and what as plateau. + relative height is relative to what?

Response: We have updated figure S1 as suggested (please see below). In the revised figure we included the classification of valley specialists, plateau specialists, and generalists along the topographic gradient. We also used distinct colors to differentiate the clay-rich plateaus and sandy valleys. Additionally, we indicated the location of the AmazonFACE experiment on the first plateau of the NS transect, noting that we avoided sampling trees in that area to prevent interference with the experiment. The relative height shown in the figure is related to the ZF-2 access road located in the plateau (we described it better in the figure and text).



Supplementary Figure 1: Topographic profile of the North - South Transect. Blue dots represent the generalist species, green dots represent plateau specialists and the orange dots represent valley specialists. Note that some of the 35 sampled individuals appear to be overlapped due to their close spatial proximity in the field.

- Fig. S2: I count 13 trees for valley specialists

Response: we have reviewed the supplementary fig 2 to represent the DBH variation in all selected individuals.

- Fig. S3: Give info only for relevant species

Response: Thank you for the observation. We are updating Figure S3 to include information only for the relevant species and will share the revised figure in the revised version of the manuscript.

- Generally: what do the confidence intervals indicate? How were they constructed?

Response: For our understanding, confidence intervals represent the range within which we expect the average of a given variable to lie, with a certain level of confidence. A 95% confidence interval indicates that, if the same sampling process were repeated many times, approximately 95% of the calculated intervals would contain the true population mean. In our analyses, we used 90%, 95%, and 99% confidence intervals. Accordingly, p-values < 0.1, < 0.05, and < 0.01 were considered statistically significant to varying degrees.

- Is there no suppl. fig. 6?

Response: We missed the counting of suppl. figures.What was previously labeled as Supplementary Fig. 7 is actually Supplementary Fig. 6. We did the corrections accordingly in the revised version of supplementary material.

- see comments in attached file for Suppl. Fig. 7 & 8.

Response: Thanks! We are already working on it.

References

Cosme, L. H. M., Schietti, J., Costa, F. R. C., and Oliveira, R. S.: The importance of hydraulic architecture to the distribution patterns of trees in a central Amazonian forest, New Phytol., 215, 113–125, https://doi.org/10.1111/nph.14508, 2017.

Costa, F. R. C., Schietti, J., Stark, S. C., and Smith, M. N.: The other side of tropical forest drought: do shallow water table regions of Amazonia act as large-scale hydrological refugia from drought?, New Phytol., 237, 714–733, 2023.

Davies, S.J., Tan, S., LaFrankie, J.V. & Potts, M.D. (2005) Soil-related floristic variation in the hyperdiverse dipterocarp forest in Lambir Hills, Sarawak. Pollination Ecology and Rain Forest Diversity (eds D.W. Roubik, S. Sakai & A. Hamid), pp. 22–34. Springer-Verlag, New York.

John, R., Dalling, J. W., Harms, K. E., Yavitt, J. B., Stallard, R. F., Mirabello, M., Hubbell, S. P., Valencia, R., Navarrete, H., Vallejo, M., and Foster, R. B.: Soil nutrients influence spatial distributions of tropical tree species, Proc. Natl. Acad. Sci., 104, 864–869, https://doi.org/10.1073/pnas.0604666104, 2007.

Pastorello, Gilberto, Lima, Adriano, Gimenez, Bruno, Longo, Marcos, Chambers, Jeff, & Higuchi, Niro (2024). Harmonized wood density data for Central Amazon species in the BIONTE experimental area in Manaus, Brazil. https://doi.org/10.15486/ngt/1898906

Russo, S.E., Brown, P., Tan, S. and Davies, S.J. (2008), Interspecific demographic trade-offs and soil-related habitat associations of tree species along resource gradients. Journal of Ecology, 96: 192-203. https://doi.org/10.1111/j.1365-2745.2007.01330.x

Soong, J. L., Janssens, I. A., Grau, O., Margalef, O., Stahl, C., Van Langenhove, L., Urbina, I., Chave, J., Dourdain, A., and Ferry, B.: Soil properties explain tree growth and mortality, but not biomass, across phosphorus-depleted tropical forests, Sci. Rep., 10, 2302, 2020.

Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F. and Jackson, R.B. (2012), Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecological Monographs, 82: 205-220. https://doi.org/10.1890/11-0416.1

Zuleta, D., Russo, S. E., Barona, A., Barreto-Silva, J. S., Cardenas, D., Castaño, N., Davies, S. J., Detto, M., Sua, S., Turner, B. L., and Duque, A.: Importance of topography for tree species habitat distributions in a terra firme forest in the Colombian Amazon, Plant Soil, 450, 133–149, https://doi.org/10.1007/s11104-018-3878-0, 2020.