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Response to Reviewer 2

In this study, the authors ask how environmental variation drives specialist and generalist species distributions in a terra-firme tropical forest in the central Amazon. They examine how tree nutrient tissue concentrations across specialist and generalist species are distributed along a topographic gradient (valley vs. plateau) that varies in soil nutrients. The authors hypothesize that specialists and generalists differ in their tissue nutrient content which could explain their distributions. In addition, they hypothesize that wood density is a strong predictor of wood nutrients, and that wood nutrients correlate with soil nutrient availability. They selected three plateau specialists, three valley specialists, and three generalists, replicating four individuals per treatment for a total of 35 individual trees, collecting samples for leaf and trunk measure carbon, nitrogen, phosphorus, calcium, potassium, and magnesium in addition to soil measurements.

In summary, they found that specialist species restricted to the valley had the highest leaf and wood nutrient content, reflecting the soil nutrient availability, and that nutrient concentrations within generalists remained relatively consistent across topography, suggesting adaptability across nutrient gradients. They also found coordinating between leaf and wood nutrients, but no relationships between wood density and nutrient content.

These are important questions as we know very little about nutrient content in wood, despite wood pools being a very large sink of nutrients in highly weathered tropical forests (e.g. Bauters et al., 2022 and Dalling et al., 2024). In addition, we know very little as to how leaf and wood tissues are coordinated, and how well tissue nutrient content reflects soil nutrient availability and bioavailability of soil nutrients to plants.

Response: We thanked Reviewer 2 for their supportive revision and constructive comments. We have carefully considered all points raised in preparing our revised manuscript and have provided a detailed, point-by-point response to each comment.

Overall, I have some major comments to improve the interpretation of the findings.

Contextualizing the importance of the study: I think that one of the main arguments for this study is that we understand that soil nutrient availability can drive species distributions, but the mechanisms are relatively unknown. In addition, nutrient stocks in leaves and wood are often understudied, but could help explain species distributions and how species are able to persist on soils with low nutrient availability via changes in nutrient-use efficiency. This background could be set up a bit better; currently the first paragraph doesn't introduce what the study is focusing on, which is on tissue nutrient concentrations.

Response: We have revised the introduction to better contextualize the motivation and relevance of our study. While we did not directly quantify nutrient use efficiency (NUE) of plant species or assess nutrient acquisition mechanisms such as fine root dynamics or AMF associations, we incorporated these considerations into the revised manuscript. We recognize that the observed variation in leaf and wood nutrient concentrations across species and habitats suggests that such strategies may play an important role in shaping species distributions along soil fertility gradients in the Central Amazon. Although the relationship between soil properties and species distributions in tropical forests has been well documented (e.g., John et al., 2007; Russo et al., 2007; Zuleta et al., 2020; Davies et al., 2005), as the reviewer pointed out, relatively little is known about nutrient concentrations in wood and leaves of tropical species growing in highly weathered soils (e.g., Bauters et al., 2022; Dalling et al., 2024). Based on that, we did a better contextualization of our study shows highlighting that at a local spatial scale in the central Amazon, soil nutrient stocks across topographically distinct environments (sandy valleys vs. clay-rich plateaus) are reflected in nutrient allocation to plant tissues—particularly in leaves and wood—among generalist and specialist species.

Data presentation: I understand the value of focusing on stocks, but there are a lot of extrapolations made to estimate these stocks (lines 214-230). To understand how soils and tissues are coordinated, it would be very helpful to see scatterplots such as in Heineman et al. 2016 with tissue concentrations, similar to Figure 6. You could make side-by-side panels with concentration data and stock data, for example. In addition, for the correlation matrix, the same could be done with concentrations rather than stock data; for example a correlation matrix between leaf and wood nutrient concentrations, soil and leaf nutrient concentrations, and wood and soil nutrient concentrations. Currently, it is not super clear based on the labels in Figures 7 and 8 what properties are being compared/correlated.

Response: We appreciate the comment and the suggestion for using scatterplots. We are currently preparing normalized scatterplots to incorporate into the manuscript. We observed strong correlations in nutrient stocks between wood and leaf for many of the analyzed nutrients, as shown in the figures below. These results reveal a clear coordination of nutrient concentrations between different plant organs (high correlations; $r > 0.90$ for some nutrients such as P, N, K, for example), which may provide valuable insights into nutrient dynamics across functional groups and contribute to our understanding of how nutrient stocks are coordinated within plants. The amount of nutrients in plant tissues (expressed on a fresh weight basis in Kg) was calculated using the detailed equations provided in the Materials and Methods section, based on the methodology proposed by Silva (2007; equations 4 to 6). The 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) (see *Section 2.6: Quantification of carbon and nutrients in leaf and wood*). This information is highly relevant for improving ecological and Earth system models. These new figures will be shown in the revised version of the manuscript as well as the relative discussion of the findings.

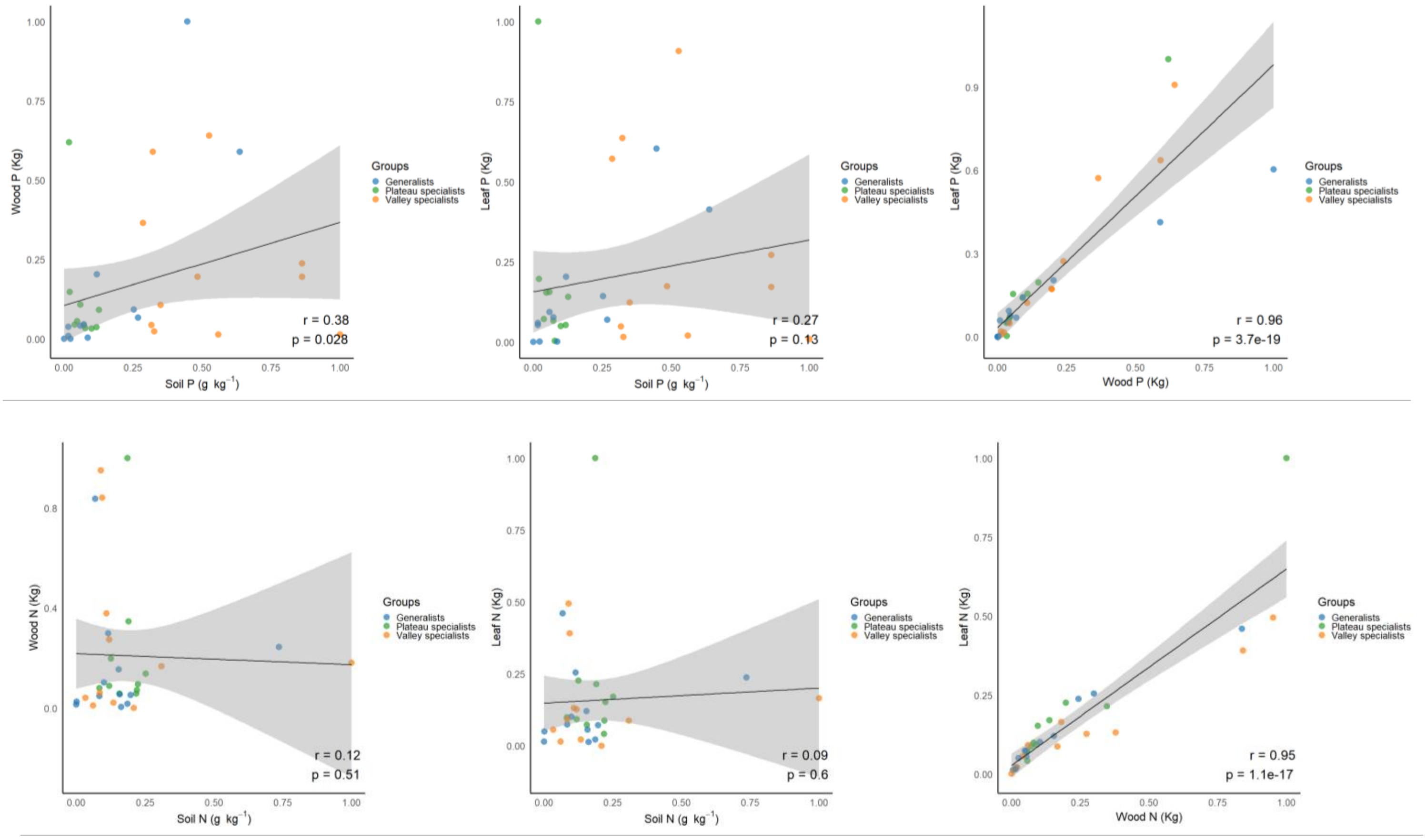

Figure X – Scatterplot showing the relationship between soil, leaf and wood nutrient stocks for some nutrients for all functional groups. This figure will be incorporated in the revised version of the manuscript.

Data presentation: It might be easier to interpret the differences between valley vs. plateau specialists compared to generalists if information from Figures 3, 4, and 5 were integrated more. For example, since there are no differences between C and Ca between the valley and plateau soils, this is important to remember when looking at Figures 4 and 5 when comparing valley and plateau specialists vs. the generalists. I might try a multipaneled figure where the soil, wood, and leaf data are presented across columns and the different nutrients across rows or something like that. Similarly, the results can also integrate the soil and tissue concentration data; for example, "Consistent with the higher Mg concentrations in the valley, wood and leaf Mg concentrations were also higher for valley specialists"

Response: We thank the reviewer for the valuable recommendation. Figures 3, 4, and 5 have been updated and reorganized into two composite figures (now Figures 3 and 4, shown below). In this revised layout, soil, leaf, and wood nutrient concentrations are presented together to improve the visualization of the difference in nutrient concentrations across topographic levels (plateau and valley) and functional types (generalists, valley specialists, and plateau specialists). Based on these updated figures, we also revised the Results section of the manuscript to reflect the new presentation, as follows: "*Valley soils presented significantly higher concentrations of P, K, and Mg compared to plateau soils. Similarly, foliar P concentrations differed significantly between plateau and valley specialists (Dunn, $p \leq 0.05$), and between generalists and valley specialists (Dunn, $p \leq 0.01$) (Fig. 3), with valley specialists showing, on average, the highest concentrations of P in both wood and leaves.*"

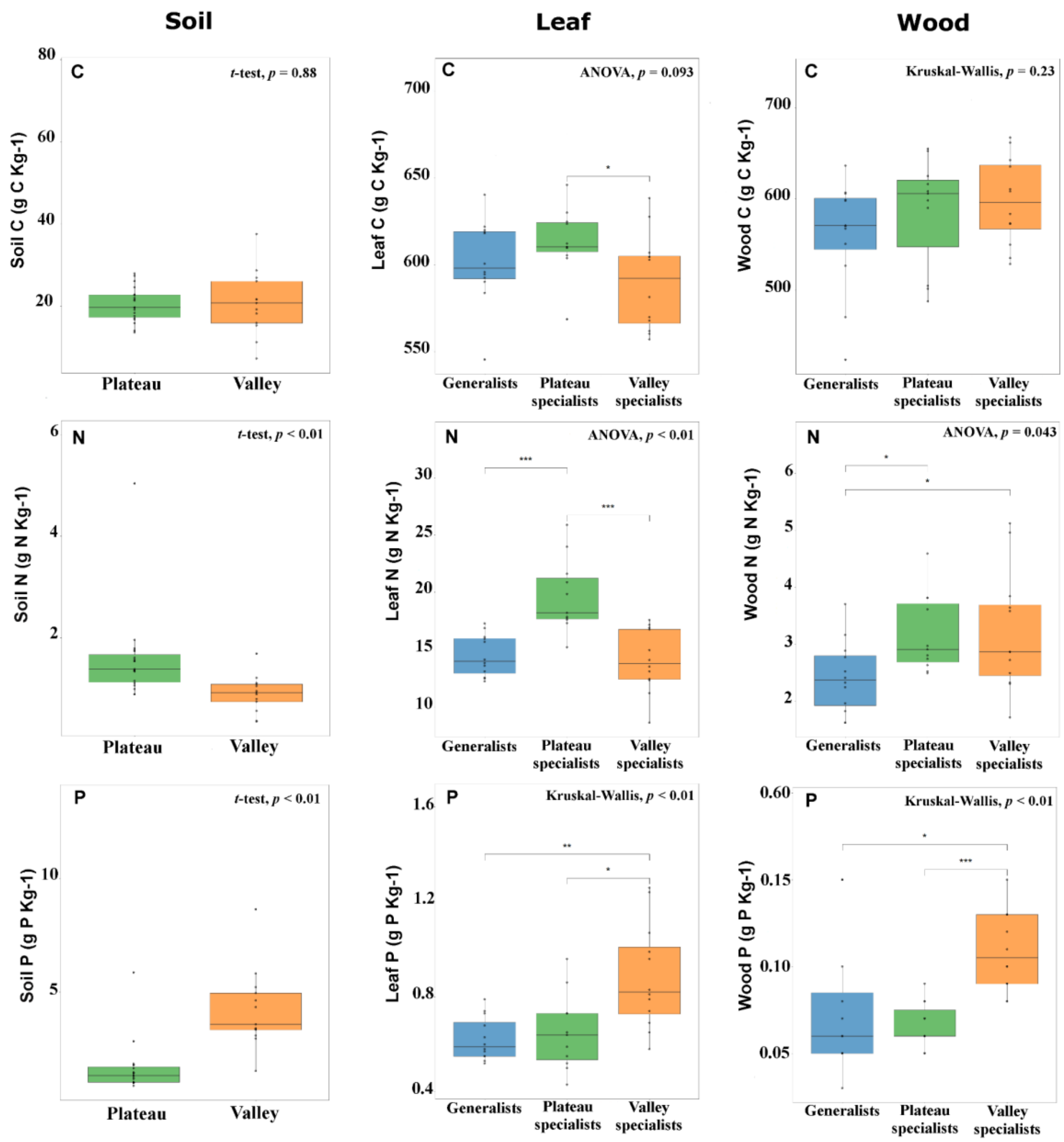

Figure 3 Carbon (C), nitrogen (N), and phosphorus (P) concentrations in soil, leaves, and wood across topographic habitats (plateau vs. valley) and among functional groups (generalists, plateau specialists, and valley specialists). Statistical tests are indicated in each panel (*t*-test, ANOVA, or Kruskal–Wallis),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

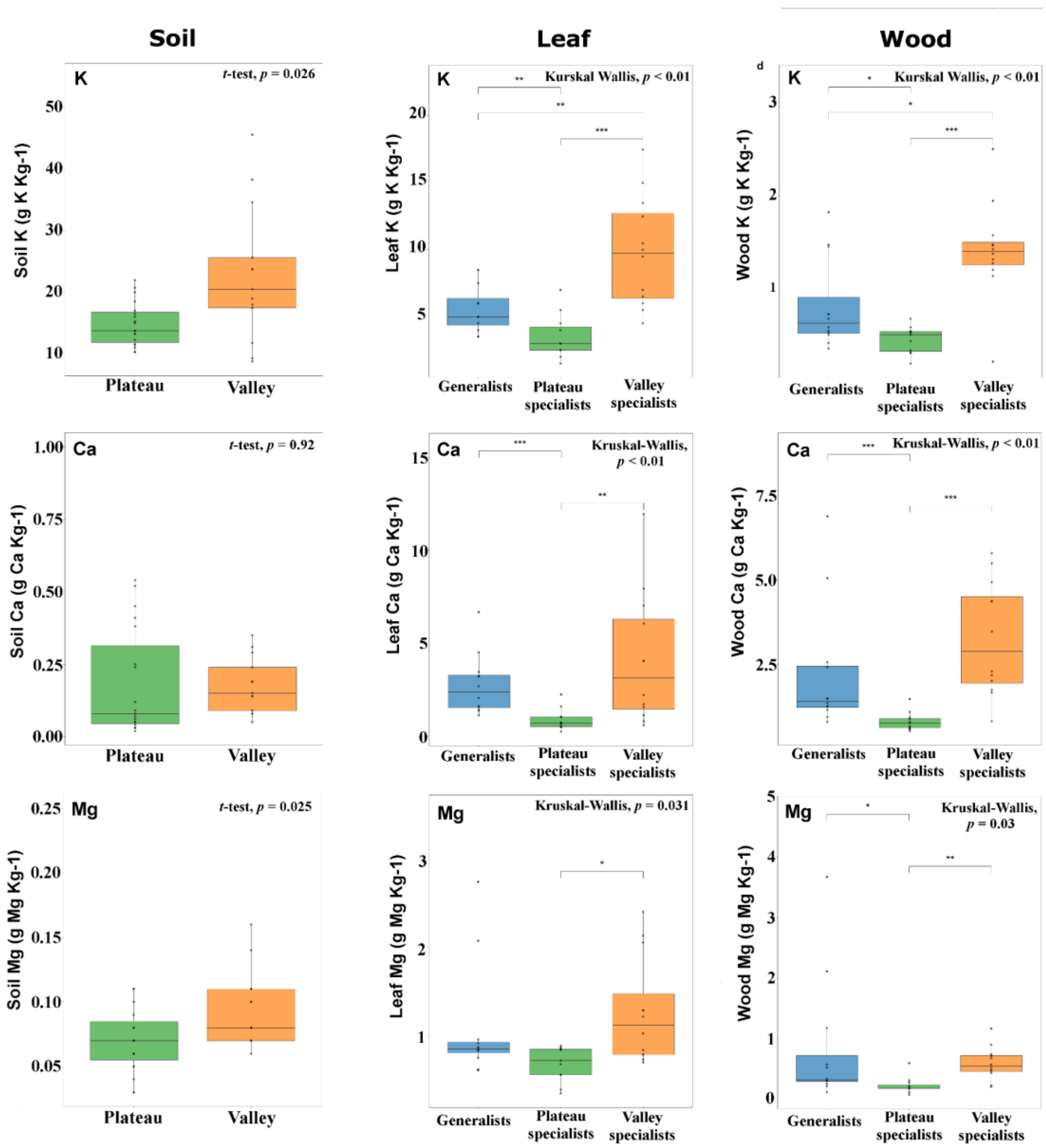

Figure 4 Potassium (K), calcium (Ca), and magnesium (Mg) concentrations in soil, leaves, and wood across topographic habitats (plateau vs. valley) and among functional groups (generalists, plateau specialists, and valley specialists). Statistical tests are indicated in each panel (*t*-test, ANOVA, or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

Explaining species selection: why were these nine species selected, and what is the relative abundance of the selected species at the site? Why were the species selected ranging in diameter classes? More context as to how specialists vs. generalists are defined would help; for example, if they are common and abundant, these characteristics may be more reflective of generalist species.

Response: Species selection in the Amazon is inherently challenging due to the extremely high tree diversity. In the Central Amazon, a single hectare can contain up to 700 tree species (Rankin-de Merona et al., 1992; Chambers et al., 2000), with roughly half belonging to hyperdominant genera and the other half to rare species (ter Steege et al., 2013). This high diversity makes it difficult to work at the species level, as the sampling effort required to obtain sufficient replicates is considerable. To partially address these issues, permanent plots networks can be used to monitor and select species at different scales (example: plots from ForestGEO). In this sense, the North–South (NS) permanent plot, which spans 5 hectares and includes 3,522 tree individuals ≥10 cm DBH that are monitored annually across a pronounced topographic gradient (valleys and plateaus) was used to categorize the different functional groups and select the species and individuals of the present study. An example of the partial distribution of species along the NS transect is shown in Supplementary Figure S3. Based on that, species were selected based on two primary criteria: (1) Topographic distribution—we defined *specialists* as species that occur only in one topographic position (either plateau or valley), and *generalists* as those found in both environments. (2) Sampling feasibility—we selected species that had at least four individuals (replicates) available for sampling. Based on these criteria, we selected nine species in total.

Given the high diversity and logistical constraints, the selected individuals naturally varied in DBH. Standardizing DBH across individuals of different species was not feasible due to the difficulty of locating similar-sized individuals in sufficient numbers, particularly for less common species. Nonetheless, the selected species include representatives of both common (hyperdominant) genera (see ter Steege et al., 2013)—such as *Eschweilera tessmannii* and *Protium hebetatum*—and less abundant taxa, providing a representative cross-section of functional types across the study area.

Nutrient availability at the site: First, it would help to contextualize general nutrient limitation at the site: are these soils low in base cations or phosphorus? This could help understand which nutrients may be more critical for structuring community composition. The setup for distinguishing valley vs. plateau soils could also be structured better, and topography as a driver of soil nutrient availability should be introduced in the introduction. In addition, the authors need to explain how the soils were measured and what extractants were used; total or "available" fractions for example, as well as the tissue concentrations: were they ashed at 500C and digested in aqua regia? Reporting the concentration rather than the stock data would also help contextualize how nutrient concentrations in soils and tissues vary across sites. For tissues, C:N ratios might also be more helpful in textualizing N rather than C and N alone, for example. Another aspect could be to examine tissue relationships with the topsoil only, and to show depth distributions of soil in the supplement to see how much the soils vary across depth rather than integrating the entire top 50 cm.

Response: We thank the reviewer for the valuable comment. Soils in the Central Amazon are known to be highly P-limited (see Cunha et al., 2022; https://www.nature.com/articles/s41586-022-05085-2), although other nutrients are also scarce. We have also rewritten the introduction and updated the methods section to clarify how soils were measured and which extractants were used. We appreciate the suggestion to consider C:N ratios, and we are currently conducting additional analyses incorporating this metric. Some initial analyses were performed using data from the topsoil layer (0–5 cm), but our preliminary correlation matrix did not reveal strong relationships between soil and plant tissue nutrient concentrations (see the figure below). The figures 7 and 8 in the original version already presented correlation matrices using total nutrient stocks, with no exciting correlations of correlations between soil and plant tissues. We are continuing to investigate this further to better understand the observed patterns, especially considering the role of topsoil, which is particularly relevant in tropical, nutrient-depleted systems where the upper soil layer contains the highest concentrations of organic matter. These updates and analyses will be reflected in the revised version of the manuscript.

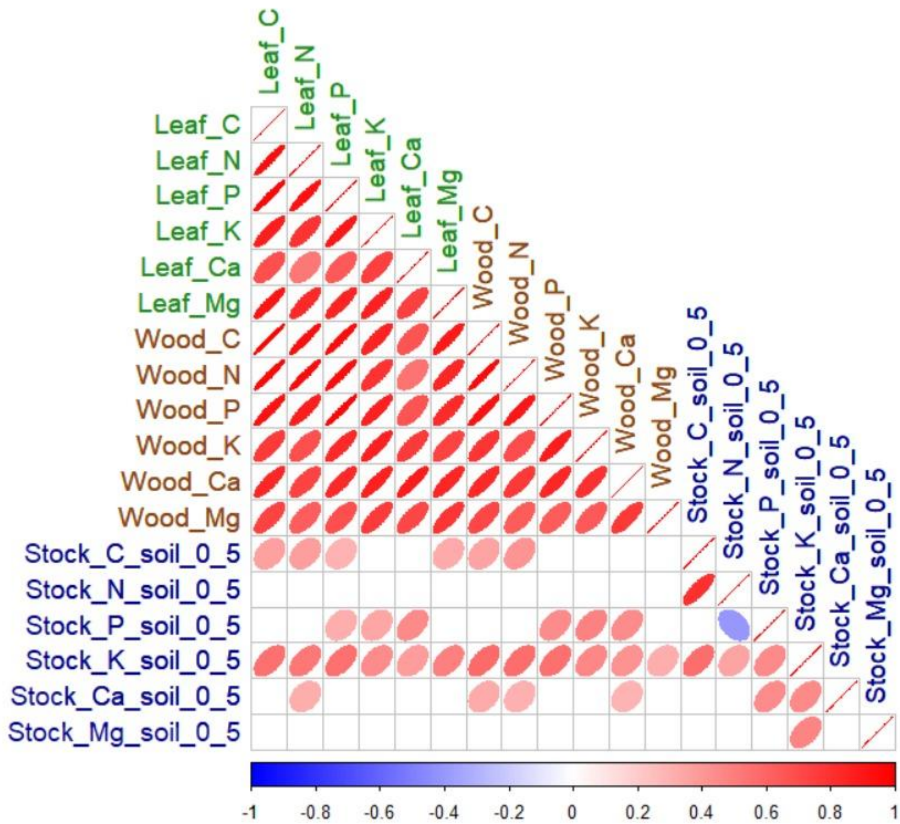

Figure XX - Example of analysis that we are currently doing to see the correlations between the total amount of carbon and nutrients in the leaf and wood and the stock of carbon and nutrients in the both valleys and plateau topsoil (first 0-5 cm).

Why was the growth rate (periodic annual increment) measured, and could an analysis be done comparing nutrient content with growth rates?

Response: This is an important question. We calculated growth rates, expressed as periodic annual increment (PAI), because growth is a key variable for distinguishing plant functional types (PFTs), particularly for fast- to slow-growing spectrum. In addition to presenting average growth rates by species, we are currently conducting further analyses to explore the relationship between nutrient concentrations and growth, similar to the correlation shown in Figure 6 (between wood density and wood nutrient concentrations). We plan to include these results as a supplementary figure in the revised version of the manuscript.

Spend the discussion more on interpreting the results. I would recommend restructuring the discussion around the main findings, discussing whether or not the findings supported your hypotheses (and if they were surprising or not), contextualizing them with previous work, and discussing the generality of these results and what they might tell us about tropical forest nutrient cycling or species distributions more broadly.

Response: We thank the reviewer for the observation. We have rewritten the entire Discussion section in the revised version of the manuscript to more clearly align with and highlight the main findings of the study.

Line by line comments:

Title: the title should include topography rather than soil texture

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

Abstract: The abstract could benefit from a hypothesis: “we hypothesized that across a nutrient gradient, generalists might differ from specialists in their stoichiometric demands for nutrients and carbon.” The abstract could also benefit from explaining the role of topography upfront: how do you expect nutrient availability to change across topography; higher nutrient availability in the valley and lower nutrient availability in the plateaus?

Response: The abstract was rewritten in the new version to better represent our hypothesis.

Line 15: Some “tree” or “plant” species?

Response: thanks. changed to “tree species”.

Line 16: “We” instead of “This study”

Response: Done. Line 20: I believe analysis and quantification are the same thing; you can just write “for total carbon and nutrient analysis”

Response: Replaced by: “for total carbon and nutrient analysis”

Line 34: perhaps “lowland” Amazon? The western Amazon is thought to be much higher in soil fertility?

Response: This is a very good point. We will revise this part, but we think “Central Amazon” is the better way to describe what we want to mean in terms of soil fertility.

Line 46: “The primary reservoir of essential nutrients for most forest ecosystems is from the soil”

Response: Thanks. We have corrected the sentence accordingly in the revised manuscript.

Line 51: another citation could be Dalling et al., 2024Dalling, J.W., Flores III, M.R. and Heineman, K.D., 2024. Wood nutrients: Underexplored traits with functional and biogeochemical consequences. New Phytologist, 244(5), pp.1694-1708.

Response: Thanks for the reference. We used Dalling in this and other parts of the manuscript.

Line 56: do you mean, serving as a long-term nutrient reserve?

Response: yes, thank you!

Line 60: perhaps “experiments testing tree responses to P limitation”?

Response: thanks for the suggestion.

Lines 60-62: this sentence is very confusing; it is not clear how maximum electron transport rates are related to foliar nutrient concentrations and wood density

Response: We have revised and improved the entire introduction section in the new version of the manuscript.

Line 92: “trunks”

Response: we changed trunks for wood throughout the manuscript.

Line 100: perhaps describe the site in a more general pattern: “the study was conducted in tropical forests in the central Amazon Basin…”

Response: We thank the reviewer for the observation. We will describe it in order to facilitate understanding for a broader audience.

Line 106: describe the elevational changes, 47-114 m?

Response: We will describe better the elevational changes in the site.

Line 133: list the ranges of sizes of the trees so we know can estimate their canopy position. Also explain why a variety of sizes were sampled?

Response: Thanks for the observation**.** We will update Table 1 to include more detailed information on the size ranges of the sampled individuals. The variation in DBHs reflects the inherent high species diversity in tropical forests and the strong influence of competition, which typically results in a reverse J-shaped distribution, where most individuals fall into the smaller diameter classes. Selecting species replicates all within the same DBH class would be extremely difficult and, in many cases, unfeasible given the limited number of individuals per species and the logistical constraints of field sampling in such remote areas in the Amazon. We also calculated the amount of nutrients in plant tissues (expressed on a fresh weight basis in Kg) using the biomass equations proposed by Silva (2007), as described in the Materials and Methods (*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.

Line 138: Brosimum rubescens should be italicized

Response: Done.

Line 141: just “associated” unless there are N fixers that are not in the Fabaceae family at your sites

Response: Ok. Done

Line 143: maybe just diameter growth instead of “PAI”? you can summarize it as DBH t2- DBH t1/ (t2-t1) instead of “current”

Response: Yes. we agree. We replaced “PAI” with “growth rate,” which is a more commonly used and intuitive term.

Table 1: might help to list the initial size or report the relative growth rate since interpreting a change in the DBH increment also is relative to the size of the tree

Response: The growth rates per species reported in Table 1 represent the average periodic diameter increment of individuals monitored since 1996 through biennial forest inventories, and annually since 2014. Since growth rates are a simple and direct variable to obtain in the field, Earth system models such as FATES (under development by the NGEE-Tropics project) can use them to define plant functional types (e.g., along a fast-to-slow growing continuum) to infer nutrient allocation strategies in plant organs such as leaves and wood. We will run the analysis to assess whether growth rates are significantly correlated with nutrient concentrations and present the results in the revised version of the manuscript.

Lines 170-178: how were the samples extracted or digested? Are the concentrations estimated as total nutrient content?

Response: The samples underwent pre-processing, during which they were dried in an oven at 65°C and sent to the chemical analysis laboratory. The main types of extraction performed for nutrient analysis included hot acid digestion, dry decomposition, and extraction by shaking.

Line 182: “sampled” instead of “opened”

Response: corrected for “sampled”.

Line 193: “Soil bulk density”

Response: corrected for “Soil bulk density”

Line 219: “o” is a typo?

Response: o” was a leftover word that has now been removed from the revised manuscript.

Line 256: “were significantly higher” rather than “presented significantly higher”

Response: changed to “were significantly higher”

Line 262: Referring to Figure 4 here?

Response: Yes, thanks for the observation. The order of figures were changed so Figure 3, 4 and 5 were transformed into Figures 3 and 4. This part will refer to Figure 3.

Lines 331-333: nutrients also accumulate in valleys because of gravity and weathering. I would be hesitant to argue that valley soils typically have higher nutrient availability because of litterfall decomposition (also lines 418-425), as the landscape erodes into the valley.

Berhe, A.A., Barnes, R.T., Six, J. and Marín-Spiotta, E., 2018. Role of soil erosion in biogeochemical cycling of essential elements: carbon, nitrogen, and phosphorus. Annual Review of Earth and Planetary Sciences, 46(1), pp.521-548.

Response: We agree with the reviewer’s observation regarding nutrient accumulation in valleys due to gravity and weathering processes. We have revised this section of the text accordingly and included references to Berhe et al. and Rodrigues et al. (2024).

Line 341-342: Soong et al. (2021) did not attribute the higher growth rates in the clayey soils solely due to the greater prevalence of AMF?

Response: We thank for the observation. We acknowledge that Soong et al. (2020) did not attribute higher growth rates in clayey soils solely to the greater AMF prevalence. Soil clay content and total phosphorus were other factors related to growth as described by Soong et al. (2020). We have revised this part of the manuscript to more accurately reflect the findings reported by Soong et al. (2020).

Lines 351-354: the growth rates might need to be presented as relative growth rates since it’s hard to interpret the growth without knowing the size of the trees

Response: Yes we agree. We will represent growth rates as the average of growth rates per species.

Lines 363 to 367: why not instead that the nutrient content mirrors the environmental variables to some extent?

Response: Yes, we agree with the reviewer’s suggestion. In this part of the text, our intention was to highlight potential trait-based adaptations, such as differences in root architecture and fine root biomass, that may allow valley specialists to acquire more nutrients. We will try to focus more in this part that to some extent nutrient content in plant tissues mirrors environmental variables. However, it is important to highlight that our study was carried out at a more localized scale, compared to the study of Bauters et al. (2022) who conducted at a much broader, pantropical scale. In our case, variations in nutrient availability along the topographic gradient may be more subtle, given that the underlying bedrock and edaphoclimatic conditions are relatively the same.

Line 413: Bauters et al. (2022) likely found correlation between nutrients in tissues probably because of the soils, not because of the internal demand

Response: Bauters et al. (2022) found that: *Variation in wood P storage appears to be linked to a dimension of evolutionary adaptation and ecological variation among species, while wood Ca, Mg, and K appear to be defined by the abiotic environment.*" The high correlation found between wood P and leaf P (r=0.96) as shown in the figure below shows a coordination between nutrient stocks (Kg). P, for instance, is highly mobile within plants, facilitating internal redistribution through processes such as foliar nutrient resorption, while it remains relatively immobile in soils (Aerts, 1996; Vergutz et al., 2012). P resorption efficiency, for example, tends to be higher in P-limited systems (Vergutz et al., 2012).

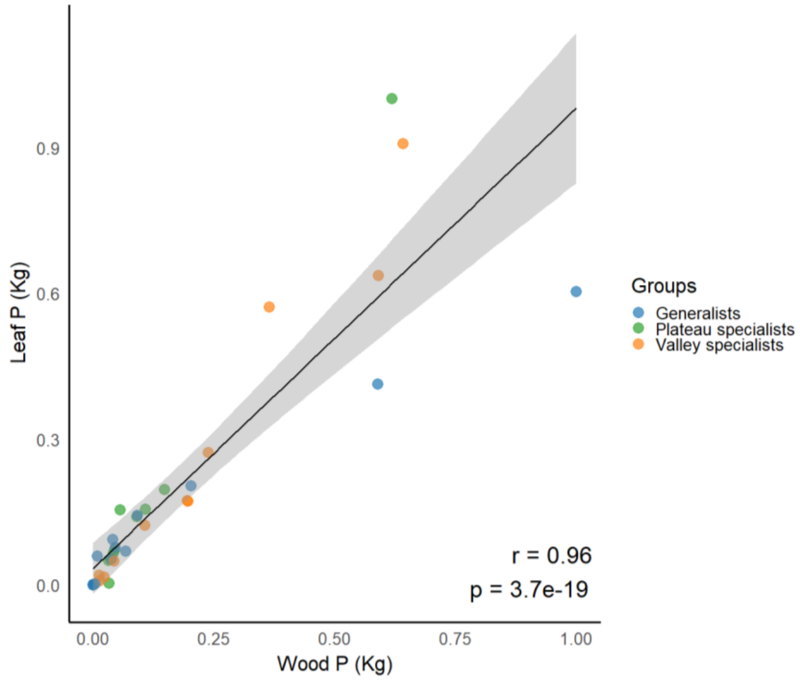

Figure XX - relationship between Leaf P and Wood P nutrient total stocks (Kg). This figure will be incorporated in the revised version of the manuscript.

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