Response to referees

Manuscript ID: JEcol-2023-0296.R1, "Starch storage strategy in the stem wood influences carbon dynamics and storage-growth trade-offs in tropical trees"

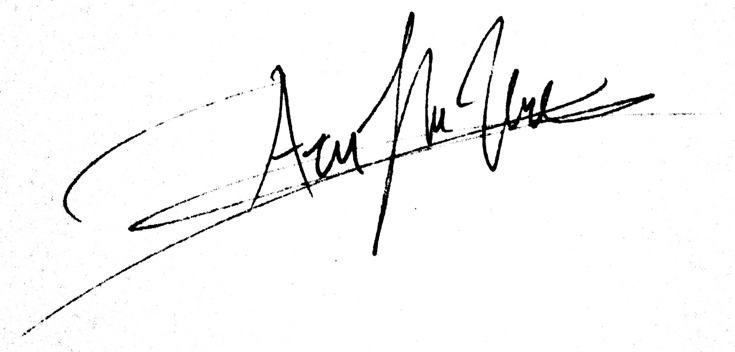
Dear Editors,

We appreciate the assessment that our manuscript has improved during the last round of revisions and appreciate the opportunity for further consideration of our paper in the Journal of Ecology. The valuable feedback provided by the Associate Editor and the reviewers allowed us to further improve the manuscript and the clarity and comprehensibility of our work.

We have carefully addressed all the new suggestions from both reviewers, which have improved the readability of the manuscript and provided more details on the quality of data collection and the statistical analysis of our results. To reflect this, we have made substantial changes to the Methods and Results sections of our manuscript, paying special attention to the description and reporting of the statistical analyses. For additional transparency, we share our data and code in an open GitHub repository, accessible through the link “https://github.com/dahera8/MPIBGC-Anatomical\_distribution\_of\_starch\_and\_storage\_growth\_trade\_offs”.

In the following sections, we provide detailed responses to each of the reviewers’ comments, as well as the specific changes made throughout the manuscript. For ease of reference, we have denoted the reviewers’ comments in italics, with our responses presented in normal text.

Sincerely,



David Herrera-Ramírez, on behalf of all coauthors

Reviewers’ comments:

*Reviewer: 1*

*COMMENTS FOR THE AUTHOR*

*Major comments:*

1. *Figures. Please label sub-panels with letters and refer to them.*

We appreciate the reviewer’s suggestions for improving the clarity and readability of this manuscript, including the Figures. We have incorporated the suggested changes.

1. *L328-335. No statistical support for text in this section. This is a problem, because the authors refer to changes in concentrations at the deepest depths. Yet, this is where concentrations are the lowest, and at or near values that are essentially indistinguishable from zero due to analytical uncertainty. This is if concentration measurements were used, yet, the histological measurements capture 80% of variation in concentrations and thus uncertainty is higher.*

We have revised this section to enhance clarity. We now provide a supplementary figure (Figure S6) showing the maximum difference in starch concentration between sampling dates at each wood depth for each species, which supports our claim that the maximum change in starch concentration occurs in the first few centimeters of sapwood and decreases with depth along with starch concentrations. We have also included the p-values resulting from the analysis of variance comparing the maximum change in starch concentration between all measured wood depths from bark to pith.

We have removed the text referring to the changes in concentrations in the deepest layers of wood because it is not central to our story and does not contribute to our conclusions. The histological method allows us to visually identify the absence or presence of starch grains. Although starch concentrations are very low when averaged over the inner stem region, we were able to see that the deepest layer of sapwood was completely devoid of starch grains during the dry season. This was not stated explicitly in the previous version of the paper.

We included the following modifications in the main text:

Lines (297-303):

“We estimated the seasonal amplitude of the starch concentration as the difference between the maximum and minimum starch concentrations measured during 2019 for each 5mm depth increment. The maximum concentration corresponds to the peak of the starch accumulation period, while the minimum concentration represents the period of starch depletion. We used analysis of variance (ANOVA) to evaluate differences in these starch changes between different depths to determine at which wood depths the greatest changes in starch concentration occurred.”

Lines (377-384):

“Quantifying starch concentrations every 5 mm of wood along the radial axis from bark to pith allowed us to estimate with high precision the radial profile of starch concentration across the sampled wood cores (Fig. 3). Starch concentrations decreased radially across the sapwood from bark to pith for all species on all sampling dates (Fig. 3). Notably, the largest differences in starch concentration between sampling dates occurred in the first 20 mm of wood for *D. microcarpa* and *O. leucoxylon,* and in the first 60 mm of wood for *S. guianensis* (Fig.3, Fig. S6, p.adj<0.05), as compared to the inner regions where starch concentrations were very low overall.”

1. *Lots of the text refers to the “wet” or “dry” season but the figures refer to monthly time periods. Consider adding labels to plots.*

We carefully revised every figure to ensure that we indicate when the dry and the wet seasons occur. To do this, we used two vertical shaded areas: one yellow shaded area to indicate the dry season and another blue shaded area to indicate the wet season. We now made sure that we refer to this appropriately in the figure captions.

1. *The results remain difficult for me to follow. Much of the results also refers to supplemental figures.*

We have revised the Results section. We have rearranged some results to follow a structure that reflects the order of the hypotheses, as follows: 1) seasonality of starch mass, growth, and respiration), 2) Accumulation and consumption of starch across different seasons, and 3) seasonal storage-growth trade-offs in the stemwood. Most of the modifications were made in the first subsection (3.1 Seasonality of starch mass, growth, and respiration), which generated the most questions from reviewers. In this section, first, we present the results on seasonality in the radial distribution of starch concentration, then the total starch mass in the entire wood core, and finally the seasonality in growth and wood respiration. We now make a clear distinction when we refer to starch concentration at different wood depth increments and when we refer to starch mass in the entire wood core. We also refer in the text to the alphabetical labeling of each figure sub-pannel as suggested in the first reviewer comment.

We recognize that it was a mistake to relegate Figure S4 to the Supplement, as it is a central part of our story. We now have moved this figure to the main text as Figure 5. We further made changes to this figure to make it more readable. We have indicated the statistical differences with different alphabetical letters, improved the sub-panel labels, and indicated on the x-axis which time period is being evaluated in each case.

*L1. Suggest replace “Starch storage strategy in the stem wood” with “Distribution of sapwood starch”. I also suggest to make this change throughout the manuscript, as the “strategy” is never defined and simply describes the anatomical distribution of starch within stem wood. At the very least I would suggest “Starch storage traits…”, but this does not well describe radial patterns in starch amount such as my first suggestion does.*

We have replaced the term “starch storage strategy in the stem wood” with “anatomical distribution of starch within the stemwood” in the title and throughout the text, as suggested by the reviewer.

*L25. Storing starch in the stemwood is not a strategy; all trees to my knowledge contain some starch in stemwood. Suggest to revise.*

We have revised and replaced the term “storage strategy” with the term “anatomical distribution of starch within the stemwood”.

*L34. Starch mass or starch amount? Mass was not measured…*

Starch content, We have corrected this.

*L40. No mention of sugars in these results.*

We now mention the soluble sugars in the abstract.

*L46. Remove mentions of mortality.*

We have revised the text to remove mentions of causality between the storage traits and mortality. We now refer to it only as an association between mortality, survival, and storage traits.

We think that the potential influence of our trait combination on tree mortality and survival is an important implication of our results and it deserves to be mentioned in the synthesis of the abstract. In the last part of the discussion, we now provide additional evidence showing the possible relationship between the anatomical distribution of starch in the stemwood and mortality. Here we show that not only the background mortality is higher in the parenchyma-storing species than in the fiber-storing species, but also the increase in mortality after high frequency fires (Figure S7).

*L130. It would be helpful to justify why these specific traits are focused on. Some would argue belowground traits are actually much more important.*

We justify the selection of the specific traits in the introduction, in lines 88-92:

“Herrera-Ramirez et al. (2021) demonstrated that these storage traits were related to stem growth and mortality rates for the species involved. Thus, further understanding of how this anatomical distribution of starch within the stemwood is related to the metabolism of trees and the dynamics of NSC would improve our mechanistic understanding of how these tropical trees regulate carbon storage to increase competitiveness and/or survival.”

And lines (102-109):

“Tree species that rely on living fibers for NSC storage (e.g., fiber-storing species) may indicate a high priority for storage formation that would compete with other carbon sinks like growth and respiration and may be related to higher plasticity of carbon metabolism, such as higher capacity for variability of seasonal carbon fluxes (Plavcová et al., 2016; Herrera-Ramírez et al., 2021). It is possible that trees with larger plasticity in carbon storage and sink fluxes may be better adapted to stressful conditions that severely reduce photosynthesis or increase carbon demand because they may be more tolerant to a wider range of environmental conditions”

We agree that belowground traits are very important and they must be studied as well. We recognize this in our Discussion (lines 681-684, see below). Unfortunately, we do not have data about root carbon fluxes for this study.

Lines (679-682):

“Nevertheless, it is important to clarify that these storage-growth trade-offs may change when other forms of growth are considered. At the whole tree level, the inclusion of other starch storage tissues, such as phloem (Rosell et al., 2021) or roots (Hillman et al., 2021) may provide further insight.”

*L138-139. Unclear.*

We rephrased it for clarity (lines 144-145): “We used phenological data on the percent cover of mature leaves in the tree crown as a proxy for carbon acquisition”.

*L159. Where is zero on the figure? It seems to be in different places for different quantities (NSC vs. others). NSC is negative? Add tick labels.*

We now indicate the 0 in each panel of the figure. We also explain in more detail in the figure caption how carbon sinks are represented in the figure.

*L195-106. Reference? Or, additional methodological description?*

We added the reference and improved the methodological description of the collection of the phenological data.

*L216. Please justify the use of this starch method in the main text (not just in the response letter). In particular, the authors state this method gives more detailed information about starch storage. Regarding the localization within specific cell types this is certainly true. But concentration methods can also be used to estimate radial profiles. Numerous examples in the lit.*

We now justify why we used the histological method in our Methods section (lines 237-239):

“This method allows us to observe and quantify spatial patterns of starch distribution in the stemwood with high resolution and identify the cell types that were used to store starch. ”

and give reasons why it would be useful in the future (lines 599-600).

“Histological methods provide a rapid and convenient method for quantifying starch storage that can support observations in a large number of species over the longer term.”

*L235. Suggest “percentage covered by starch approximate”*

Done.

*L236. Report the slope in the main text (0.88?)*

Thank you for the suggestion, we included the slope and the p-value of the slope in the text.

*L276. At what depth? Is this total starch? Or??*

We have carefully reviewed the Methods section to clarify when we are referring to starch concentration at certain wood depths as opposed to referring to total starch mass in the entire wood core.

In this specific case, it reads as follows (lines 315-317):

“We also estimated the relative change of the total starch mass of the entire wood core between seasons, by dividing the absolute starch mass change by the starch mass in the final month (Equation 1). ”

*L281. how is a non-parametric confidence interval calculated? Citation or more explanation required*

We have rephrased this section, in order to be clearer and more specific about the confidence interval that we estimated and we added the respective references (lines (321-327).

“We evaluated whether the changes in starch mass between seasons were different than zero by building 95% non-parametric confidence intervals for the mean using the adjusted bootstrap percentile (BCa) interval method (Davison and Hinkley 1997). For this purpose, we used the ‘boot’ package available in R (Canty and Ripley 2022). ”

*L296. How? Also, software??*

We now describe how we assessed heteroscedasticity and normality in the text and add a respective reference (lines 344-346):

“The heteroscedasticity of the residuals was checked by plotting the fitted values of the model against the residuals, while the normality assumption was checked using a Q-Q plot (Fox 2015).”

furthermore, we added a section in the methods (2.6. Data analysis software, lines 371-374) where we cite the software used to perform the calculations, statistics and figures that we present in the manuscript.

*L301. What does “in parallel” mean here*

It means that all the incubations were running at the same time and under the same conditions. We now deleted this word as it seems to add some confusion to the readers and it is not critical for the method.

*L303-304. Reference*

We added the reference as suggested: Muhr, J., Trumbore, S., Higuchi, N. and Kunert, N. (2018), Living on borrowed time – Amazonian trees use decade-old storage carbon to survive for months after complete stem girdling. *New Phytologist*, 220: 111-120. <https://doi.org/10.1111/nph.15302>

*L307. Why not mass-specific respiration rate?*

For the respiration incubations, we did not measure the mass (grams of dry wood) of each individual wood core, we only estimated the wood core volume based on its dimensions. We now mention in the text that we estimated the volume-specific respiration rate.

*L311. Any references for this approach?*

Yes, we added the reference in the text:

Fournier, L.A. 1974. Un método cuantitativo para la medición de características fenológicas en árboles. Turrialba 24(4):422-423.

*L337. Again is this total starch mass?*

Yes, we modified this section to add clarity to this respect. We took more care when referring to the total starch mass in the entire wood core, or when referring to starch concentration at a given wood depth.

*L353. “evergree”. Also, label heartwood boundary? Report sample size (number of trees per type and date) in caption.*

Thanks, the typo has been corrected. The information about the number of trees per species and per time point has been added in the caption.

*L360. How is individual measurement uncertainty at a given depth in the profile propagated to these total starch masses?*

We estimated the mean total starch mass in the entire wood core per individual as a linear combination of the mean starch mass measured at each wood depth. Within each 5mm depth increment, we measured the percentage of starch cover within 50 images, each with an area of 1mm x 1mm. For each 5mm depth increment, we calculated the mean and standard deviation of the fraction of the total area covered by starch grains. This was converted to starch content (grams of starch/grams of dry wood) according to the calibration between area coverage and extracted starch developed for these species in Herrera-Ramírez et al., 2021. These were combined with the average wood density for each species (derived from local sampling) and the wood core volume to estimate the starch mass in grams within each 5 mm depth of the wood core. The mean total starch is the sum of the 5 mm increments, and the reported error was obtained by calculating the mean standard deviation across all depths.

*L340. Why is this in the supp?*

We have relocated this figure in the main text, where it provides important information for the reader. As with other main text figures. In order to improve readability, we have incorporated additional details such as clear labeling of the dry and wet seasons and labeling for the statistical comparison with distinct alphabetical letters.

*L344/Fig. 5. I do not understand the significant difference being described and it is not descrbed at all in the fig caption. Nor does L291-292 provide sufficient information to understand what is being compared. Each month uniquely? If so, multiple comparisons adjustments are required. But, I can’t figure out from the text how many comparisons were made.*

We have rewritten the paragraph to better describe the comparisons (lines 336-339):

“To assess the significance of variations in monthly radial growth rates within each species, we used Wilcoxon signed-rank tests with a 95% confidence level. This test compared the growth rates between individual months. To ensure the robustness of the results, p-values were adjusted following the Holm method (Holm 1979). ”

We have also extensively modified the figure caption to give the information necessary to better understand the figure content and allow an unambiguous interpretation of the results.

*L395. P is supposed to be less than 0.05 I think.*

This p-value is exactly 0.0505 as it is reported in Fig. 7.

*L573. But guianensis had the most starch during the wet season.*

We agree with the reviewer that there is no evidence of starch consumption during the wet season for *S. guianensis* and therefore we observed the highest starch content during the wet season for this species. Despite this, we would like to point out in the text the potential impact of flowering and fruiting on the demand for carbon for metabolism. This may impair the accumulation of starch and may even force these species to consume starch during the wet season.

Reviewer: 2

*COMMENTS FOR THE AUTHOR*

*The authors sufficiently addressed my comments and the manuscript is much improved and overall clearer to read. See below for some further comments on the revised manuscript that should be addressed.*

We would like to sincerely express our sincere gratitude to the reviewer for the consistent effort and attention to detail during the last two reviews. We have gone through all of the reviewer’s suggestions line-by-line and have included our response in the text below.

*Line by line comments:*

*L 47: should be "semi-deciduous"*

Correct, we have made the change.

*L 62: I think you don't need to say "dry and hot conditions" and "droughts". Maybe choose one?*

Right, we have just kept the term “droughts”.

*L 98-103. This added text may not be necessary, since it is phrased similarly in lines 130-133.*

We have eliminated this phrase.

*L144: Change "Then," to "Thus,"*

Changed

*L 152: Change "will" to "would" for consistency.*

Changed

*L 292-293: It is not clear what is being described in this sentence.*

We apologize for the remaining lack of clarity in our manuscript. We have now comprehensively reviewed the manuscript and edited all instances that caused confusion. This phrase was reworked in order to be clearer and more specific (lines 344-344):

“To evaluate seasonal trade-offs between growth and storage, we calculated three-month cumulative growth, with the three months corresponding to the core of the wet and dry seasons and the two transition periods as defined above. We estimated Pearson’s correlations and linear regressions between the cumulative growth and starch mass changes for each season.”

*L 293: I'm not sure "estimated" is the correct word to use here.*

We changed it to “calculated”.

*L 318-319: Remove "Then in relative terms we expect" and replace with something like "In general, we assume a higher flux..."*

Changed.

*L 333: Should be "sapwood"*

Changed.

*L 334: Remove "totally"*

Removed.

*Fig. 5: Is the shading around the solid line 95% confidence intervals? If so, add to figure caption.*

Yes it is, we now mention it in the figures caption.

*L 402-404: Did you try this for D. microcarpa too? If not, why not?*

Yes, we have tried this for *D. microcarpa* too, but no significant correlation was identified. We think this is not relevant to the story as the trade-offs were present in the simultaneous time period.

*L 449 (Fig. 7 caption): Should be "non-significant*"

Yes, we apologize, we have changed this.

*L 453 (Fig. 7 caption): "scratch" should be "starch"*

Changed.

*L 459-460: Rephrase second part of sentence. Perhaps "...leaf habit, which we summarize in our conceptual framework"*

Changed.

*L 468-470: This sentence is hard to follow; perhaps re-phrase for clarity? Could you say something like "Carbon availability was balanced by starch and..."*

We modified this phrase to make it clearer. We incorporated the suggested change and modified the phrase (lines 558-561):

“This species had the largest starch consumption during the dry season, when carbon availability was low and radial growth was almost absent, and low overall consumption during the wet season, when carbon availability and growth were higher (Fig. 5, Fig. 6).”

*L 472: Could you instead just say "when carbon availability was higher due to greater leaf area and growth increased"? It is not very clear as it is currently written.*

Yes, changed as suggested.

*L 474: Replace "with trees growing" with "when trees grew"*

Replaced

*L 476: Add "presented in our conceptual figure" to the end of the sentence.*

Added

*L 483: Missing word? Perhaps "but we did observe a significant consumption"*

Yes, we have corrected the phrase as suggested.

*L 499: It would be helpful to cite Figure 4 here too.*

Cited

*L 507: Change to "dependent on..."*

Changed

*L 513-516: I think that just including this in the methods is enough, and it doesn't need to be justified in the discussion as well.*

We agree with the reviewer and have eliminated the repetitive mention of why we did not include soluble sugars in the analysis of NSC seasonality in our tree species.

*L 516: Remove "So far,"*

Removed

*L 526: Might rephrase to "which could reduce their vulnerability to carbon starvation" to be clearer.*

We have rephrased this sentence, Thank you for the suggestion.

*L 538: By wood growth rates, do you mean radial growth rates? If so, say that.*

We mean radial growth rates, we have incorporated this through the text.

*L 539: It seems like there should be a citation for this sentence.*

We have included a citation here that helps to support our hypothesis. The citation included is:

Rowland, L., Ramírez-Valiente, J. A., Hartley, I. P. and Mencuccini, M. (2023), How woody plants adjust above- and below-ground traits in response to sustained drought. *New Phytologist*, 239: 1173-1189. <https://doi.org/10.1111/nph.19000>

*L 548: Replace "Then," with "Thus,"*

Replaced

*L 551: Add "Overall" to the start of the sentence so that the respiration discussed here is not confused with the respiration seasonality discussed in the previous paragraph.*

Added

*L 567: Photosynthetic limitation due to what? Due to greater water availability?*

We have added these details in the text (lines 652-654):

“We hypothesize that trees may recharge the carbon storage pools during the wet season due to favorable growth and less photosynthetic limitation due to more water availability and lower air temperatures (Dietze et al., 2014).”

*Figure S5: Consider also using dashed lines to indicate non-significant correlations in this figure too.*

We apologize again for the shortcomings in this revision. We have included many details in our figures and we have modified Figure S5 as suggested by the reviewer.