

## **Reviewer #2:**

### **Main comments**

The manuscript entitled “Molecular-level carbon traits of fine roots: unveiling adaptation and decomposition under flooded condition” focused on the differences of chemical traits of soil-grown and water-grown fine roots of a tropical tree. The authors extracted compounds in root samples and characterized the chemical compositions using targeted and non-targeted analysis techniques. Targeted analyses such as GC-MS can provide more accurate information of chemical compositions (e.g., specific compounds), but may also lose many information since a lot of compounds cannot be identified using this method. I am glad to see that state-of-the-art analyses were used in root studies, which should advance mechanistic understanding of root acclimation and associated biogeochemical processes under changing environments. This study should raise broad interests to the readers at BG. Overall, this manuscript is well written with sufficient data to support the main findings. The experiments are also well designed. It is amazing to see that a single tree has different habitats for the growth of roots in natural conditions. Similar studies may need to design split-root systems. I am really enjoyed reading through the manuscript, although I still have some minor comments.

Re: We sincerely thank you for the helpful and constructive comments and suggestions regarding our manuscript. Following the comments, we have thoroughly revised our manuscript. Here, we provide our point-by-point responses. The line numbers in this response letter refer to the revised manuscript with tracked changes.

### **Specific comments**

Line 15-18: This sentence is too long, please split it into short ones.

Re: It has been split into two sentences: “*Here, we used a sequential extraction method, starting from non-polar to polar solvents, to obtain dichloromethane-and-methanol-extractable ( $F_{DcMe}$ ), base-hydrolyzable ( $F_{KOHhy}$ ), and CuO-oxidizable ( $F_{CuOox}$ ) fractions from fine roots of *Dysoxylum binectariferum* naturally grown in soil and water. Subsequently, we characterized them using targeted gas chromatography-mass spectrometry and non-targeted Fourier transform ion cyclotron resonance mass spectrometry.*” (Page 1, Line 15–19)

Line 24-25: Is it necessary to emphasize the techniques as a highlight at the end of the abstract. I do think more emphasizes should be on the research question per se, although combination of diverse techniques contributed to the findings.

Re: As per your recommendation, we have removed the sentence concerning techniques from the abstract. This adjustment aims to ensure that the abstract succinctly communicates the primary focus of our research. We appreciate your input and believe that this change enhances the clarity and effectiveness of our abstract.

Line 29-31: long sentence.

Re: It has been split into two sentences: “*Additionally, with the highest productivity and turnover rate among all underground plant organs, fine roots considerably contribute to organic matter formation and stabilization in soils. Thus, they drive the cycling and distribution of carbon and nutrients in terrestrial ecosystems (Dijkstra et al., 2021).*” (Page 1, Line 29–32)

Line 31: the fine-root tissue chemistry

Re: It has been revised accordingly.

Line 81-83: Is the first hypothesis necessary? As commented above, this is not a methodological paper to me, although I understand you also want to test the feasibility by combining different analytical methods. Otherwise, you can test it using any experimental systems, but why focusing on the flooded vs. the non-flooded condition? I suggest removing this hypothesis since the data you provided can show the validation.

Re: Thank you for your thoughtful review and constructive feedback. Upon carefully considering your comments, as well as those of the other reviewers, we have decided to remove the technical hypotheses and retain only the scientific hypotheses. By removing it, we aim to provide a clearer and more direct presentation of our ecological hypotheses. This revision has been made in the manuscript: “*We tested two main hypotheses: 1) compared to SGR, WGR should contain more aromatics, especially in bound-compounds to cope with flooding stress; and 2) the WGR would have lower decomposition rate than the SGR in both aerobic and anoxic conditions.*” (Page 3 L73–75)

Line 150-152: please elaborate the statistical analysis further. This section is too simply described in current version.

Re: The statistical section has been revised: “*Significant differences between SGR and WGR were assessed using a two-way independent-samples T test. Furthermore, significant differences among the three fractions were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. All statistical analyses were conducted using IBM SPSS Statistics 23. Statistical difference was considered when  $P < 0.05$ .*” (Page 5, Line 145–148)

Line 323: delete ‘to say’.

Re: It has been revised accordingly.

Line 320: it is a little bit weird to me to see the discussion of hypothesis three in the section of ecological implications. Why not move this part of discussion in a separated section? Or is the subtitle of ecological implication necessary here? When talking about ecological implication, we typically put it into a broader scenario, rather than discussing a part of main results at this stage.

Re: Thank you for your insightful comments. We have revised the subtitle “Ecological implication” to “Impact of molecular-level carbon traits of fine roots to decomposition”.

Line 344-347: Okay, when reading to the very end, ‘Together, ...’, it sounds like all these results are just for validating the complementary information by combining GC-MS and FT-ICR MS analyses? If this is the case, please reconsider my previous comments about this concern. If not, please rephrase the last sentence in the conclusion and consider my suggestion above, which may cause confusion to readers.

Re: Thank you for your insightful comments. We have carefully revisited the concluding statement in our manuscript's conclusions section in light of your concern. While our study primarily focuses on addressing the research question, we acknowledge that the previous wording may have inadvertently emphasized the validation aspect too strongly. As per your suggestion, we have rephrased the last sentence in the conclusion to better align with the main focus of our study. The revised statement now accurately reflects the emphasis on addressing the research question rather than solely validating the complementary information from combining GC-MS and FT-ICR MS analyses. We appreciate your feedback and believe that this adjustment will enhance clarity for readers.

*“Together, we incorporated the molecular-level carbon traits of fine roots into trait-based ecology, which could improve our understanding of plant functioning and biogeochemical progress in response to global environmental change.” (Page 16, Line 355–357)*