Reviewers' Comments:

Reviewer #1:

Main comments

The manuscript by Wang and co-authors on 'Molecular-level carbon traits of fine roots: unveiling adaptation and decomposition under flooded conditions' describes how soil changing soil oxic/anoxic conditions affect the molecular composition of a tree species naturally growing in changing water-logged/non-water logged systems, and highlights the species' adaptability of their chemical composition. The authors did not only compare roots from plants growing under different water/non-water logged conditions, they also used a combination of analytical methods (targeted GC-MS, non-targeted FTICR, as well as solid state NMR) to verify their applicability to grasp potential changes occurring at the chemical/compound specific composition. Moreover, they tested, whether the differences in root chemical composition also influenced their decomposability under different environmental conditions. The environmental conditions indeed changed the chemical root composition, with anoxic conditions leading to higher production/contents of polyphenolic compounds (with a high degree of unsaturation and aromaticity) and contained more non-structural compounds, and moreover, those roots decomposed faster.

The results of this study are very interesting and a good contribution for describing root traits beyond commonly measured parameter and show the adaptability traits. Moreover, the authors show that a combination of methods is needed to get a good coverage of non-structural carbon compounds in roots that could respond to changing environmental conditions. Overall, the manuscript clearly structured and well written.

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.

I would however clearly separate the technical question from the ecological question in the hypotheses section. The different methods that are now embedded as hypothesis i), but are in my opinion the tools to test the more ecological hypotheses ii and iii). I would suggest to turn the sequence at the end of the introduction around.

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 L72–74)

I appreciated the scheme in Figure 1, though it could be an idea to add, if known, some description of the expected C pool that could be extracted with the different fractionation steps (Question 1). Was there an effect on root morphology detectable (Question 2)? Was there any effect on root N or other nutrient contents (Question 3)?

Re: **Question 1**: Thank you for the helpful feedback and comments. Following your suggestion, we have added the description of three expected fractions in Figure 1.

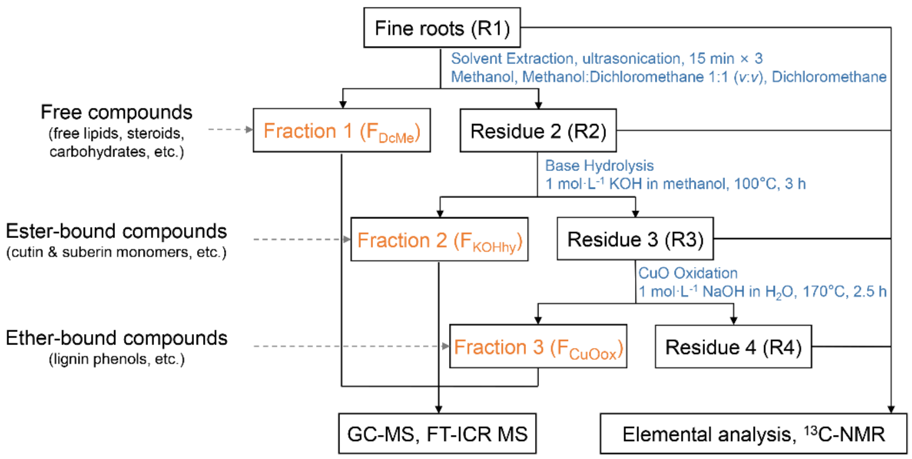

***Figure 1** Schematic chart of the sequential extraction of organic compounds from fine roots to obtain different operational fractions and residues….*

Question 2 & 3: According to your comments, we have added Table S6 in SI to show the morphology (root diameter, specific root length, and root tissue density) and chemical elements (C, N, C:N) of different fine-root samples. SGR has a higher specific root length and nitrogen content, with a lower average diameter and carbon nitrogen ratio than WGR ($P < 0.05$).

***Table S6** Morphology and chemical properties (means ± standard errors) of different fine-root samples. The P-value indicates the significance level of a two-way t-test between soil-grown roots (SGR) and water-grown roots (WGR).*

	Units	SGR	WGR	*P*-values
Average diameter	mm	1.18 ± 0.02	1.70 ± 0.10	0.003
Specific root length	$m\ g^{-1}$	6.00 ± 0.22	2.63 ±0.20	< 0.001
Root tissue density	$g\ cm^{-3}$	0.15 ±0.01	0.17 ± 0.00	0.101
Carbon content	$mg\ g\ root^{-1}$	390 ±4	440 ± 2	< 0.001
Nitrogen content	$mg\ g\ root^{-1}$	18.2 ± 0.10	16.3 ± 0.03	0.013
Carbon nitrogen ratio	-	27.0 ± 0.35	21.4 ± 0.80	< 0.001

It has revised in manuscript: "*To investigate the influencing factors regulating fine-root decomposition, we detected the morphological traits and nitrogen content in origin roots. The results showed that SGR has a higher specific root length and nitrogen content, with a lower average diameter and carbon nitrogen ratio than WGR ($P < 0.05$; Table S6)*." (Page 13, Line 241–244)

Moreover, there should be more detailed description of the statistics in the methods section, whether the data was tested or transformed for normality or homogeneity of variance or if non-parametric tests were applied (Question 1). Another idea to show overlaps of compounds extracted with the different methods could be venn-diagrams for instance (Question 2).

Re: **Question 1**: Thank you for your insightful comments on our statistical approach. In this study, the sample size in each group was determined based on the results of three repeated experiments. It is worth noting that the data error observed in most repeated experiments was minimal. All data were found to be normally distributed according to the Shapiro-Wilk test. Therefore, it is not necessary to perform data transformation.

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)

Question 2: As you mentioned, the overlaps of FT-ICR MS-detected formulae from sequentially extracted fractions have been shown in Venn diagrams of Figure S4 in SI.

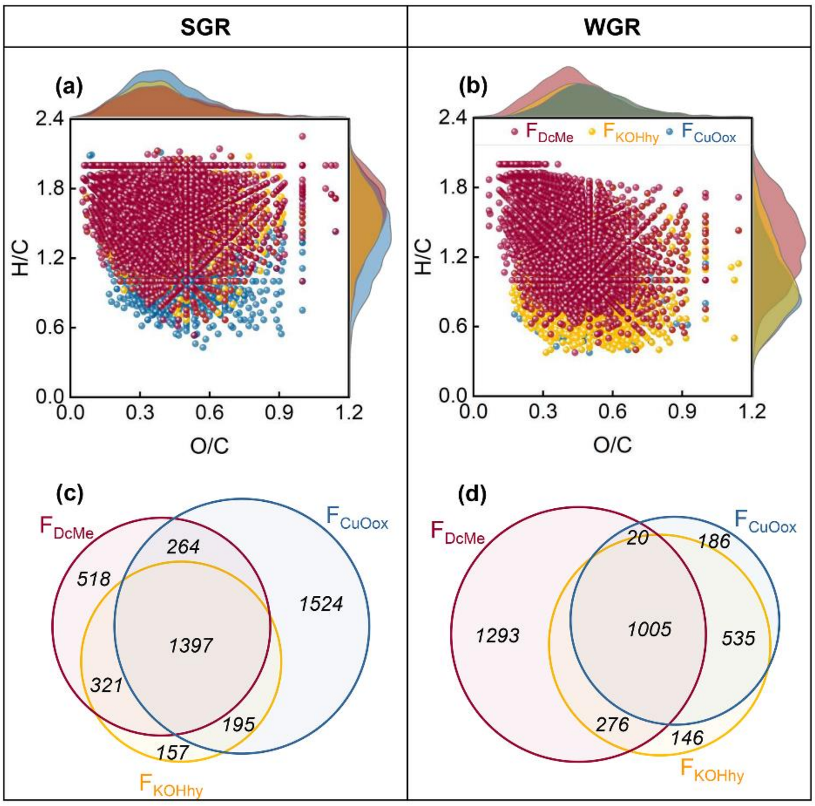

***Figure S4** van Krevelen and Venn diagrams of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)-detected formulae …*

The results are clearly stated, I am just not sure why the authors decided to use ‘formulae’ and not ‘compounds’? I think that would be easier to understand and follow, if that was changed (Question 1). Moreover, I would simplify and stick to C compounds or organic compounds and avoid carbonaceous (Question 2). In the first section 3.1 of the results there could be also some statistical tests added (or point to a Table with statistical results testing the different fraction distributions) (Question 3).

Re: **Question 1**: We utilized the term ‘formulae’ instead of ‘compounds’ because in our study, the mass spectrum obtained from FT-ICR MS comprises numerous mass peaks, each representing a molecular formula. Identification of mass peaks involves calculating the elemental composition based on the exact relative atomic masses, referencing the basic chemical guidelines and databases of naturally occurring molecules, thus assigning molecular formulae to the mass peaks. It is important to note that a single formula may correspond to multiple isomers or compounds, hence 'formulae' was chosen to accurately reflect this aspect of our analysis.

Question 2: Following your suggestion, the term "*carbonaceous*" has been replaced with "*carbon compounds*" throughout the main text. (Page 1, Line 14; Page 2, Line 34, 35, 36, 38; Page 13, Line 254, 257)

Question 3: According to your suggestion, we have added Table S1 with statistical results testing the different fraction distributions. And the section 3.1 of the results has been revised: "*Each sequential extraction step could extract considerable proportions of biomass and carbon for all root samples.* F_{KOHhy} *and* F_{CuOox} *had the similar (P > 0.05) and highest biomass fractions, accounting for 34% and 32% of the total biomass, respectively, … Although WGR had near twice biomass and carbon fractions in* F_{DcMe} *of SGR (P < 0.05), the two classes of roots were similar on both biomass and carbon content in the other three fractions (P > 0.05; Table S1). The proportion of ash content in SGR was approximately twice that in WGR (P < 0.05; Fig. 2c; Table S1)*." (Page 6, Line 151–158)

Table S1 *Biomass fractionation and fractional carbon contents based on sequential extraction, and ash content of in soil-grown roots (SGR) and water-grown roots (WGR). Different letters indicate the statistically significant differences (P < 0.05) among the four fractions. Asterisks indicate the statistically significant differences (P < 0.05) of a certain fraction between SGR and WGR.* F_{DcMe}: *dichloromethane and methanol extractable fractions;* F_{KOHhy}: *base-hydrolyzable fractions;* F_{CuOox}: *CuO-oxidizable fractions.*

	SGR								**WGR**								
	F_{DcMe}		F_{KOHhy}		F_{CuOox}		Residue		F_{DcMe}			F_{KOHhy}		F_{CuOox}		Residue	
Biomass (%)	8 ± 0	d	34 ± 0	a	33 ± 1	b	25 ± 1	c	13 ± 0	c	*	33 ± 1	a	31 ± 4	a	23 ± 4	b
Carbon (%)	4 ± 1	b	15 ± 0	a	16 ± 0	a	4 ± 0	b	8 ± 0	b	*	16 ± 0	a	15 ± 1	a	5 ± 1	c
Ash (%)	16 ± 2 *								8.6% ± 1.3%								

More detailed comments

Line 12: carbonaceous organics is a bit counterintuitive – the definition of organic is that they should contain C, while carbonaceous suggests rather inorganic C sources.

Re: “carbonaceous” has deleted in the manuscript accordingly. (Page 1, Line 14)

Line 16: what was the hypothesis or expectation how different environmental (oxic – anoxic) should cause in roots (Question 1)? Why were those two extraction methods selected to get different fractions (Question 2)?

Re: **Question 1**: Plants can adapt to hypoxia by changing the morphology and anatomical structure of the root system. It has been revised in the abstract: “*Flooding is known to regulate the physiology and morphology in plant roots; however, its impact on molecular-level characteristics of carbon compounds (carbon traits) in fine roots remain largely unexplored*”. (Page 1, Line 12–14)

Question 2: Carbon compounds are highly diverse in fine roots. Therefore, direct measurement often suffers from low resolution due to limitations inherent in the detection method. To overcome this challenge, it becomes imperative to isolate and characterize components within the sample. In this study, we adopt a systematic approach, employing a sequential extraction method that progresses from non-polar to polar solvents. This methodology ensures comprehensive analysis, allowing for a more nuanced understanding of the intricate chemistry present within fine roots. It has been revised in the abstract: “*we used a sequential extraction method, starting from non-polar to polar solvents, to obtain*…”. (Page 1, Line 15–16)

How were the decomposers selected? Was this maybe also a result of communities present under oxic conditions vs. anoxia?

Re: To obtain the same decomposers in two incubation conditions of our decomposition experiment, A horizon soil near SGR and surface sediment near WGR were collected and homogenized based on a 1:1 fresh mass ratio. Then, both SGR and WGR were incubated in the mixed matrix under anoxic or aerobic conditions. In addition, given the understanding that identical initial microbial communities undergo substantial alterations when subjected to varying environmental conditions, this study primarily focuses on elucidating the decomposition characteristics exhibited by two distinct types of fine roots under identical incubation conditions. The result showed that organic carbon decomposability was consistently higher in WGR than in SGR regardless of the incubation conditions, and WGR in anoxic condition had similar decomposability to SGR in aerobic condition (Fig. 7). Therefore, we suggested that "*although flooding provided an anoxic condition that slowed down root decomposition, the adaptive strategy of developing more non-structural labile components in water-grown roots accelerated root decomposition, thereby counteracting the effects of anoxia.*" (Page 1, Line 23–25)

Line 29: check reference, it should be McCormack 2015

Re: It has been revised accordingly. (Page 1, Line 29)

Line 30: what other underground organs do plants use?

Re: In addition to fine roots, which primarily serve absorption functions, the underground organs of trees encompass coarse roots, tasked with facilitating transportation, storage, and structural support within the plant.

Line 30: delete the comma after 'and'

Re: It has been revised accordingly. (Page 1, Line 31)

Line 34: I think it should be C containing organic compounds (but could be just organic compounds (as per definition they should contain C). and maybe already give examples from the start of the MS).

Re: Thank you for your suggestion. The term "*carbonaceous*" has been replaced with "*carbon compounds*" throughout the main text. (Page 1, Line 14; Page 2, Line 34, 35, 36, 38; Page 13, Line 254, 257)

Line 35: See e.g. the reviews by Freschet, which could be of interest to the authors: doi:10.1111/nph.17572, or doi:10.1111/nph.17072

Re: We appreciate the suggestion related to the two important papers, and we have carefully studied them and cited them in our revised manuscript to promote the understanding of carbon traits (Page 2, Line 35)

Line 37: ancestry meaning 'phylogenetically conserved'?

Re: Yes, the cluster analysis of the previous study revealed a strong phylogenetic pattern in lipid composition from fine roots, with showing the lipid signatures of species from the same genus were consistently more similar to each other than to species from other genera. However, the authors did not test phylogenetic signal. Hence, it is described as: "*a high level of heterogeneity in carbon compounds of fine roots, associated with … ancestry*" (Page 2, Line 37)

Line 50: not sure if this should be a technical paper?

Re: Thank you for your comment. Upon carefully considering your comments, we have decided to remove the technical paragraph in Introduction. We main discussed the technical analyses in section 4.1 of Discussion. This helps to enhance the simplicity and scientific focus of our manuscript.

Line 60: do some plant species adapted to water saturated situations have undergone trough a selection towards different compounds?

Re: Yes. As an example, we subsequently represented a previous study that demonstrated the increased contents of suberin and lignin from the roots of rice (*Oryza sativa* L.) under flooding conditions (Kotula et al., 2009). Another study based on histochemistry and microscopy suggested apoplastic barriers with suberin lamellae and secondary lignification in the cortex of fine roots could promote adaptation of *Metasequoia glyptostroboides* to aquatic environments (Yang et al., 2019).

References

Kotula, L., Ranathunge, K., Schreiber, L., and Steudle, E.: Functional and chemical comparison of apoplastic barriers to radial oxygen loss in roots of rice (*Oryza sativa* L.) grown in aerated or deoxygenated solution, J. Exp. Bot., 60, 2155-2167, 2009.

Yang, C., Zhang, X., Wang, T., Hu, S., Zhou, C., Zhang, J., and Wang, Q.: Phenotypic plasticity in the structure of fine adventitious metasequoia glyptostroboides roots allows adaptation to aquatic and terrestrial environments, Plants (Basel), 8, 501, 2019.

Line 83: this is not a real hypotheses, but a technical question?

Re: Thank you for your suggestion. 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 L72–74)

Line 93: I think this is very important information that there are many plants growing at the waterline, this has not totally come across in the introduction. It sounded like a greenhouse experiment so far.

Re: Thank you for this valuable suggestion. We have added the content in the manuscript: "*Numerous plants naturally thrive at the waterline within tropical forests, offering an exceptional opportunity to examine and verify the responsiveness of plant fine roots to environmental fluctuations.*" (Page 3, Line 64–65)

Line 99: based on the previous studies, what should be the main components extracted in each fraction that would need such a highly work- and chemical intense fractionation scheme? It might be nice to add what is known what compounds the different fraction are supposed to contain.

Re: It has been added in the manuscript: "*Extraction of the roots with organic solvents followed by alkaline hydrolysis can obtain the free compositions (such as free lipids, steroids, and carbohydrates) and ester-bound compositions (cutin and suberin monomers), respectively. The CuO oxidation yields ether-bonded compositions (preferentially lignin phenols).*" (Page 3, Line 91–93).

In addition, we have added the description of three expected fractions in Figure 1.

Line 105: derivatized.

Re: It has been revised accordingly. (Page 4, Line 98)

Line 150: It would be great if the statistical section could be extended. Were all data normal distributed, were data transformed if they were not?

Re: Thank you for your insightful comments on our statistical approach. In this study, the sample size in each group was determined based on the results of three repeated experiments. It is worth noting that the data error observed in most repeated experiments was minimal. All data were found to be normally distributed according to the Shapiro-Wilk test. Therefore, it is not necessary to perform data transformation.

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 156: based on the figure 2a it looks as if the F_{KOHhy} and F_{CuOox} fraction are very similar both in sized and C content. Would be great if there is some statistical support to detect if they are similar or different.

Re: According to your suggestion, we have added the Table S1 in SI to show the statistical analysis for the biomass and carbon fractionation, and ash content. It shows that there is no significant difference both in biomass and carbon content between F_{KOHhy} and F_{CuOox} fraction of all roots except for biomass in SGR. Therefore, the manuscript has been revised: "*F_{KOHhy} and F_{CuOox} had the similar (P > 0.05) and highest biomass fractions, together accounting for 34% ± 1% and 32% ± 3% of the total biomass, respectively* ..." (Page 6, Line 152–153)

Line 158: give exact numbers here for the residual biomass after all digestion steps, and moreover, test also if there were differences between the WGR and SGR.

Re: It has been revised accordingly: "*There was approximately 24% of biomass residue after the three-step extraction. … Although WGR had near twice biomass and carbon fractions in F_{DcMe} of SGR (P < 0.05), the two classes of roots were similar on both biomass and carbon content in the other three fractions (P > 0.05; Table S1).*" (Page 6, Line 154–158)

Figure 4: please give a clear description of the y-axis – in the text it is referred to C here to OC

Re: Thank you for your comment. All concentrations should be organic carbon (OC)-normalized. It has been added in the caption of Figure 4: "*The Y axes represent organic carbon (OC)-normalized concentrations.*" And the unit "*mg g C^{-1}*" has been replaced with "*mg g OC^{-1}*" throughout the main text.

Line 214: and/or

Re: It should be "and".

Line 218: what is meant with the common formulae? Common compounds? assignable compounds? classified compounds?

Re: We utilized the term 'formulae' instead of 'compounds' because in our study, the mass spectrum obtained from FT-ICR MS comprises numerous mass peaks, each representing a molecular formula. Identification of mass peaks involves calculating the elemental composition based on the exact relative atomic masses, referencing the basic chemical guidelines and databases of naturally occurring molecules, thus assigning molecular formulae to the mass peaks. It's important to note that a single formula may correspond to multiple isomers or compounds, hence 'formulae' was chosen to accurately reflect this aspect of our analysis.

The term ‘common formulae’ refers to those formulae shared among the three replicates. This excludes any unique formulae present in only one or two of the three replicates.

Figure 5 it is difficult to see if the treatments/categories overlap, it would help to reduce the transparency of the dots.

Re: The dots in Figure 5 have been revised accordingly.

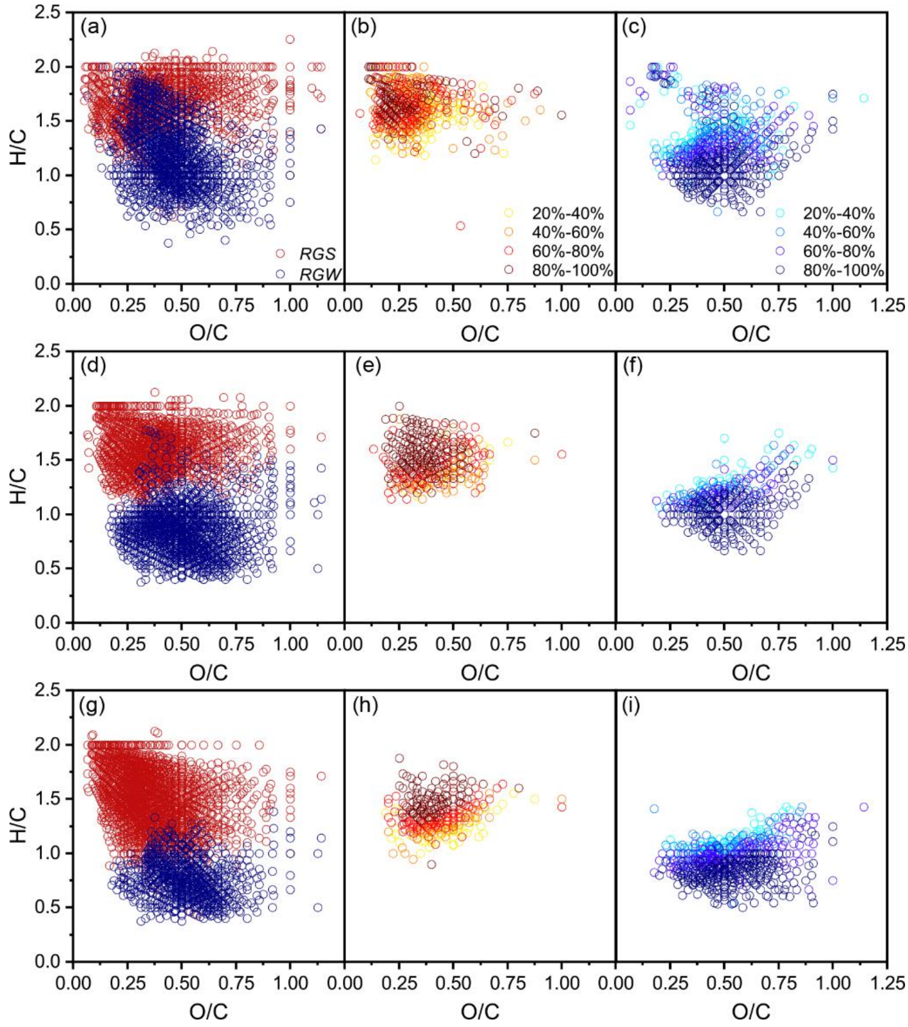

__Figure 5__ Comparison of van Krevelen diagrams for Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)-detected specific formulae of soil-grown roots (SGR, in red) and water-grown roots (WGR, in blue) from …

Line 266: change to targeted and non-targeted approaches

Re: It has been revised accordingly.

Line 280: were there changes in N content or other micronutrients observed? In certain environments those can be important for decomposers and decomposition rates?

Re: Thank you for your insightful comments. We have added the Table S6 in SI to show the morphology and chemical elements (C, N, C:N) of different fine-root samples. SGR has a higher specific root length and nitrogen content, with a lower average diameter and carbon nitrogen ratio than WGR ($P < 0.05$), which theoretically suggests that SGR has a higher decomposition rate than WGR. However, our decomposition experiment exhibited the organic carbon decomposability was higher in WGR than in SGR. Therefore, molecular-level carbon traits of fine roots may play a key role in unveiling decomposition under flooded condition.

It has revised in manuscript: "*To investigate the influencing factors regulating fine-root decomposition, we detected the morphological traits and nitrogen content in origin roots. The results showed that SGR has a higher specific root length and nitrogen content, with a lower average diameter and carbon nitrogen ratio than WGR ($P < 0.05$; Table S6)*." (Page 13, Line 241–244)

"*Although conventional wisdom held that high specific root length and lower carbon nitrogen ratio commonly correlated with fast decomposition rates (Guo et al., 2021), our decomposition experiment exhibited the organic carbon decomposability was higher in WGR with lower specific root length and higher carbon nitrogen ratio than in SGR (Fig. 7, Table S6). Therefore, the commonly used parameters like morphological traits and stoichiometric indicators can not well predict the decomposition characteristics of fine roots under flooded conditions.*" (Page 15, Line 327–332)

Line 286: Does less ash indicate more organic compounds being lost during ignition, right? Why is this in contrast to the previous sentence?

Re: Yes, WGR has less ash but more organic compounds than SGR. The previous sentence also shows "*compared with SGR, WGR contained a high proportion of free compounds and free carbon.*" (Page 14, Line 283)

Line 291: but the compounds are specific to different stressors: e.g. suberin has several times been reported to be increased in response to water stress see e.g. doi: 10.3390/metabo11110735

Re: Thank you for your suggestion and recommendation. We agree with your view about the specific response of suberin in plants to water stress. Also, some previous study indicated that suberin would be increased in response to flooding stress (Soukup et al., 2007; Yamauchi et al., 2018; Yang et al., 2019). Therefore, we have cited the reference and revised the manuscript: "*many recalcitrant heteropolymers, including lignin and suberin phenolics, are biosynthesized via the phenylpropanoid pathway and can be directly regulated by environmental stressors (Dong and Lin, 2021), including flooding (Yamauchi et al., 2019), drought and salt stresses (De Silva et al., 2021).*" (Page 14, Line 292–293)

References:

Soukup, A., Armstrong, W., Schreiber, L., Franke, R., Votrubova, O., 2007. Apoplastic barriers to radial oxygen loss and solute penetration: A chemical and functional comparison of the exodermis of two wetland species, *Phragmites australis* and *Glyceria maxima*. New Phytologist 173, 264-278.

Yang, C., Zhang, X., Wang, T., Hu, S., Zhou, C., Zhang, J., Wang, Q., 2019. Phenotypic plasticity in the structure of fine adventitious *Metasequoia glyptostroboides* roots allows adaptation to aquatic and terrestrial environments. Plants (Basel) 8, 501.

Yamauchi, T., Colmer, T.D., Pedersen, O., Nakazono, M., 2018. Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress. Plant Physiology 176, 1118-1130.

Line 309: one could also suggest that in soil plant roots need more and stronger structures supporting a lot of lignin-phenolics, to endure the more harsh conditions.

Re: This is a good point, but it is not fully supported by our data. In this study, there are no significant difference in lignin-derived phenol contents between SGR and WGR.

Line 320: it would be great if a sentence about potential differences and impacts on decomposer communities could be discussed see e.g. https://doi.org/10.1016/j.soilbio.2020.10779 as well as the conditions for those decomposers.

Re: Thank you for your valuable suggestion and recommendation. We have cited the reference and revised the manuscript accordingly: "*Moreover, environmental stressors can alter the structure and metabolic activity of the decomposer community (Chuckran et al., 2020). For example, flooding of soils induced the function increase of CH4 production, S cycle, and Fe cycle (e.g., Bathyarchaeota, Bacteroidetes, and Geobacter) and the function decrease of environmental remediation and biological control (e.g., Blastococcus and Roseiflexus) (Luo et al., 2023), which consequently regulated organic carbon sequestration and biogeochemical cycling.*" (Page 15, Line 321–325)

Line 343: , 'counteracting' the reducing effect .. or 'and counteract'

Re: It has been revised accordingly: "*flooding would accelerate short-term root decomposition to counteracting the effect of anoxic conditions.*" (Page 16, Line 353)