nature portfolio

Peer Review File

Microbial responses to changing plant community protect peatland carbon stores during Holocene drying

Corresponding Author: Professor Xianyu Huang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In this manuscript, the authors use paleoecological macrofossil, FTIR, and advanced biomarkers geochemical data from a peatland in southern China to reconstruct vegetation composition, peat chemical composition and microbial metabolism in order to understand controls of carbon accumulation and organic matter protection mechanism. The authors find that the high carbon accumulation rates during the mid-Holocene at 8-6 ka correspond with high woody material, high TOC, high aromatics, and variable biomarker-based proxies. They conclude that vegetation change to woody plants dominance was caused by a dry climate, resulted in an increase in aromatics, and reduced bacterial heterotrophy, and eventually increase carbon protection and enhance carbon storage.

The study addresses an important research topic due to the large carbon stock in peatlands and its vulnerability to climate change and human disturbance and potential complex biogeochemical processes involved. The manuscript is well organized and written. I have several comments and suggestions as listed below.

1. Regional comparisons of peatland vegetation change to woody dominance is not convincing. The tree pollen records from other three peatlands as shown in Fig. 2b-d mostly reflect regional vegetation change or vegetation change from surrounding uplands, not local vegetation on the peatland that would produce litter inputs to peats. For example, there are no trees or low abundant shrubs, if any, in peatlands around Hongyuan, as they are dominated by Carex and/or Kobresia. We would need plant macrofossil data to support the presence and abundance of trees/woody plants on peatlands.

2. The study site is strongly influenced by East Asian summer monsoon, and it should reflect that general hydroclimate pattern showing stronger monsoon and greater precipitation amount in the mid-Holocene than in the late Holocene. However, the proxy curves labeled as Hydroclimate in Fig. 2g-l show large and high-frequency fluctuations that lack long-term trends as shown in other typical summer monsoon records. These proxies may reflect local peatland moisture conditions, but not necessarily regional hydroclimate. Correlations with regional climate change as shown in Fig. 2 are generally not convincing, and I believe that such a direct connection with climate change is not really necessary for this study. If you can show that a drying peatland induces woody plant expansion and that climate change and human activities could cause peatland drying, then the proposed mechanism would apply and most parts of story hold. I just think the whole regional woody expansion and climate correlations are not convincing, including the other curves that I did not comment on here.

3. Data synthesis of carbon accumulation rates of woody vs. non-woody peatlands needs to be re-analyzed. As now, the synthesis analysis treats tropical peatlands (swamp forests) and northern peatlands, including permafrost-affected peatlands, in the same way and calculation. Tropical swamps from SE Asia and South America are quite different ecosystems compared to the study peatland located in subtropical mountains (so the climate could be similar to non-permafrost northern peatlands). I think they need to be analyzed separately in comparing woody vs. non-woody peats. Also, the authors need to specify exactly how many peatlands and peat cores and samples/measurements are used in the analysis of each type of peatlands.

Specific comments:
L1: “Plant-microbe interactions” in the title seems to be inaccurate and misleading. In the soil microbial and ecological literatures, plant-soil microbe interactions refer to two way “interactions” that include how plants, especially root extrudes,

influences soil carbon stability, such as through priming effect, and how microbial responses might influence mineralization and nutrient availability to plants. In this manuscript, the influence appears to be one way about how change in vegetation composition and soil organic matter input affect microbial activities.

L27: need a specific number of peatlands, not “over 150 peatlands”

L41: The claim of peatland carbon stock of >1000 GtC has been criticized in regarding to its incorrect algorithm and uncritical use of records in databases.

L120: a swamp can be a peatland as well, but it is different from bogs or fens commonly found in northern peatlands. Are there more detailed macrofossil results, such as at species or section level for Sphagnum macrofossils? In addition to Sphagnum, is there any other mosses present? Oftentimes, Sphagnum and mosses are better preserved and more identifiable in peats than herbaceous and woody remains. A forest swamp could have abundant understory mosses, but not the same Sphagnum species often found in peatlands.

L123-129: Tree pollen mostly reflect regional vegetation or vegetation in the surrounding upland, rather than local vegetation on peatlands. See comments above.

L162-166: too much speculations, and they are not necessary here.

L167-171: The reference Jia et al. 2015 is about sedimentary record from a maar lake, not peatland, and C3 and C4 plants are not relevant here.

L187-197: The discussion and evidence presented for a drying climate in the mid-Holocene at 8-6 ka is not convincing.

L216-218: not a good idea to discuss too much on a single-data point. Could it be a measurement outlier?

L323-330: As aCAR values are determined by (calculated from) peat accumulation rates, bulk density and carbon content (formula in line 580). Carbon content (Fig. 3i) and accumulation rate (Fig. S10) at 8-6 ka seem to show limited variations, so I wonder if the spikes in aCAR during 8-6 ka are mostly due to high bulk density. Also, bulk density mostly reflects the degree of decomposition, that is, highly decomposed peat becomes dense as decomposition reduces the size of organic materials. The authors should consider to discuss and compare their bulk density results.

L333-335: Correlation may not represent causation. See comments above about aromatics, BD, and aCAR relationship. Also, primary production could play a major role in causing the high aCAR at 8-6 ka, not just decomposition. If this is the case, then decomposition and microbial role are secondary. How to evaluate the relative contribution of production and decomposition to aCAR?

L350-357: Fig. S7 shows several correlation relations between aCAR and other variables, and the authors should be cautious in inferring causation. For example, the positive relationship between aCAR and aromatics in Fig. S7f could be due to that the high calculated aCAR at values above 40 g C/m2/yr at 8-6 ka is due to high bulk density, and the peat layer with high bulk density is highly decomposed with concentrated recalcitrant aromatics. Thus it may not be that high aromatics causes high aCAR, but high decomposition results in high bulk density and high aCAR as well as high aromatics. Greater primary production would compensate high decomposition to result in high aCAR values.

L390-393: how is this calculated?

L436-437: aCAR calculations need to separate northern (including permafrost-affected) peatlands from tropical peatlands. See comments above.

L465: I wonder how the peatland has changed after 6 ka. As presented in Fig. 3i, there are dramatic decrease in TOC after 6 ka, and this is at a shallow depth of ~40 cm. Could recent ~40 cm peat represent disturbance that caused a wet successional change from a rapidly-accumulating peatland/ swamp? Are there any human activities/disturbance at the peatland or in the watershed? It would be useful to indicate in the manuscript the presence or absence of disturbance at the site.

L466: The term subalpine is probably inaccurate here, as it refers to a climate/vegetation zone not far below the treeline. At only 1600-1800 m elevation, I don’t think it is anywhere closer to treeline at such a low 25 N latitude location. Also, it would be useful to provide climate information, such as mean annual temperature. Such an information would also help the authors decide that the study peatland is more like northern or tropical peatlands.

L597: Need the exact number of peatlands.

L612-614: The assumed values for TOC and bulk density induce great uncertainties. These sites should be removed from the analysis.

Fig. 4. It appears that this figure simply summarizes the correlation coefficient (r value) in the one-to-one correlation analysis in the text, but I’m not sure this is appropriate and useful. Also, relative abundance of aromatics not only influence microbial metabolism, but also the product of microbial-induced decomposition of organic matter, as greater decomposition removes more carbohydrate and concentrates aromatics. Suggest to redraw or delete.

Reviewer #2

(Remarks to the Author)
The topic addressed in this study is highly relevant. The investigation of plant succession towards more woody plants in peatlands with drier climate conditions and its influence on the microbial community is of great importance for understanding long-term carbon cycling in these ecosystems.
The study presents strong and well-supported correlations between vegetation dynamics, microbial metabolic processes, and carbon sequestration potential. In particular, the combination of vegetation data, metabolic indicators (such as the relative abundance of carbohydrates and aromatics), and long-term carbon storage rates is a noteworthy strength of this work. The authors succeed in linking changes in plant community composition—especially an increase in woody plant cover—with altered decomposition patterns and carbon dynamics.
The data set is comprehensive and convincingly connects site-specific analyses with broader climatic and ecological data sets. The use of a wide range of parameters enables a multi-dimensional perspective on ecosystem responses to environmental change, producing a rich and holistic picture of peatland development, vegetation history, and diagenesis.
Of particular interest is the quantification of the impact of woody plant increase on soil carbon storage (line 390), providing an exact value (10% woody plant increase leading to 16.9 g C/m²/yr change), which adds significant value for climate modeling and ecosystem management.
My main concern relates to the microbial community investigated. While the study centers on bacterial communities, it is essential to consider that microbial decomposition in soils involves not only bacteria but also archaea and, importantly, fungi. Especially in peat soils, where the conditions shift from aerobic to anaerobic, fungi can become dominant decomposers under drier conditions. A potential shift from bacterial- to fungal-dominated decomposition in line with increased woody plant presence and drying could substantially affect carbon cycling. Therefore, I strongly recommend including markers for fungal biomass and activity, such as PFLAs 18:2ω6,9 or 16:1ω5. This would allow a more comprehensive interpretation of the microbial community dynamics.
Specific comments:
- L64ff: Please explain why the microbial community is destabilized by changes and why this leads to increased degradation. This concept should be more clearly defined.
- L83ff: Can you disentangle the effects of warming (and thus increased oxygen availability) from vegetation change? Because from my point of view vegetation always respond to changing climate conditions.
- L175ff: Since hydrology changes have to have an impact on vegetation changes, it seems implausible to assume climate change without concurrent vegetation change. Please clarify this assumption.
- L223: I support the suggestion that increased fungal competition under warm and dry conditions could explain some of the contradictory findings. Also, the observed 13C bulk data may reflect microbial community shifts (e.g., fungi typically show enriched 13C signatures compared to bacteria). These aspects would greatly benefit from fungal data to ensure robust conclusions.
- L290: Are there data available on CO_2 fluxes at sites with varying aromatic compound levels? This could strengthen the argument that higher aromatic content in woody-dominated sites leads to greater carbon recalcitrance.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have done a really good job in addressing my comments on the earlier version of the manuscript. The response to other reviewer's comments also appears to strengthen the manuscript. I have no further comments, but just a few editorial suggestions that I think can be addressed during proof corrections stage.

Editorial suggestions:
Line numbers are as in tracked change PDF file.

L35: change "fewer" to "less" or "decreased"?

L 396: change to "bulk density mostly varies between 0.43 and 0.66 g/cm3" (this probably works better than the alternative phrase "ranges from … to…"; however, "ranges between … and…" is incorrect)

L401: change "between 14-12 ka" to "between 14 and 12 ka"

Reviewer #2

(Remarks to the Author)
All of my previous comments have been fully and thoroughly addressed.
In particular, the additional analysis on biomarkers specific to fungal abundance significantly strengthens the conclusions of the manuscript and represents a substantial improvement.
The discussion on CO_2 fluxes is coherent and well-reasoned.

From my perspective, the manuscript is now ready for publication.

****

Response to comments from the Reviewer #1:

Reviewer #1:

In this manuscript, the authors use paleoecological macrofossil, FTIR, and advanced biomarkers geochemical data from a peatland in southern China to reconstruct vegetation composition, peat chemical composition and microbial metabolism in order to understand controls of carbon accumulation and organic matter protection mechanism. The authors find that the high carbon accumulation rates during the mid-Holocene at 8-6 ka correspond with high woody material, high TOC, high aromatics, and variable biomarker-based proxies. They conclude that vegetation change to woody plants dominance was caused by a dry climate, resulted in an increase in aromatics, and reduced bacterial heterotrophy, and eventually increase carbon protection and enhance carbon storage.

The study addresses an important research topic due to the large carbon stock in peatlands and its vulnerability to climate change and human disturbance and potential complex biogeochemical processes involved. The manuscript is well organized and written. I have several comments and suggestions as listed below.

Reply: We thank the reviewer for the positive assessment of our manuscript and for the constructive comments provided. These comments have greatly helped us clarify key points and improve the manuscript.

1. Regional comparisons of peatland vegetation change to woody dominance is not convincing. The tree pollen records from other three peatlands as shown in Fig. 2b-d mostly reflect regional vegetation change or vegetation change from surrounding uplands, not local vegetation on the peatland that would produce litter inputs to peats. For example, there are no trees or low abundant shrubs, if any, in peatlands around Hongyuan, as they are dominated by Carex and/or Kobresia. We would need plant macrofossil data to support the presence and abundance of trees/woody plants on peatlands.

Reply: We agree with the reviewer that the pollen records cited from other peatlands primarily reflect upland vegetation and cannot be directly interpreted as evidence of woody plant expansion within peat-forming communities. In the revised manuscript, we have deleted almost all of the corresponding discussion of regional woody expansion and clearly stated that these pollen records most likely reflect regional upland vegetation dynamics rather than local peatland vegetation. Due to the lack of plant macrofossil data from these sites, we are currently unable to determine whether mid-Holocene woody expansion occurred more broadly across southern Chinese peatlands. As such, the relevant pollen curves (previously shown in Fig. 2) have been removed from the main figure and are now presented as background information in the Supplementary Materials (Supplementary Fig. S1). We now restrict our interpretation of woody plant expansion to the ZGT peatland, based on our macrofossil evidence. Changes made in Lines 131-143 of the revised manuscript.

2. The study site is strongly influenced by East Asian summer monsoon, and it should reflect that general hydroclimate pattern showing stronger monsoon and greater precipitation amount in the mid-Holocene than in the late Holocene. However, the proxy curves labeled as Hydroclimate in Fig. 2g-I show large and high-frequency fluctuations that lack long-term trends as shown in other typical summer monsoon records. These proxies may reflect local peatland moisture conditions, but not necessarily regional hydroclimate. Correlations with regional climate change as shown in Fig. 2 are generally not convincing, and I believe that such a direct connection with climate change is not really necessary for this study. If you can show that a drying peatland induces woody plant expansion and that climate change and human activities could cause peatland drying, then the proposed mechanism would apply and most parts of story hold. I just think the whole regional woody expansion and climate correlations are not convincing, including the other curves that I did not comment on here.

Reply: We appreciate the reviewer's insightful comments and agree that the regional paleohydroclimate correlations in the original version were overextended. In the revised manuscript, we have removed the broad discussion of East Asian summer monsoon intensity and deleted the regional hydroclimate proxy curves from the main figure (previously Fig. 2h-2o). We now focus on local signals from the ZGT peatland to support our interpretation of localized mid-Holocene drying. Specifically, we interpret the rise in plant-wax $\delta^{13}C$ values and the increase in hopanoid flux between 8-6 ka as evidence for lower water tables and drier conditions at the site (revised Fig.2b and 2c). We have revised the main text (Lines 181-224) to reflect this shift in focus and no longer attempt to correlate our site with regional-scale monsoon records. As suggested by the reviewer, we now present a more self-contained argument that links drying within the ZGT peatland to woody plant expansion.

3. Data synthesis of carbon accumulation rates of woody vs. non-woody peatlands needs to be re-analyzed. As now, the synthesis analysis treats tropical peatlands (swamp forests) and northern peatlands, including permafrost-affected peatlands, in the same way and calculation. Tropical swamps from SE Asia and South America are quite different ecosystems compared to the study peatland located in subtropical mountains (so the climate could be similar to non-permafrost northern peatlands). I think they need to be analyzed separately in comparing woody vs. non-woody peats. Also, the authors need to specify exactly how many peatlands and peat cores and samples/measurements are used in the analysis of each type of peatlands.

Reply: We thank the reviewer for this valuable suggestion. In the revised manuscript, we have re-analyzed the global synthesis by separating tropical and northern peatlands, recognizing their distinct vegetation and climatic characteristics. The updated dataset includes 128 northern peatlands (166 cores, 372 measurements) and 27 tropical peatlands (30 cores, 148 measurements), with all entries lacking TOC or bulk density excluded. Our results continue to support the conclusion that woody-dominated periods are associated with significantly higher aCAR values in both climate zones (Lines 507-536 and Supplementary Fig. S9a and S9b).

We agree with the reviewer that tropical swamps are quite different ecosystems compared to the study peatland and northern peatlands. We have added a brief discussion noting that the substantially higher aCAR values observed in tropical woody peatlands may result from a combination of ecological factors, including sustained woody dominance, high primary productivity, and the accumulation of recalcitrant organic matter (Lines 523-533). This highlights that although the association between woody vegetation and carbon accumulation appears broadly consistent, the underlying mechanisms may differ between tropical and northern peatland systems.

Specific comments:
L1: "Plant-microbe interactions" in the title seems to be inaccurate and misleading. In the soil microbial and ecological literatures, plant-soil microbe interactions refer to two way "interactions" that include how plants, especially root extrudes, influences soil carbon stability, such as through priming effect, and how microbial responses might influence mineralization and nutrient availability to plants. In this manuscript, the influence appears to be one way about how change in vegetation composition and soil organic matter input affect microbial activities.
Reply: We agree that the original title may have overstated bidirectional plant-microbe interactions. We have revised the title to: Microbial responses to changing plant community protect peatland carbon stores during Holocene drying.

L27: need a specific number of peatlands, not "over 150 peatlands"
Reply: We have revised the sentence to specify the exact number: 155 peatlands.

L41: The claim of peatland carbon stock of >1000 GtC has been criticized in regarding to its incorrect algorithm and uncritical use of records in databases.
Reply: We have removed the >1000 GtC estimate and now cites a more widely accepted range of 500-700 GtC (Lines 44-46).

L120: a swamp can be a peatland as well, but it is different from bogs or fens commonly found in northern peatlands. Are there more detailed macrofossil results, such as at species or section level for Sphagnum macrofossils? In addition to Sphagnum, is there any other mosses present? Oftentimes, Sphagnum and mosses are better preserved and more identifiable in peats than herbaceous and woody remains. A forest swamp could have abundant understory mosses, but not the same Sphagnum species often found in peatlands.
Reply: We agree this is an important distinction. In our plant macrofossil analysis, we identified two *Sphagnum* species: *S. palustre* and *S. subsecundum*. No other moss taxa were observed or preserved in the peat samples. We have clarified this point in the revised text to better distinguish our site as a *Sphagnum* bog with woody shrubs, rather than a forested swamp.

L123-129: Tree pollen mostly reflect regional vegetation or vegetation in the surrounding upland, rather than local vegetation on peatlands. See comments above.

Reply: We agree and have clarified that these pollen records reflect regional upland vegetation. The related records have been removed from Fig. 2 and are now presented only in the Supplementary Information.

L162-166: too much speculations, and they are not necessary here.

Reply: We agree that the original version contained too many speculative explanations. In the revision, we have removed unnecessary mechanisms.

L167-171: The reference Jia et al. 2015 is about sedimentary record from a maar lake, not peatland, and C3 and C4 plants are not relevant here.

Reply: We have removed the reference Jia et al. (2015) and the related discussion from the revised manuscript.

L187-197: The discussion and evidence presented for a drying climate in the mid-Holocene at 8-6 ka is not convincing.

Reply: We appreciate the reviewer's concern and have revised the manuscript to clarify that our interpretation of drying during 8-6 ka is based on local evidence from the ZGT peat core, including enriched $\delta^{13}C$ values of plant waxes and increased hopanoid flux. We no longer attempt to infer regional climate trends and have removed broader paleohydrological comparisons from the main text and figures.

L216-218: not a good idea to discuss too much on a single-data point. Could it be a measurement outlier?

Reply: We have revised the text to avoid overinterpreting the single-point peak at 7.3 ka. We now acknowledge that localized variability or analytical uncertainty cannot be excluded.

L323-330: As aCAR values are determined by (calculated from) peat accumulation rates, bulk density and carbon content (formula in line 580). Carbon content (Fig. 3i) and accumulation rate (Fig. S10) at 8-6 ka seem to show limited variations, so I wonder if the spikes in aCAR during 8-6 ka are mostly due to high bulk density. Also, bulk density mostly reflects the degree of decomposition, that is, highly decomposed peat becomes dense as decomposition reduces the size of organic materials. The authors should consider to discuss and compare their bulk density results.

Reply: Thank you for this thoughtful comment. In the revised manuscript, we have added and discussed bulk density data from the full ZGT core (now shown in Fig. 2n). During the 8-6 ka interval, we find that bulk density ranges between 0.43 and 0.66 g/cm^3, with a notable single-point peak of 0.85 g/cm^3 around 7 ka, coinciding with the aCAR maximum. We acknowledge that this may have partially contributed to the elevated aCAR values. However, the bulk density during 8-6 ka is very similar to that of other periods and shows no significant difference ($p = 0.12$), and even lower than values observed during the last deglacial (0.8-1.0 g/cm^3 between 14-12 ka) (Fig. 2n). We therefore interpret that while compaction may have influenced aCAR to

some extent, the main drivers were elevated TOC and reduced microbial degradation linked to vegetation and organic matter changes (Lines 393-402).

L333-335: Correlation may not represent causation. See comments above about aromatics, BD, and aCAR relationship. Also, primary production could play a major role in causing the high aCAR at 8-6 ka, not just decomposition. If this is the case, then decomposition and microbial role are secondary. How to evaluate the relative contribution of production and decomposition to aCAR?

Reply: We thank the reviewer for raising this point. As clarified in the following response, we have incorporated bulk density data (Fig. 2n) and addressed the relationship among aromatics, BD, and aCAR. We agree that primary production may have played a key role in the observed aCAR increase. In fact, the paragraph following our correlation analysis explicitly acknowledges the influence of elevated productivity during woody plant expansion.

L350-357: Fig. S7 shows several correlation relations between aCAR and other variables, and the authors should be cautious in inferring causation. For example, the positive relationship between aCAR and aromatics in Fig. S7f could be due to that the high calculated aCAR at values above 40 g C/m2/yr at 8-6 ka is due to high bulk density, and the peat layer with high bulk density is highly decomposed with concentrated recalcitrant aromatics. Thus it may not be that high aromatics causes high aCAR, but high decomposition results in high bulk density and high aCAR as well as high aromatics. Greater primary production would compensate high decomposition to result in high aCAR values.

Reply: We appreciate the reviewer's thoughtful caution regarding the interpretation of correlations and agree that such relationships should be interpreted with care. In the revised manuscript, we have added bulk density data for the ZGT core (Fig. 2n). During the period of elevated aCAR (8-6 ka), bulk density did not increase and remained comparable to other periods (Fig. 2n), indicating that high bulk density is unlikely to explain the high aCAR. Crucially, the complexities in inferring causation is why our microbial biomarker and isotopic data are critical to interpretation. They suggest suppressed microbial heterotrophic degradation during this interval. Therefore, we interpret the increase in aromatics as primarily resulting from woody plant expansion-driven changes in organic matter inputs, particularly the accumulation of lignin-rich woody residues, rather than decomposition processes.

L390-393: how is this calculated?

Reply: The estimated changes in carbon accumulation were calculated based on regression equations between aCAR and key variables (e.g., woody plant abundance, organic composition, ε values). We calculated the effects by assuming a 10% increase in woody plant abundance and applying regression equations to estimate the resulting aCAR changes via enhanced primary productivity, shifts in organic matter composition, and microbial metabolism.

L436-437: aCAR calculations need to separate northern (including permafrost-affected) peatlands from tropical peatlands. See comments above.

Reply: We have separated northern and tropical peatlands (revised Fig. S9a and S9b, and Lines 516-522).

L465: I wonder how the peatland has changed after 6 ka. As presented in Fig. 3i, there are dramatic decrease in TOC after 6 ka, and this is at a shallow depth of ~40 cm. Could recent ~40 cm peat represent disturbance that caused a wet successional change from a rapidly-accumulating peatland/ swamp? Are there any human activities/disturbance at the peatland or in the watershed? It would be useful to indicate in the manuscript the presence or absence of disturbance at the site.

Reply: Thank you for this suggestion. In the revised manuscript, we have clarified that the uppermost peat layers are highly decomposed and may have been affected by recent land use. By extension, we also acknowledge that the post-6 ka decline in woody vegetation and aCAR may reflect possible anthropogenic disturbance (Lines 475-478).

L466: The term subalpine is probably inaccurate here, as it refers to a climate/vegetation zone not far below the treeline. At only 1600-1800 m elevation, I don't think it is anywhere closer to treeline at such a low 25 N latitude location. Also, it would be useful to provide climate information, such as mean annual temperature. Such an information would also help the authors decide that the study peatland is more like northern or tropical peatlands.

Reply: We thank the reviewer for this helpful clarification. In the revised manuscript, we have removed the term "subalpine" and added local climate information: The site experiences a mean annual temperature of 14.2 °C and a mean annual precipitation of 1890 mm (Han et al., 2016).

L597: Need the exact number of peatlands.

Reply: Changed to 155 peatlands.

L612-614: The assumed values for TOC and bulk density induce great uncertainties. These sites should be removed from the analysis.

Reply: We have removed these sites in the revised version.

Fig. 4. It appears that this figure simply summarizes the correlation coefficient (r value) in the one-to-one correlation analysis in the text, but I'm not sure this is appropriate and useful. Also, relative abundance of aromatics not only influence microbial metabolism, but also the product of microbial-induced decomposition of organic matter, as greater decomposition removes more carbohydrate and concentrates aromatics. Suggest to redraw or delete.

Reply: We thank the reviewer for the suggestion. In the revised manuscript, we have updated Fig. 4 to reflect a bidirectional relationship between microbial heterotrophic metabolism and peat organic composition (aromatics and carbohydrates), acknowledging the point that microbial decomposition can also lead to aromatic enrichment. This clarification better reflects the feedback loop between microbial

activity and organic matter quality, while retaining the core function of the figure as a synthesis of our key findings.

****

Response to comments from the Reviewer #2:

Reviewer #2:

The topic addressed in this study is highly relevant. The investigation of plant succession towards more woody plants in peatlands with drier climate conditions and its influence on the microbial community is of great importance for understanding long-term carbon cycling in these ecosystems.

The study presents strong and well-supported correlations between vegetation dynamics, microbial metabolic processes, and carbon sequestration potential. In particular, the combination of vegetation data, metabolic indicators (such as the relative abundance of carbohydrates and aromatics), and long-term carbon storage rates is a noteworthy strength of this work. The authors succeed in linking changes in plant community composition—especially an increase in woody plant cover—with altered decomposition patterns and carbon dynamics.

The data set is comprehensive and convincingly connects site-specific analyses with broader climatic and ecological data sets. The use of a wide range of parameters enables a multi-dimensional perspective on ecosystem responses to environmental change, producing a rich and holistic picture of peatland development, vegetation history, and diagenesis.

Of particular interest is the quantification of the impact of woody plant increase on soil carbon storage (line 390), providing an exact value (10% woody plant increase leading to 16.9 g $C/m^2/yr$ change), which adds significant value for climate modeling and ecosystem management.

My main concern relates to the microbial community investigated. While the study centers on bacterial communities, it is essential to consider that microbial decomposition in soils involves not only bacteria but also archaea and, importantly, fungi. Especially in peat soils, where the conditions shift from aerobic to anaerobic, fungi can become dominant decomposers under drier conditions. A potential shift from bacterial- to fungal-dominated decomposition in line with increased woody plant presence and drying could substantially affect carbon cycling. Therefore, I strongly recommend including markers for fungal biomass and activity, such as PFLAs 18:2ω6,9 or 16:1ω5. This would allow a more comprehensive interpretation of the microbial community dynamics.

Reply: We thank the reviewer for this very positive evaluation of our study. We agree that fungi play a critical role in peatland decomposition processes, particularly under drier conditions, and appreciate the reviewer's emphasis on incorporating fungal dynamics. In response, we have re-analyzed our ZGT peat core samples to include the fungal biomarker 18:2ω6,9 fatty acid, and have added its abundance to the revised main figure (Fig. 2e). We also added a dedicated discussion of fungal

abundance trends during woody plant expansion in the main text (Lines 254-269).

In addition, although the low concentrations of 18:2ω6,9 in some intervals prevented us from obtaining reliable $\delta^{13}C$ values for this compound, we examined $\delta^{13}C$ values of saturated C18 fatty acid, which is a possible degradation product of 18:2ω6,9 and may be derived from both fungi and bacteria. The $\delta^{13}C$ values of C18:0 are presented in the Supplementary Materials (Fig. S4), and we include further discussion in the main text (Lines 283-292 and 363-369).

These new lines of evidence suggest that fungal abundance increased sharply during the onset of mid-Holocene drying and woody expansion but gradually declined thereafter, likely due to the accumulation of recalcitrant woody residues. Correlation analyses (Fig. S7e and S7f) further show that $\delta^{13}C$ values of C18:0 are associated with organic matter composition, indicating that microbial metabolism - including that of fungi - was shaped by vegetation-mediated shifts in peat organic matter composition.

We believe these additions substantially improve our interpretation of microbial community dynamics and more comprehensively address the reviewer's concerns regarding fungal involvement in peatland carbon cycling.

Specific comments:

- L64ff: Please explain why the microbial community is destabilized by changes and why this leads to increased degradation. This concept should be more clearly defined.

Reply: We thank the reviewer for pointing this out. In the revised manuscript, we have clarified this statement (Lines 67-69) by more clearly explaining recent evidence showing that shrub expansion can reduce bacterial diversity and alter keystone taxa, thereby leading to a more unstable microbial network structure (Xue et al., 2023).

- L83ff: Can you disentangle the effects of warming (and thus increased oxygen availability) from vegetation change? Because from my point of view vegetation always respond to changing climate conditions.

Reply: We have clarified that the observed woody plant expansion was a response to climatic changes, rather than an independent driver, by revising the relevant sentence to emphasize the causal relationship between warming, drying, and vegetation shifts (Lines 87-90).

- L175ff: Since hydrology changes have to have an impact on vegetation changes, it seems implausible to assume climate change without concurrent vegetation change. Please clarify this assumption.

Reply: We agree that hydrology and vegetation changes are often tightly coupled, and we do not assume that climate change occurred without concurrent vegetation change. Our original statement aimed only to interpret the proxy in question, but we

recognize that this may have caused confusion. We have removed the sentence from the revised manuscript. To clarify, our interpretation emphasizes that mid-Holocene warming and drying facilitated the observed woody plant expansion at the ZGT peatland.

- L223: I support the suggestion that increased fungal competition under warm and dry conditions could explain some of the contradictory findings. Also, the observed 13C bulk data may reflect microbial community shifts (e.g., fungi typically show enriched 13C signatures compared to bacteria). These aspects would greatly benefit from fungal data to ensure robust conclusions.

Reply: Thank you for your support. In the revised manuscript, we have added fungal data to address this point, including 18:2ω6,9 concentrations and $\delta^{13}C$ values of C18 fatty acids (Lines 254-269, 283-292). We observe that C18 fatty acids - potentially derived from fungi - are more 13C-enriched than bacterial hopanoids (Lines 283-285), and they show isotopic enrichment during the early phase of woody plant expansion followed by depletion. This pattern may reflect shifts in microbial community composition and metabolism linked to vegetation and hydrological changes.

- L290: Are there data available on CO_2 fluxes at sites with varying aromatic compound levels? This could strengthen the argument that higher aromatic content in woody-dominated sites leads to greater carbon recalcitrance.

Reply: We appreciate this insightful suggestion. While we currently do not have direct CO_2 flux measurements from the ZGT peatland to assess the relationship with aromatic compound content, existing research supports the notion that higher aromaticity is associated with reduced CO_2 emissions due to enhanced carbon recalcitrance. In particular, Normand et al. (2021) demonstrated that peatlands with greater aromatic carbon content exhibited lower aerobic CO_2 production, highlighting the role of organic matter chemistry in regulating carbon fluxes. We have now cited this study in the revised manuscript (Line 345) to strengthen our discussion on carbon recalcitrance under woody vegetation expansion.

Reference:

Normand, A. et al. Organic matter chemistry drives carbon dioxide production of peatlands. Geophys. Res. Lett. 48, e2021GL093392 (2021).

****

Response to comments from the Reviewer #1:

Reviewer #1 (Remarks to the Author):

The authors have done a really good job in addressing my comments on the earlier version of the manuscript. The response to other reviewer's comments also appears to strengthen the manuscript. I have no further comments, but just a few editorial suggestions that I think can be addressed during proof corrections stage.

Reply: We thank the reviewer for supporting our revised manuscript.

Editorial suggestions:

Line numbers are as in tracked change PDF file.

L35: change "fewer" to "less" or "decreased"?

Reply: Changed.

L 396: change to "bulk density mostly varies between 0.43 and 0.66 g/cm3" (this probably works better than the alternative phrase "ranges from … to…"; however, "ranges between … and…" is incorrect)

Reply: Changed.

L401: change "between 14-12 ka" to "between 14 and 12 ka"

Reply: Changed.

****

Response to comments from the Reviewer #2:

Reviewer #2 (Remarks to the Author):

All of my previous comments have been fully and thoroughly addressed.

In particular, the additional analysis on biomarkers specific to fungal abundance significantly strengthens the conclusions of the manuscript and represents a substantial improvement.

The discussion on CO_2 fluxes is coherent and well-reasoned.

From my perspective, the manuscript is now ready for publication.

Reply: We thank the reviewer for acknowledging the improvements in our revised manuscript.