Exploring micro-scale heterogeneity as a driver of biogeochemical transformations and gas transport in peat.

Kohl et al., in review for Biogeosciences

Response to reviewers

Reviewer comments are printed in italics, our responses in normal font.

Reviewer 1

> *The manuscript explores the impact of micro-scale heterogeneity on biogeochemical processes and gas transport in peat soils using a 13C pulse-chase assay and X-ray microtomographic imaging. While the topic is relevant, several critical issues warrant rejecting this manuscript in its current form.*

We appreciate the reviewer's critical assessment of our work and will improve the manuscript based on their comments in a revision of the manuscript.

Overall, it appears that the reviewer's expectation regarding rigor of our relatively small study by far exceeded our own. We aim to publish this manuscript primarily to document and share our methodological approach, rather than present definitive outcomes. We will therefore adapt the manuscript to better manage the readers expectations. The reviewer's comments also indicate that additional clarity is needed regarding the novelty of our study - a novelty that appeared evident to reviewer 2.

> *Literature Review:*
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> *The manuscript lacks comprehensive references to existing literature, predominantly citing the authors' previous works. A thorough literature review is essential for contextualizing the study and demonstrating its novelty. The claim that this is the first investigation into spatial heterogeneity in biogeochemical transformation rates appears overstated. Relevant literature includes DOI: 10.1016/j.geoderma.2022.116224, 10.1007/s00374-022-01673-6, 10.1016/j.scitotenv.2023.165192, https://www.ssrn.com/abstract=4092466, 10.1016/j.soilbio.2022.108565, 10.5194/bg-18-1185-2021, 10.1007/s11104-021-04871-7, 10.3389/fenvs.2018.00017, 10.1038/ngeo2963, and 10.1111/gcb.14855.*

The reviewer references a body of literature that is primarily focused on predicting or explaining N_2O production in mineral soils based on the pore architecture. We will incorporate literature suggested by the reviewer, however, we think that these studies are only partially relevant to our manuscript which deals with methane production in peat soil. N_2O production in mineral soil and CH_4 production in peat soil are significantly distinct systems. Most importantly, the relevant pore sizes CH4 production in peat soils tend to be orders of magnitude larger than those relevant for N2O production in mineral soil.

Also, in most of the mentioned studies the linkage between pore architecture and biogeochemical transformation rates remains limited to correlative analysis of pore structure properties vs. whole-sample N_2O production (Kim et al. (2021) plant and soil is a noteworthy exception).

The novelty in our study, in contrast, was our aim to make spatially resolved measurements of actual transformation rates (expressed as the production of 13CH4/13CO2 after injections at distinct depths). The lack of such measurements in the studies mentioned by the reviewer actually supports our claim of novelty.

> *Experimental Design:*
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> *The study's experimental design lacks clarity or is simply wrong for answering all hypotheses. There were seven replicates for two treatments, but it's unclear how samples were labeled and analyzed at different depths. The division of replicates across three depths at multiple time points compromises the factorial design and diminishes the robustness of replication. A mixed effect model can deal with small sample numbers. However, the nested design, with depths and time points nested within field samples, requires careful statistical handling (need to be included in the error term), which the current sample size may not support adequately.*

In the revised manuscript, we will provide a figure depicting our experimental approach. The limited replication is a consequence of our novel measurement approach, where further increasing the number of replicates was not feasible, thus requiring the nested design. Nevertheless, we think that our replication is appropriate for the study. In the revision, we will clarify this by documenting the mixed effects model in greater detail.

> *The introduction fails to provide a strong motivation or hypothesis for analyzing different depths. Key parameters are introduced without sufficient context, making many results appear as unstructured data dumps rather than addressing specific research questions.*

The motivation for injecting at different depths was to elucidate the spatial heterogeneity of biogeochemical transformations within peat cores. We will improve the justification of our research questions.

> *The discussion is weak, lacking depth and failing to integrate findings with existing literature. The conclusion that heterogeneity is significant at the core and within-core scales but not at the stand scale is unsupported by the study design, as the core scale and stand scale are conflated. There are no real replicates on the plot scale.*

With stand scale, we mean pit-to-pit variety within stands, not stand-to-stand variety. We will clarify this in the revision, and we will also improve the integration of our findings with existing literature. Again, this comment indicates that we did not appropriately manage the reader's expectation, in what we intended to publish primarily to document our measurement approach.

> *Essential details about the scanning procedure, such as panel size, exposure time, and number of projections, are missing, hindering reproducibility. Additionally, the rationale for cropping images to 90 mm diameter instead of the full 100 mm is unclear, especially considering the impact of fissures and cracks on diffusion, which is evident in the radial porosity analyses.*

We will provide these details in a revision of the manuscript.

> *The term "pore network modeling" is misleading. The study employs image analysis algorithms to extract features like porosity and connectivity, but does not engage in actual modeling of pore networks.*

We use the term '*pore network model'* to refer to the mathematical abstraction of uCT images, first into a set of pores and connecting throats (as spheres and tubes), and then further into network graphs. A pore network model is thus a simplified depiction of the actual porespace the same way that a map is a simplified model of the actual landscape. This is a common use of the term in the scientific literature.

Using these graphs allows us to use the mathematical tools provided by network theory to analyse pore networks This would not be possible with depictions of the pore space in its actual complexity. We therefore argue that pore network analysis is the appropriate term in this study.

> *Minor Comments*
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> *There are several language mistakes / missing words*
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> *Abstract: The sentence "Greater peat air-filled porosity was and pore network metrics could not explain the fraction of label converted to CO2, but greater porosity as well as higher clustering coefficients and betweenness centrality were associated with slower CO2 emissions" needs correction. Clustering coefficients and betweenness centrality should be introduced for reader comprehension.*
>
> *Introduction: The first two sentences are misplaced. Begin with the broader relevance of the study.*
>
> *Line 18: Contrary to the authors' claim, there is a growing body of literature using X-ray CT and other methods to explore pore heterogeneity in soil functions.*

Line 27: Replace with "anaerobic."

Line 172: Clarify that certain parameters are critical for transport properties but are not transport properties themselves. Provide references.

Figures: Ensure all y-axis titles in Figures (e.g., Fig. 2, Figs. 4-6) have correct subscripts. The physics of porous media should be correctly attributed (Line 36). Consider moving Fig. 2 to supplementary information.

Statistical Analysis: Include a reference for the mixed effect model and details on testing assumptions. The p-value in Fig. 8(e) should be clarified.

We address these comments in a revision of the manuscript.

<u>Reviewer 2</u>

This study performs a lab experiment on peat cores to quantify the effect of peat pore structure on the production and transport of CH4 and CO2. The study is unique because few studies on peat have addressed these processes at these scales. The experimental setup and data analysis are sound and well explained. The study could not provide a definitive linkage between pore structure, gas transport and gas production, but this does not detract from the study's overall value. I have one concern about the study and list several minor corrections that I recommend the authors should address:

We thank Reviewer 2 for their positive feedback on our work.

It appears that the shrinkage of the peat has affected your analysis of air-filled porosity near them edge of your samples. Although you explain this in the text, the plot in Figure 3f can be misleading. Could you either indicate on Figure 3f where the edge effects begin or subset them data that does not include the edges and replot Figure 3f.

Shrinkage was indeed a minor issue during our study. We therefore excluded the horizontal edges from further analysis. We will update Figure 3f to indicate this more clearly.

Minor corrections
Line 5: remove “the”
Line 12: consider rewriting “air-filled porosity was” as it does not read smoothly
Line 31: add Wright et. al 2018 as a reference for peat pore structure affecting methane emissions in the field
Methane ebullition from subtropical peat: Testing an ebullition model reveals the importance of
pore structure, W Wright, JA Ramirez, X Comas - Geophysical Research Letters, 2018

Line 39: instead write “Despite the progress in”

Figure 2: line 2 should be CH4. Throughout all plots, axis labels should be formatted as CO2 and CH4. Remove brackets from axis labels.

Line 108: complete subscript for Vmol and tcycle, and add symbol for 20 °C

Line 112: complete subscript for cmin and cmax

Line 117: remove bold

Line 119: complete subscript for Rref and CH4

Line 175: typographical error: effecst

Figure 3: add to caption that the air-filled pore space is displayed in black and peat in white, and also add a scale bar

Figure S1: consider adding a legend to easily understand the colors. Marker sizes are difficult to distinguish so make the figure larger on a 2 x 2 layout. Line 3 in caption typographical error: “[he”

Line 207: is this the correct units: µmol hh-1 ?

Figure 4: add subscripts and superscripts to y-axis labels. Not sure what you mean by “Letters in panel(b) indicate significant differences between the injection rounds” because no letters appear in the panels. For the methane flux plots, consider adjusting the min and max values of the y-axis for complete visualization of the data (i.e negative fluxes).

Line 213: make sure subscripts and superscripts are applied throughout the remainder of the manuscript (e.g. CH4). Is this correct µmol hh-1, if not, please also correct throughout the manuscript.

Line 224: please report the temperature values throughout the duration of the experiment.

Line 226: typographical error: trying

Figure 5: add subscripts to y-axis labels

Figure 6: add subscripts to y-axis labels. Explain in the caption the meaning of the letters (e.g. ab) within the plots.

Line 243: replace til with until

Line 256: remove bold

Line 259: typographical error: (13CO2

Line 262: first sentence reads awkwardly and needs rewriting

Figure 7: subscripts are needed throughout labels and there is no reference to plot d in caption

Line 281: not clear what “time 5” means and replace slowly with slower
Line 291: figure 9 does not exist
Line 292: Fig. Snn?
Line 295: Table Snn?
Line 300: remove “a”
Line 303: “small number of essential...” should be “small number of pores are essential...”?
Figure 8: time on the y-axis label needs units. In the caption provide explanation for asterisks
and n.s.

We address these comments in a revision of the manuscript.