We thank the editor and both referees for their careful evaluation of our manuscript and for their constructive feedback, which has helped us to further improve its clarity and robustness. We provide our point-by-point responses below (in blue). All changes to the manuscript are highlighted in yellow in the file *"Manuscript-Version3_SpatialHeterogeneity_MarkedChanges"*. We hope that the revised version meets the requirements for publication.

Associate editor decision: Publish subject to technical corrections

By Erika Buscardo

Public justification (visible to the public if the article is accepted and published):
Dear Victoria Martin,

We have received the comments by the two original reviewers. They both agree that the revised manuscript had improved after revision and one of them reckons that some concerns should be addressed before the manuscript is ready for publication.
I have also read your revised manuscript and have some suggestions to improve overall the manuscript. You introduction is rich in references and very didactical and the reader may discern implicit hypotheses. The paper could be significantly improved if you proposed hypotheses to explicitly test the potential effects that polygon morphology and soil layers may have on soil properties, microbial community composition / structure / function.

Dear Erika Buscardo,

Thank you very much for your thoughtful feedback and the suggestions provided. However, we do not agree that formulating hypotheses would significantly improve the manuscript. Presenting post hoc hypotheses in a paper as if they were a priori hypotheses, when they were actually informed by the results, may be widespread practice but is problematic (also from a philosophy-of-science perspective). We therefore refrain from formulating post hoc hypotheses, even if they would improve the readability. We believe, however, that it is entirely justified to frame our research in the form of research questions, particularly because our work is exploratory rather than confirmatory in nature, and the systems we were investigating are still relatively poorly understood, making it difficult to formulate specific hypotheses a priori.

There are minor points that should be addressed.
L120 incorrect use of term 'brine'?

Thank you for the remark. Although is occasionally used in permafrost research (e.g., Gilichinsky et al., 2003; Waldrop et al., 2025), we have revised the wording in the respective sentence for clarity (now L122).

L241 fungal taxa were identified by sequencing the ITS1 region

Thank you for noting this. We have corrected the statement (now L244).

L616-18 revise sentence

We have revised the sentence accordingly (now L618–621).

L630-31 incomplete sentence

Thank you for the remark. Please note that we have corrected the sentence, plus slightly modified the surrounding paragraph (now L648-651).

L641 I reckon that ‘finding’ / ‘result’ would better fit instead of ‘pattern’

We have adjusted the wording accordingly (now L659).

L 719 What about the temporal dimension and the potential implication of climate change on carbon and nutrient cycling? It may be worth considering it as a limitation of the study or propose it for future research?

Thank you for the remark. We have now included a statement acknowledging the lack of temporal resolution as a limitation of the study (now L755–757).

Check the use of acronyms throughout the manuscript including in figure legends and table captions. Table captions and figure legends should be stand-alone so that they can be understood without recourse to the main text (what? where? why?). Citations should be checked throughout the manuscript (e.g. L75; L233; L304).

Thank you for highlighting this. We carefully checked all references and corrected those mentioned (L75; L233 → L235; L304 → L313). We also revised acronyms throughout the manuscript and implemented minor corrections in the figure legends (Fig. 2 and Fig. 3). In addition, all table captions and figure legends were revised to ensure they are fully stand-alone and self-explanatory.

Referee #1

Martin et al. have carefully considered most of the comments and have either addressed them or provided detailed explanations. The manuscript is largely improved compared to the previous version, and the FungalTraits inference is a welcome addition. I have very few textual comments left and consider the manuscript ready for publication after addressing those.

Although the authors argue that rarefaction is an appropriate method for normalizing heterogeneous read numbers among taxa, we believe this approach carries the risk of removing rare taxa that may be ecologically significant. Rarefaction, while widely used, is far from uncontroversial (McMurdie & Holmes 2014 comes to mind..), and the authors did not clarify whether the rarefaction they used were a single random draw or averaged across multiple random rarefactions at the chosen depths. The concern here is particularly because the decrease in alpha diversity over depth could be linked to the decrease in sequencing depth over depth shown in the tables presented in the authors' response.

The authors ruled out that DADA2 ASV clustering is inappropriate for fungal ITS by saying it would likely not affect the results. One would happily trust their word on this, but the phrasing suggests they have not looked into it. Demanding to redo the analyses would be excessive, but acknowledging the limitations of their approach is reasonable.

Fungi exhibit high variability in copy number, ranging from a few to several hundred (https://doi.org/10.1111/mec.14995), and bacterial 16S rRNA gene copy numbers are also well known to vary substantially across taxa. Although relative abundance was normalized using ddPCR measurements, the reported read counts may reflect copy numbers originating either from the same taxon or from different taxa. This issue was addressed in Vandeputte et al. (2017), to which the authors refer, but it is not discussed explicitly here. The database referenced by Vandeputte et al. (2017) contains information on bacteria and archaea. It remains unclear which database the authors used to account for fungal ribosomal copy number variation. Therefore, abundance estimates based on copy number, in any form, present inherent challenges. Differences in the quality and completeness of reference data for ribosomal copy numbers, particularly between well-characterized systems such as the human gut microbiome and comparatively understudied environments such as Arctic permafrost, further complicate interpretation. In this context, the authors refer to their estimates as "absolute abundance," although the underlying assumptions do not fully support that designation. Describing the measurements as semi-quantitative would have been a more precise and methodologically consistent way to frame the results.

We thank the reviewer for evaluating the revised manuscript.

Comment to rarefaction and α-diversity:
We acknowledge that rarefaction is subject to ongoing debate and may lead to the exclusion of rare taxa that could be ecologically relevant (e.g., McMurdie & Holmes, 2014). In our study, we applied rarefaction to standardize sequencing depth across samples. We would like to clarify that rarefaction was performed as a single random subsampling step without replacement, as was indicated in both the main text and the Supplementary Information ("using the rarefy_even_depth() function implemented in phyloseq with replacement = FALSE"). However,

we do agree that averaging across multiple random rarefactions would provide a more robust estimate.

We have now ensured that this aspect, and the general limitations of rarefaction are explicitly addressed in the manuscript (L275–281). Regarding the reviewer's concern about the potential influence of rarefaction on the observed decrease in α-diversity with depth, we note that, although methodological effects cannot be fully excluded, the observed pattern of decreasing microbial biomass and diversity with increasing soil depth is well established in tundra ecosystems (see also L700–704).

Comment to DADA2 and fungal ITS:
We agree that ASV-based analyses of ITS data require careful interpretation. We have now explicitly acknowledged this limitation in the Methods section, noting that intra-genomic variability may influence taxonomic resolution (now L267-268).

Comment to ddPCR derived abundance estimates:
We have revised the terminology to ddPCR-informed abundance estimates" or "ddPCR-informed abundance estimates" throughout the manuscript. In addition, we have expanded the Methods section to explicitly acknowledge that taxon-specific ribosomal gene copy number variation was not corrected for, and that the resulting estimates should be interpreted as proxies of microbial abundance rather than direct measures of cell numbers (L292-296).