

We thank the reviewer for the comments and provide our point-by-point responses below (in blue).

The authors compare biogeochemical and microbial community properties among ice-wedge polygons in Arctic tundra and identify disparate compositions in saturated, low-centered polygons and organic horizons across all topographic features. Their contemporary analysis of SOM quality (from pyrolysis GC-MS), microbial community composition, and hydrolytic soil enzyme activities provides an integrated description of SOM turnover potential that both expand on previous reports from individual polygonal features and provide a self-consistent dataset for future modeling. I offer some suggestions for structural improvements that may help focus this manuscript.

Major comments

The Introduction section conscientiously reviews almost 75 years of permafrost soil science, but it could be better focused on a hypothesis or specific, mechanistic research question. The final paragraph of this section (lines 122-131) includes a list of questions about biogeochemical correlations across polygons and soil layers but does not describe a strategy to demonstrate causal relationships that would support the desired mechanistic insights. Is there an intrinsic model of the biogeochemical processes motivating this research that could be explicitly described and tested here to enhance the impact of this impressive work?

We thank the reviewer for this constructive comment and agree that the Introduction can be strengthened by more explicitly articulating the conceptual framework that motivates the study. While our approach is observational, it is guided by a mechanistic framework in which polygon morphology and soil layers represent the two dominant axes of environmental organization in ice-wedge polygon tundra that impose recurring constraints on edaphic properties and biogeochemical processes.

Polygon microtopography structures lateral gradients in surface hydrology, organic matter quality (via differential vegetation cover) and soil types, while vertical gradients along the soil profile impose additional physicochemical controls through changes in temperature, texture, pH, oxygen availability, and plant-derived organic matter inputs. Together, these gradients shape soil organic matter properties and microbial community characteristics across scales, including microbial abundance, diversity, and community structure, and constrain pathways of organic matter transformation and decomposition.

To address the reviewer's suggestion, we will revise the final part of the Introduction to make this conceptual framework explicit. First, we will clarify that jointly analyzing terrain-scale (polygon morphology) and pedon-scale (soil layers) variability allows us to test whether these two spatial dimensions exert predominantly independent or interacting controls.

Second, we will clarify that the mechanistic insight sought in this study concerns how cross-scale spatial organization generates distinct biogeochemical environments via differential hydrological regime, redox conditions, and organic matter inputs. These environments, in turn, shape microbial communities and constrain soil organic matter processing. Identifying these environmental linkages allows us to infer how spatial heterogeneity directs biogeochemical functioning in Arctic

lowland tundra and may inform scalable representations of its heterogeneity in ecosystem and land-surface models.

We believe that making this framework explicit will better motivate the study, clarify its mechanistic underpinnings, and strengthen the link between our analyses and their relevance for ecosystem-scale representation and modeling.

Section 3.1 & Figure 2. What factors from the pyrolysis GC-MS analyses contributed to the variation among SOM contents in LCP and organic soil samples? Were specific features heavily weighted in the principal components that can be identified to gain insight into the composition? Are these the same factors identified in LME analysis (Figures S3 & S4)?

We thank the reviewer for this question and the opportunity to clarify our analytical approach. First, we would like to emphasize that Figure 2 illustrates differences in chemical structure of SOM, rather than differences in SOM content. The fingerprints are based on the clr-normalized abundance of 534 pyrolysis-GC/MS products (“peaks”) and thus reflect compositional information rather than SOM quantity.

We agree that inspection of PCA loadings can provide insight into which pyrolysis products contributed most strongly to the observed ordination patterns. To address the reviewer’s request, we therefore examined the PCA loadings in detail. Separation among polygon types (Figure 2a) occurred primarily along PC2, with high-loading peaks including, for example, phenolic and N-containing compounds (e.g., phenol, 1H-imidazole, 1H-pyrrole-3-carbonitrile) as well as long-chain aliphatic compounds (e.g., 1-hexadecanol, 2-nonadecanone), and compounds of unknown origin (referred to as “Peaks 184, 1600, 1678, and 1720”). Differentiation among soil layers (Figure 2b) was mainly reflected on PC1 and similarly reflected contrasts between aliphatic hydrocarbons (e.g., 1-Nonene, -Nonadecanone, Octadecane, Nonadecane) and more compounds of unknown origin (referred to as “Peaks 934, 959, 1563, and 1720”).

However, we note that the interpretation of individual pyrolysis products is often ambiguous, as detected compounds may originate from different or the same precursor molecules. For this reason, we consider individual peak loadings as difficult to interpret and of limited value to most readers. Instead, we deliberately focused our main analysis on compound-group summaries, which provide a more robust and interpretable representation of SOM composition. Accordingly, all detected pyrolysis products were grouped into SOM compound classes (Supplementary Table S3), and their relative and absolute abundances were analyzed using linear mixed-effects models (Supplementary Figures S3 and S4). Importantly, we want to note that both PCA and compound group abundance patterns describe the same underlying differences in SOM chemistry, but at different levels of resolution.

Figure 4. Were the final community members related to known saprotrophs, endophytes, or ectomycorrhizal fungi? This distribution affects the interpretation of their potential physiological roles. The FungalTraits database may be useful here.

We agree that assigning fungal taxa to functional guilds (e.g., saprotrophic, ectomycorrhizal, endophytic) can provide valuable ecological context for interpreting the observed community patterns. Although functional inference from Arctic ITS1 datasets can be challenging (high taxonomic uncertainty at lower ranks and limited trait coverage is common) we explored the trait-based annotation using the FungalTraits database and will incorporate these data into our revised manuscript.

To balance ecological interpretability and assignment confidence we suggest assignments at the genus level appropriate, as higher taxonomic ranks often encompass multiple or ambiguous ecological strategies. Using this approach, approximately 18% of fungal ASVs could be reliably assigned to functional guilds (whereas 82% remained unclassified). Among the assigned ASVs, the most prevalent guilds were ectomycorrhizal fungi and saprotrophs (including litter-, wood-, and soil-associated taxa), followed by root endophytes. Additional lifestyles (e.g., parasitic, lichenized, or specialized saprotrophic fungi) were present but contributed less than 1% of total ASVs.

This trait-based annotation revealed differences in the distribution of guilds (e.g., ectomycorrhizal fungi) across polygon types and soil layers, which we will incorporate into the revised manuscript to strengthen our ecological interpretations. Nevertheless, we also need to acknowledge that the large majority of taxa could not be reliably assigned, and that overall, rather large uncertainty that is associated with functional inference in Arctic fungal communities. Summary Tables of the FungalTraits database-assignments will be provided via Zenodo Repository.

Section 4.1 The Discussion should be based on interpreting the present Results. Consider omitting text that speculates on tundra processes without connecting to Results. Alternatively, sections like lines 555-560 that discuss potential Archaea-mediated biochemical processes could be bolstered by reference to specific community composition results. See also lines 613-630

We agree that the respective sections could build more on explicit community composition results. We plan to revise the sections and to implement the following changes:

L551-554: We will incorporate insights from the FungalTraits analysis, demonstrating that LCP soils (2.2 %) indeed hosted a lower proportion of ectomycorrhizal fungi compared to FCP (7.8 %) and HCP (6.8 %) soils.

Lines 555–560: We will highlight archaeal abundance patterns across soil types, and their eminent role in LCP communities. LCP soils accounted for 67% of all ddPCR-corrected archaeal reads in the dataset, compared to 29% in FCP and 4% in HCP soils. Moreover, also the relative abundance of archaea (expressed as a fraction of the total prokaryotic community) was substantially higher in LCP soils (7%) than in FCP (1%) and HCP soils (0.2%). The paragraph will then better connect to the discussion of archaeal-mediated processes in peaty environments.

L555-560: note numerically that archaeal abundance was elevated in LCPs soils compared to FCP and HCP soils. Specifically, LCP soils harbored 67 % of all dd-PCR corrected reads in the dataset that were assigned to archaea (FCP soils 29 %, HCP soils 4 %). Also, the relative abundance of

archaea (expressed as fraction of their total prokaryotic community, respectively) was notably high in LCP (7 %) soils (FCP 1 % and HCP soils 0.2%).

Lines 613–630: revise the respective paragraph to more tightly connect the discussion of permafrost microbial communities to the results presented in this study. We reduce the general discussion of microbial adaptations to the physical and ecological constraints imposed by the frozen conditions but will connect abundance patterns of specific phyla with those environmental conditions, instead (Supplementary Table 8). For example, we will highlight the strong association of Cloacimonadota, Caldisericota, and Campylobacterota with the permafrost layer, phyla linked to anaerobic organic carbon turnover, including fermentation and sulfur and nitrogen redox processes. In addition, we will refer to the elevated abundance of Firmicutes, which may benefit from dormancy and spore-forming strategies in the freeze–thaw transition zone.

The Conclusions section is unusually long, and it includes significant Discussion text. This section should not introduce any new material; therefore, authors should carefully consider omitting any sentences that include citations. As Josh Schimel recommends in his excellent book "Writing Science" (Oxford) this section should concisely reiterate the from Methods & Results, answer key questions posed in the Introduction, and demonstrate how those answers have advanced understanding of the topic. I suggest shortening and rewriting this section to address those three items.

We thank the reviewer for pointing this out. We will shorten and rewrite the Conclusion section and remove the discussion-style material.

First, we will remove interpretations about potential future trajectories or Arctic lowland tundra ecosystems under climate change, including the topics of polygon transitions, thermokarst, active layer deepening, vegetation change, and rhizosphere priming, and relocate this content to the Discussion section.

Second, we will limit the revised Conclusions to:

(i) briefly restate the importance and strength of the used study approach which considers both major axes of spatial heterogeneity in lowland tundra soils (i.e., polygon types and soil layer) simultaneously.

(ii) directly answering the questions posed in the Introduction, by summarizing that polygon-specific signals persisted across soil layers, and layer-specific patterns were consistent across polygon types, whilst interactive effects seemed comparatively minor. However, we also conclude from the patterns that we observed that most edaphic variability across both spatial scales emerged from the gradients in redox conditions and vegetation-associated organic matter inputs.

(iii) outlining that a very limited number of spatial “units”, (i.e., polygon types and soil layers), is sufficient to effectively capture a disproportionate share of edaphic, microbial, and biogeochemical variability in Arctic lowland tundra soils. Therefore, we advocate that differentiating polygon types and major soil layers, with LCPs, and organic topsoils as potential priority units, can provide a

simplified but practical framework for parameterizing and scaling soil processes across this geomorphologically complex ecosystem.

Minor comments:

Introduction: It is not necessary to cite the same paper multiple times in a paragraph, particularly in successive sentences.

We acknowledge the comment, and will remove double citations within the same paragraph, accordingly.

Line 62. While HCP and FCP soil may be 'dry' compared to 'LCP' soil, both soils have high SWC compared to typical agricultural or temperate ecosystem soils. This affects diffusion, substrate availability, and microbial growth and should be noted.

We agree that Arctic lowland tundra soils (across all polygon types) generally may all have relatively high soil water content compared to temperate or agricultural soils. Our focus, however, is on relative hydrological differences among polygon types (emerging from their differential microtopography), which strongly regulate oxygen availability, soil organic matter characteristics, microbial community composition, and biogeochemical processes. Certainly, water availability is not a limiting factor for substrate diffusion and substrate accessibility in these soils, but that it's rather the permafrost table that imposes the strongest physical constraint for (L94-95).

When discussing the establishment of predominantly aerobic versus anaerobic microbial communities in drained versus inundated terrain (L78), we also highlight the comparatively lower energy yield of anaerobic respiration compared to aerobic respiration (L545–548), which of course also affects microbial growth rates. We will hence also incorporate the link between microbial growth and redox conditions, accordingly.

Section 2.3. What was learned from the isotopic signature analysis performed here?

Stable carbon and nitrogen isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) can provide useful contextual information on soil organic matter (SOM) processing stage and the nitrogen cycle. In our dataset, however, isotopic differences between soil layers and polygon types were relatively subtle and did not reveal strong or consistent trends, which is why we didn't discuss them in the main text of the manuscript. To address the reviewer's comment, we here briefly summarize what can and cannot be inferred from the isotopic data in this study.

Soil $\delta^{13}\text{C}$ values showed only minor variation across polygon types and soil layers (from approximately 27 ‰ to –26 ‰). Although vegetation composition differs markedly among LCPs and FCPs/HCPs, but bulk SOM $\delta^{13}\text{C}$ values were largely similar likely due to the fact that all plants in the Arctic are C3-plants and differences in water use efficiency are not pronounced. Soil $\delta^{13}\text{C}$ values were also not statistically different across soil layers, suggesting that differences in decomposition state were not strongly expressed in bulk SOM $\delta^{13}\text{C}$, likely due to extensive cryoturbation activity in this area. This interpretation is also consistent with the largely uniform C:N ratios across layers and confirms that Pyr-GC/MS analyses may reveal finer-scale differences in SOM composition.

Soil $\delta^{15}\text{N}$ values may provide contextual information on nitrogen cycling, with higher $\delta^{15}\text{N}$ values representing a more open N cycling (N losses) versus lower $\delta^{15}\text{N}$ values indicating tighter N cycling (stronger N retention). However, soil $\delta^{15}\text{N}$ is a comparatively poor indicator of vegetation-derived imprints and organic matter origin, as site-specific soil properties and multiple interacting edaphic processes produce signals that are difficult to disentangle.

In our study, $\delta^{15}\text{N}$ values across polygon types and soil layers were consistently close to 0‰, indicating overall low N losses and a predominantly closed N cycle. This is consistent with the notion of N limitation typical of Arctic tundra ecosystems. In polygon tundra ecosystems, leaching losses are largely restricted by the permafrost table, prolonged frozen conditions throughout the year, and poor drainage due to the flat terrain. Also, gaseous N losses via denitrification are comparatively low, due to limited nitrate availability and the predominance of organic nitrogen in tundra ecosystems. Across polygon types, LCP soils exhibited $\delta^{15}\text{N}$ values close to 0‰ (~0.34 ‰), whereas FCP and HCP soils were slightly more enriched (~1.3–1.6 ‰). Organic-rich tundra soils, particularly peaty and waterlogged systems such as LCPs, commonly display $\delta^{15}\text{N}$ values near 0‰ while the less organic-rich soils of FCPs and HCPs showed slightly higher $\delta^{15}\text{N}$ values. Across soil layers, organic and mineral topsoils showed slightly higher $\delta^{15}\text{N}$ values than permafrost, while cryoturbated material exhibited intermediate values. Given the narrow overall range of $\delta^{15}\text{N}$ values observed in this study, we believe that these differences should be interpreted cautiously and do not support inferences regarding specific nitrogen sources or transformation pathways. Instead, the $\delta^{15}\text{N}$ data primarily constrain the system-level interpretation by indicating generally tight nitrogen cycling with only minor N losses. We therefore consider it important to report $\delta^{15}\text{N}$ values in the Supplementary table 1, while deliberately refraining from overinterpreting them.

To better integrate the isotopic results into the revised manuscript, we will (i) explicitly report soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ patterns in the Results section, and (ii) incorporate a short interpretation into the Discussion. Specifically, we will highlight that while bulk soil C:N ratios and $\delta^{13}\text{C}$ values varied little across soil layers, Py-GC/MS analyses revealed more fine-scale differences in SOM quality.

Lines 87-88. What were the bulk densities of the sampled soils? I could not readily find this information by searching the main text or supplemental materials. Did the SIPRE corer cause compression of the soil layers in the thawed LCPs?

Bulk density values have now been added to Supplementary Table 1 and are briefly described in the Results (Section 3.1). These values are also included in the updated CSV dataset deposited on Zenodo. However, please note, that bulk density measurements are missing for five cryoturbated samples, due to the irregular geometry and discontinuous nature of cryoturbated pockets.

Regarding potential compression during coring: yes, the SIPRE corer caused some compression of the active layer in LCPs. To account for the compression during coring, we measured active layer thickness independently using a soil probe, which allowed us to adjust our interpretation of the extracted cores. When field conditions permitted, we also extracted active layer blocks using a spade (Supplementary Fig. 1e) and compared them to our interpretation of cores.

Importantly, our analyses are based on (sample-wise disaggregated) soil horizons rather than absolute depth, so potential active layer compression does not affect our sample classification or conclusions.

Section 2.5 Was the depth of sequence coverage from multiplexed SSU rRNA and ITS2 gene amplicon sequencing sufficient to characterize the community diversity in all samples?

Thank you for the question. As mentioned in the Supplementary Material in L145, we removed samples that were characterized by less than 500 (raw) reads. This specifically meant the removal of 2 samples from the bacterial and archaeal dataset (16S rRNA gene), because of insufficient sequence coverage (2 x FCP_cryoturbated). As well as the removal of 3 samples from the fungal dataset (ITS1 gene region), because of insufficient sequence coverage (1 x FCP_cryoturbated, 1 x FCP_permafrost, and 1 x LCP_permafrost).

We will transfer the section that explains how the amplicon datasets were treated prior to downstream analyses from the Supplement to the Materials and Methods section of the revised manuscript.

Line 510-511. Some discussion of the role of pyrolysis GC-MS results in inferring organic matter quality is warranted here.

We acknowledge the suggestion and will extend these lines accordingly. Specifically, we will state that Pyrolysis-GC/MS is a spectrochemical method that has been successfully applied in permafrost studies (e.g., Folhas et al., 2025; Verret et al., 2025), providing compound-level details that bulk soil indices may not capture. Indeed, in our study, polygon types showed similar soil C:N ratios and bulk soil $\delta^{13}\text{C}$ signatures (Supplementary Table 1), while pyrolysis-GC/MS fingerprinting was able to reveal pronounced, but finer-scaled differences in SOM quality among polygon types and soil layers (Fig. 2(a,b)). This highlights the added value of the Pyrolysis-GC/MS method for resolving compositional variability that is not apparent from bulk metrics alone.

References: Citations to non-journal articles like the "Field Book for Describing and Sampling Soils" need to be updated with sufficient information for the reader to find the resource.

Thank you for noticing the mistake. The citation has been updated.

References:

Folhas, D., Couture, R.-M., Laurion, I., Vieira, G., and Canário, J.: Natural organic matter dynamics in permafrost peatlands: Critical overview of recent findings and characterization tools, *TrAC Trends in Analytical Chemistry*, 184, 118153, <https://doi.org/10.1016/j.trac.2025.118153>, 2025.

Verret, M., Naeher, S., Lacelle, D., Ginnane, C., Dickinson, W., Norton, K., Turnbull, J., and Levy, R.: Preservation and degradation of ancient organic matter in mid-Miocene Antarctic permafrost, *Biogeosciences*, 22, 5771–5786, <https://doi.org/10.5194/bg-22-5771-2025>, 2025.