

Dear Editor and Reviewer,

We thank the reviewer for their time and careful evaluation of our manuscript. We agree that there are several conclusions that may be overextended based on our experimental design, interpretations, and statistical analyses. We have included our responses to these comments in red below. Line numbers refer to the new line numbers following edits.

Sincerely,

Patrick Neuberger, on behalf of all coauthors

1. The authors attribute bacterial community shifts in disturbed plots directly to permafrost thaw. However, using a site prepared by an excavator adds confounding mechanical horizon inversion. Since the upper active layers were removed and possibly moved next to the thermokarst pond, the overlap in indicator taxa could reflect physical soil mixing rather than biological recruitment. We acknowledge that the site's preparation may involve both thaw exposure and horizontal disruption, and so does not perfectly replicate natural erosion or active thermokarst slumping. However, our intention was to simulate localized thermokarst disturbance conditions, where thaw and physical soil displacement commonly occurs simultaneously. As well, our observation that indicator OTUs increased gradually across the disturbance gradient is inconsistent with bulk mixing being the primary contributor to community changes. To address these concerns, we have made the following amendments:
We have included a section in the discussion which explicitly discusses soil mixing as a potential contributing mechanism in Discussion section 5.1, **lines 484-489** "Both in natural retrogressive thaw slumps and anthropogenic excavation, physical soil displacement and thaw exposure are co-occurring processes. Our study was chosen to simulate these localized thermokarst disturbance conditions, where thaw and soil displacement occur simultaneously. The distinction between physical mixing and biological dispersal may be less meaningful under field conditions, since both processes occur simultaneously." and Discussion section 5.3 **lines 551-558** "The mechanical disruption of soil horizons during site preparation may have introduced active layer microorganisms into formerly permafrost horizons, meaning that the overlap between disturbed soil and active layer communities may reflect physical translocation rather than biological recruitment. However, if bulk redistribution of active layer microorganisms were caused by physical mixing, we would expect to see an equivalent level of active layer indicator OTUs across all disturbed samples. Instead, our study observed the progressive accumulation of active layer indicator OTUs along the disturbance gradient."
2. The ecological succession narrative may be confounded by the presence of relic DNA. Table 1 shows many samples have over half non-viable cells, but community profiling (Figures 4–6) is based on bulk DNA. Since permafrost preserves DNA from dead cells, observed shifts after disturbance could be relic DNA artifacts rather than true biological changes. Without RNA data or viability dyes, it is difficult to verify that permafrost communities are supplanted (L. 440). We agree that DNA-based amplicon sequencing cannot distinguish between viable, dormant, and non-viable cells. To address this, we also conducted a paired analysis comparing community profiles of PMA treated and non-PMA treated active layer, permafrost, and disturbance gradient samples. We found that while there are marginal changes in alpha-diversity, general trends in community composition and alpha-diversity remain true without PMA treatment. This work has been included in the supplemental section of this manuscript, which supports our interpretation that observed microbial compositional shifts reflect viable microbial populations rather than

changes in relic DNA. We have also included a reference to the supplemental section on **lines 219-220**.

3. The conclusion that microbial restructuring is independent of soil chemistry may be an artifact of limited sampling frequency and variable selection. Because the study did not measure rapid-response drivers like redox potential or DOC quality, and relied on a single time point, it might have missed transient abiotic pulses following physical disturbance. Consequently, community shifts shown in Figure 4 may be driven by unmeasured environmental gradients rather than a purely biological or stochastic process. I suggest toning down the last sentence in the abstract, as well as in L. 579, stating microbial changes are induced by biotic filtering. Something more towards “playing a dominant role” under the current experimental analyses of the measured edaphic variables."

Variables such as redox potential and dissolved organic carbon may have influenced microbial restructuring but were not measured in this study. We have revised **lines 22-24** of the manuscript to read: "Disturbances may induce a strong microbial community change in permafrost-affected soils before micronutrient, organic carbon, and pH shifts as measured in this study.". In addition, we have revised **lines 593-595** to now read: "Our results suggest that biotic filtering plays a dominant role in microbial community structuring under the current experimental parameters measured in this study."

4. The RDA model in Figure 2 reports an exceptionally high coefficient of determination ($R^2 = 0.99$) using 17 predictors against a relatively small sample size, which raises concerns regarding overfitting and model inflation. The authors can apply variance partitioning to confirm that the observed lack of separation isn't simply a result of the high predictor-to-sample ratio. Furthermore, variance partitioning would help disentangle the unique contributions of soil chemistry from spatial coordinates (PCNM/MEM) and depth covariates, providing a more robust test of whether community shifts are truly independent of the edaphic environment.

We have re-evaluated the model using the adjusted R^2 and have performed variance partitioning using `varpart()` in R to confirm that community shifts operated independently of edaphic environments. These analyses identified collinearity of multiple parameters, and we have opted to include only 6 parameters in the model SOM (%), pH, Cu (mg/kg), TC (mg/kg), NO₃ (mg/kg), and NH₄ (mg/kg).

We have updated **lines 274-278** the methods to include the following: “A forward selection of the model using `ordiR2step` in `vegan` was used to determine the most appropriate variables for the RDA. In addition, we used variance partitioning to remove the possibility of collinearity of parameters included in the model. The parameters included in the model are: SOM (%), pH, Cu (mg/kg), TC (mg/kg), NO₃⁻ (mg/kg), and NH₄⁺ (mg/kg).” As well, the final R^2 has now been corrected to 0.5268742 in the results section on **line 331**, and Figure 2 has been updated to match this new model.

5. The statistical inference regarding disturbed surface diversity in Figure 3 is potentially confounded by the grouping of undisturbed and disturbed samples (A-F) into a single category. Best practices in disturbance ecology require the separation of control (undisturbed, A) and treatment sites (disturbed, B-F) to avoid masking or inflating effects.

Samples A-F do not represent independent control and treatment sites but are instead single points along a spatial gradient. Site A does represent the least disturbed reference sample. However, A-F were sampled as spatially linked positions, within the single thaw feature, rather than as independent control and treatment replicates. Therefore, separating site A and statistically comparing it against sites B-F would result in an unreplicated comparison and would not provide a robust test of disturbance effects. Our objective was therefore not to compare control versus treatment sites, but rather to evaluate patterns of community diversity across the thaw gradient,

relative to reference active layer and permafrost communities.

We have revised the manuscript to clarify this aspect of the study design on lines 133-135: "Site A represents the least disturbed reference sample along a spatially-linked position, leading to a single thaw feature, rather than as independent controls and experimental treatments".