Dear Editor and Reviewer,

We extend our gratitude for your dedicated time invested in reviewing our manuscript and for sharing your invaluable insights. Your positive feedback is deeply appreciated. Your constructive comments have significantly contributed to the enhancement of our work.

According to your comments, we have thoroughly revised the manuscript. We incorporated the necessary changes and refinements throughout the revised manuscript. To facilitate your review, we have provided detailed responses to each comment in blue color below. Additionally, you can refer to the tracked changes in the manuscript for a comprehensive overview of the revisions made.

Once again, we sincerely acknowledge the value of your time and your expert evaluation of our work. We hope that these revisions have fortified the manuscript's lucidity, precision, and overall quality.

Best Regards,

Ze Ren on behalf of all co-authors.

RC2: 'Comment on bg-2023-85', Anonymous Referee #2, 28 Aug 2023 reply

In the study by Ren et al, the authors investigate microbial community assemblages in different degraded environments, degraded permafrost soils, thermokarst lake sediments and lake water, with the aim to identify dispersion and assembly processes. Although the communities differed among the environments, they nevertheless shared 41% of OTUs which suggests that and taxa disperse among the systems.

The manuscript is very clearly structured and well written, and the introduction provides a good overview of the topic. Moreover, due to the increasing possibility of enhanced thermokarst lake formation the authors elucidate and highlight microbial colonization pathways of those newly formed ecosystems.

We greatly appreciate your positive comments and constructive suggestions regarding our study. Your comments and suggestions have been meticulously reviewed and integrated into the manuscript to improve its quality. Please refer to the responses below and revisions in the revised manuscript for details.

As pointed out by reviewer 1, more information on DNA extraction and sequencing should be included, as well as on the assumptions made in the base-SEM. Moreover, the rationale for assembly processes could be explained a more detailed, especially the definitions of homogenous and heterogenous selection and how their contribution, and dispersal

limitation were estimated, as well as how deterministic and stochastic processes (and what deterministic processes would that be) were defined? Could this maybe elaborated more in the introduction already

For DNA extraction and sequencing, more details are provided in the revised manuscript, such as: “The Magen Hipure Soil DNA Kit (Magen, China) was used to extract DNA from soil (0.5 g frozen soil), sediment (0.5 g frozen sediment), and water (membrane filter) samples according to the manufacturer's protocols. Extraction blanks were routinely performed in parallel.”, “Next generation sequencing of the amplicon products was conducted on an Illumina Miseq Platform (Illumina, San Diego, CA, USA). Automated cluster generation and 250/300 paired-end sequencing with dual reads were performed following the manufacturer’s instructions.”. We also added more details about the PCR and sequence processing. Please refer to the revised manuscript for detail.

For the assumptions in SEM, we added: “In model building, the SEM incorporated prior knowledges: (a) location and climate factors potentially influence all the studied bacterial communities, (b) physicochemical factor of each habitat potentially influences the corresponding bacterial communities, and (c) permafrost soil potentially influences thermokarst lake sediment and water, while lake sediment and water interact with each other.”

For assembly processes, we added more details in the METHODS section: “Because homogeneous selection results in communities that share greater phylogenetic similarity, the proportion of homogeneous selection was calculated as the fraction of pairwise comparisons with $\beta NTI < -2$. On the other hand, heterogeneous selection, leading to communities with lesser phylogenetic similarity, was measured as the fraction of pairwise comparisons with $\beta NTI > +2$. Because homogeneous dispersal results in communities exhibiting greater taxonomic resemblance, the extent of its impact was measured as the proportion of pairwise comparisons with $-2 < \beta NTI < 2$ and $RC_{Bray} < -0.95$. Conversely, communities constrained by dispersal limitation display lesser taxonomic similarity, and the measure of dispersal limitation was derived from the fraction of pairwise comparisons with $-2 < \beta NTI < 2$ and $RC_{Bray} > 0.95$. Finally, the fraction of the pairwise comparisons with $-2 < \beta NTI < 2$ and $-0.95 < RC_{Bray} < 0.95$ was identified as “undominated”.

We have had the explanation of deterministic and stochastic processes in the INTRODUCTION.

The discussion still has large stretches with results being repeated, which could be streamlined and put into a larger context, by relating to other findings.

In the revision, we have deleted the repeated information. In addition, we added more discussion by relating to other studies. Most of the revisions were made in the 4.2 and 4.3 sections. Please refer to the revised manuscript for detail.

More specific comments:

Across the manuscript: the abbreviations for the three studied ecosystems are not very intuitive (Permafrost soil and lake sediments are PCBs and SCBs), maybe they could be simplified?

Thanks for this great suggestion, we have simplified the bacterial communities in permafrost soil, lake sediment, and lake water as PB, SB, WB, respectively.

Fig. 1 the letters in particular in Fig. 1a and b are very small and hard to read, please increase size.

We made the revision on this figure and others figures with the same issue.

Fig. 6: How exactly was habitat niche breadth determined (based on OTU distribution?) and I am wondering if maybe Fig. 5 and 6 be merged into one, as they seem a bit redundant (as also the nmds is displaying the Bray Curtis distances, if I understood correctly.

Thanks for this suggestion. We combined Fig 5 and 6 together.

For the niche breadth, we added more details in the METHODS section: “In order to determine the habitat niche occupied by each taxon, we utilized the "spaa" package (Zhang, 2016) in R to calculate the Levin's niche width (Levins, 1968). The formula of niche breadth is $B_i = 1/\sum_1^n p_i^2$, where B_i represents the niche breadth of OTU_i across the communities, n is the total number of communities, and p_i is the proportion of OTU_i in each community.”. In the METHODS, we also clarified that: “The NMDS was based on the Bray-Curtis distance using the relative abundance of OTUs.”

Line 501 and following lines: this is a very general statement, are there any studies that could here focus more on bacterial differences in thermokarst lakes, or at least in cold/permafrost ecosystems?

In the revision, we reorganized the whole section, “4.3 Environmental influences”. We added some statement by citing other studies about permafrost microbes, such as Mackelprang et al, 2017; Romanowicz and Kling, 2022; Fu et al, 2023. Please refer to the revised manuscript for detail.

“For example, Actinobacteria and Gemmatimonadota have a negative, while Gemmatimonadota has a positive relationship with organic carbon and nutrients in

permafrost (Romanowicz and Kling, 2022; Fu et al, 2023), in line with our results. The fact that different bacterial phyla exhibited varied responses to changes in organic carbon and nutrient further emphasizes the intricate interplay between microorganisms and their environment. Due to their ecological strategies, metabolic features, and environmental preferences, bacteria in permafrost respond differentially to nutrient status and other stressors, driving adaptive changes in community composition and function (Mackelprang et al, 2017).”

- Fu L, Xie R, Ma D, Zhang M, Liu L. 2023. Variations in soil microbial community structure and extracellular enzymatic activities along a forest‐wetland ecotone in high‐latitude permafrost regions. Ecology and Evolution, 13: e10205-n/a.
- Mackelprang R, Burkert A, Haw M, Mahendrarajah T, Conaway CH, Douglas TA, et al. 2017. Microbial survival strategies in ancient permafrost: insights from metagenomics. The Isme Journal, 11: 2305-2318.
- Romanowicz KJ, Kling GW. 2022. Summer thaw duration is a strong predictor of the soil microbiome and its response to permafrost thaw in arctic tundra. Environmental Microbiology, 24: 6220-6237.