

## ***Interactive comment on “Co-occurrence patterns in aquatic bacterial communities across changing permafrost landscapes” by J. Comte et al.***

### **Anonymous Referee #2**

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### General Comments

I thoroughly enjoyed reviewing the manuscript from Comte et al. describing the diversity of the bacterioplankton and the patterns of co-occurrence among bacteria, phytoplakton, autotrophic phytoplankton, and zooplankton in thermokarst ponds across five valleys along a North-South permafrost degradation gradient in Nunavik. This study set out to determine whether there were habitat preferences shown in the diversity and co-occurrence patterns of bacteria along the permafrost gradient and, if so, whether these preferences related to differences in phylogenetic structure. This was accomplished by using 16S rRNA gene pyrosequencing to describe the bacterial community, and the collection of physico-chemical and biotic measurements, along with the characterization of the phytoplankton and eukaryotic plankton community in the lakes.

The authors found differences in bacterial community composition across valleys strongly related to landscape type, but also showed a high degree of taxonomic relatedness within and across valleys, due to a few dominant OTUs. They also showed strong local effects (pond-by-pond) on the beta-diversity of the bacterial communities and were able to identify indicator OTUs for each of the landscape types and dissolved organic carbon, phytoplankton biomass, and salinity as the main explanatory variables for the changes in diversity. The authors argue that these lines of evidence suggest habitat preference on the local scale, and in the less abundant bacteria, but with a higher similarity among the highly abundant bacterial OTUs across the landscape types. They suggest this is driven by the high variability within valleys and the DOC and salinity concentrations found in the lakes. The co-occurrence networks did find distinctions between the valleys in co-occurrence patterns, and also pointed to DOC as having a significant, if complex relationship to the bacterial co-occurrence patterns. The co-occurrence networks also suggested potentially important inter-relationships among the bacteria and identified highly connected, potential "keystone" OTUs in the network.

Overall, I think this paper was clearly written, the study was carefully done, and the data was thoroughly analyzed. I think that this study provides an interesting look into the patterns of microbial community assembly in spatially separated thermokarst lakes and provides insight into the assembly rules that generate these communities. I have a few suggestions, but I do not think that they involve any major changes in the manuscript, and I have not identified any fatal flaws. I think that these results are interesting: that the main differences in the microbial ecosystem are taking place among the less abundant OTUs, including inferred interactions and keystone OTUs. I commend the authors for a study well done.

My main concern is that MIC lacks sign, or direction, so it does not provide information on whether there is a positive vs. negative correlation (or co-occurrence vs. non-co-occurrence). In the Reshef et al. 2011 paper where MIC is described, some of their

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strongest relationships identified in their mice microbiome datasets were what the authors referred to as "non-coexistence". Using MIC allows for the identification of many of these relationships, but without looking at the relationships it's tough to interpret what they mean. The authors do some interpreting, particularly with Chitinophagaceae, but it would be strengthened if they showed examples of these relationships to bolster their point. I believe the authors have found potential relationships that point to ecological function, I just want to know what kind of relationships they've found. This would also allow the authors to suggest further hypotheses regarding function that could be tested in these or similar systems.

### Specific Comments

I would find a map or at least a schematic of the sites a helpful addition either in the main text or in the supplemental materials.

In some instances, the authors seem to be arguing for the taxonomic ID of OTUs to suggest ecological function, when they have evidence in their study which would allow them to infer function. I think this can be resolved through a change in emphasis in the discussion, rather than any major analytical change.

The identification of potential keystone species is interesting in these thaw-ponds and lakes, but there is a lack of detail about likely function that I believe the authors may be able to infer from their networks and data. I also suggest that the authors emphasize that the "keystone" OTUs identified as hubs were not merely the abundant OTUS, but were rare, potentially important actors of the particular ecosystem. This might be worth pointing out in the abstract. In addition it may be worthwhile to look for any overlap in indicator OTUs for the different habitats and highly connected nodes. I think this may provide deeper insight into these thermokarst microbial ecosystems. It's possible the data is not there for this, but the authors may be able to suggest ways to resolve these types of connections.

### Technical Corrections

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Pg 11 L17 The sentence "The discrepancy between dw4000 and duw4000 patterns...of a few but highly abundant OTUs within different valleys" Is a bit confusing. I think replacing it with "... of a small number of highly abundant OTUs within different valleys." is clearer and doesn't change the meaning. But in any case it should be re-worded.

Pg 19 L 23 "autrophic eukaryotes" should be autotrophic.

Table 3. I cannot tell the difference between the text in bold and the normal text. I see the p-values, but not the bold distinction

Table 4. I can't see the grey shading mentioned in the figure legend.

Figures 3 and 4 ... Node size is not described in the legend. In the Supplemental section it's related to degree, but it should be stated here as well.

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**BGD**

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