

Response to referees' comments on "Bacterial production in subarctic peatland lakes enriched by thawing permafrost."

We thank the reviewers for the thorough review of our manuscript and their many helpful suggestions, which will improve the manuscript. We have carefully considered each comment, and the the manuscript is being revised accordingly. The following list addresses the raised points, describes our view, and explains the changes we propose to implement in the manuscript.

Reviewer 1 comments

1) The manuscript would benefit from some clearer statements of goals, questions, and rationale, from the abstract to the end. It reads very much like a descriptive study and somewhat of a list of analyses and results. I think anything you can do to more clearly articulate goals, questions and rationale will improve the impact of your paper.

The aims and goals will be clarified in the final paragraph of the introduction and a paragraph will be added at the beginning of the discussion to better guide readers through the study objectives, findings and rationale.

2) There is a great deal of data in this paper, which is nice but it makes it a bit hard to follow main points and also seems that much of the data are underused in some cases. You may want to distill some of this down further to improve clarity of presentation. For example, I'm not sure the Fig 4 really illustrates anything key, and Fig 8 has some results that don't really seem to be discussed much. I would suggest trying to highlight the real nuggets, or at least guide the reader more through it.

A paragraph has will be inserted at the beginning of the discussion to improve clarity. Fig. 4 has been moved to Supplemental Materials, however Fig. 8 is considered to be a highly important experiment discussed within this paper. We have made efforts to better highlight its importance and to guide the reader.

3) I found it very unclear what the lake categories were in the statistics (I understand the location designation, but you allude to SAS versus the other two lake categories). How were the categories established and why? To address a particular question?

A clarifying sentence will be to the methods to better explain the categories used, and lake categories will also be mentioned specifically during presentation of statistical results. We will also aim to make the statistical categories more clear by improving our communication of the study rationale as per the first comment.

4) Main points: I would suggest you try to revise to make these clearer. For example, your final paragraph gets to the really interesting inferences and implications- it would

be great to see some of that earlier and better developed.

Sentences will be added throughout the discussion to better highlight these points, along with the addition of a paragraph at the beginning of the Discussion (as explained above).

Specific comments: Line 21: define CT Line 34: delete one of the “no” Line 145: remove “,” Line 167: should it be ug/L? Line 218: change sub-title to Bacterial abundance to match later terms Line 330: give p-value, and in this section, averages for all categories should be provided, not just for SAS lakes Line 339: can you provide some stratification data? Line 343: change “2and” to “and”. Line 389: insert “bacterial” before “cell” Line 407: include “%” to the value of 50.1. Line 488: change “Less 30%” to “Less than 30%”. Line 501: change “resulted” to “result” Line 523: may want to be consistent with notation, different in Results, and I think the usage here (all x106) is better Line 529: change “from.” to “from” Line 555: include citation after “limitation”

These changes will all be made. We thank you for your careful reading and for listing these errors so explicitly.

Table 1: I would suggest switching the order of chemical constituents so that they are organized from highest to lowest, not lowed to highest.

This will be changed as per the comment.

Figure 8: the order of the treatments listed in the legend should match the order that they appear in the figure.

This will be changed as per the comment.

Reviewer 2 Comments

The authors sample numerous lake and adjacent terrestrial sites, from a series of different geographic regions. Overall, I found it hard to follow the naming conventions used in the manuscript, and found that I often had to flip back and forth with section 2.1, where the site abbreviations were laid out. I would urge the authors to consider a way that they might make their naming conventions more intuitive throughout the manuscript. Simply taking care to always refer to a site as ‘peat thermokarst’ ‘mineral thermokarst’ or ‘rock basin’ (or similar), in addition to the less intuitive abbreviations would be very helpful.

More references to site types, as described by the reviewer, will be added throughout the paper and to figure captions to better facilitate reader understanding, and to better guide readers through the results from the different geographic locations.

In addition, I wondered about the size fractionation results, and the fact that the fractionated samples (particularly at <35 and occasionally <3 microns) often show greater values than the total fraction. Of course, this is a concern, because (unless I am misreading the treatment) the various fractions represent just a subset of the 'total' fraction. The authors do spend a fair bit of time discussing some potential methodological reasons for these results in their Discussion. However, I wonder whether it would be best to limit the presentation of data to the 'total' and < 1 micron fraction, which ends up being the focus of the discussion, anyway. These results are also more intuitive, and seem more sensible overall. Alternatively, perhaps it is the difference in counting methods (flow cytometry vs. microscopy) between the total and fractionated samples that is leading to this offset in results. In any event, I would suggest a re-consideration of the presentation of this data.

The fact that the <35 and <3 micron size-fractions show greater values than the total sample provides support for demonstrating the presence of particle-attached bacterial communities in these lakes. Presentation of the four size fractions adds substantial information and helps to differentiate between lakes across the various subarctic regions.

Finally, I noticed a few typos and grammatical errors in the manuscript.

The manuscript will be reviewed carefully and typos and grammatical errors will be corrected.

Line 29-31: It wasn't clear to me, in the abstract, that you were sampling three lake types – until this point the abstract addresses the peat thaw lakes only. More clarity on this point in the abstract would be helpful.

Reference to other lake types will be added to the abstract to clarify the extent of sampling.

Line 33: The enrichment experiment seems to out of nowhere a bit here. Perhaps just changing the wording slightly to say "An in situ experiment . . . relative to a control treatment" would make it clear to the reader that this hadn't been previously introduced in the abstract.

This change will be made, along with further steps to better guide reader through the results as indicated by reviewer 1.

Line 85: “marine snow”: This is true, but marine snow is not terrestrially derived, and so the carbon quality is likely quite different from what you’re investigating. Perhaps re-work the text slightly to reflect the fact that the importance of ‘snow’ for microbes in marine environments doesn’t necessarily suggest that particles (and permafrost- derived particles) will be similarly important in Arctic lakes. A good reason for this study!

A sentence will be added to clarify this key difference between marine snow and permafrost-derived particles.

Line 134: Six, rather than five sites? Or perhaps I am mis-understanding the site descriptions?

The sites studied include five permafrost regions and one group of reference rock-basin lakes. We count this as a total of six sites. We will clarify this within this sentence, and also add references to the site types as suggested by this reviewer to facilitate better understanding of the geographical regions and lake types studied.

Line 140: Two sets of lakes, or two lakes in the SAS valley? If two sets, then perhaps change subsequent wording to read “the SAS1 set of lakes was to the south . . .” or similar.

This sentence refers to two sets of lakes, for a total of 5 SAS lakes studied. This sentence will be clarified as suggested by the reviewer.

Line 163: 0.2 m? Units are missing here.

Units will be added as per the reviewer comment.

Line 168: Could you provide more detail on how the active layer was sampled? A full core to 60 cm? Or was a simple shovel used? Understanding the depth of sampling, and method of sample acquisition would be useful.

More detail will be added to the description of active-layer sampling.

Line 172: Define CDOM.

This will be changed as per the reviewer comment.

Line 200: It would be useful to have a description of why these depths were chosen, relative to the active layer depth of 60 cm that is stated earlier. Related to this, as I read through parts of the Discussion, I felt it would be useful to have a better understanding of how thermokarst in this regions plays out. For example – how likely is it that soils (or, leachates) from 1.25 – 2.77 m depth are going to be available for transport to nearby lakes with thermokarst in this region? Is it reasonable to expect thaw or slumping to affect soils at this depth?

A sentence describing why these depths were chosen will be added to the Methods in this section. The second aspect of this comment, regarding the justification of whether thawing or slumping at this depth and interactions will lake water will be added to the discussion, with reference to Fig. 2.

Line 201: Could you provide the soil: water ratio? Was it constant?

No, the soil:water ratio was not constant. Instead, the results have been standardized to account for the differences in thawed volume of the permafrost core sediment and the amount of ultra-pure water that was added to samples. The nutrient and carbon concentrations provided are potential contributions from the melted ice released from the permafrost soil. This detail about the permafrost core nutrient and carbon concentrations will be added to the Methods section of the manuscript.

Line 248: Bacterial community specific growth rates. You're able to calculate a slightly different growth rate here than the more typical (per cell) value that's seen more commonly in the literature, because of the availability of average cellular carbon data. I might specify the units here to clarify that your cell-normalized growth rates are expressed in a slightly different way (d-1), and also state your abbreviation (BG). I also notice that through the text, you sometime refer to this as 'specific growth rates' or 'community growth rates' or 'community specific growth rates'. I think you mean the same thing by all of these? If so, it would be good to standardize the wording, or – better yet! – just consistently use "BG". In addition, provide your BP and BA abbreviations in this paragraph, and use these consistently in the text.

The units will be added and the terms 'specific growth rates', 'community growth rates', and 'community specific growth rates' will be changed to the abbreviated terms as recommended by the reviewer.

Line 328-338: Do you use the Mann-Whitney test for all of these comparisons? These look like comparisons between three groups to me, which suggests an ANOVA (or, non-parametric analog) followed by post-hoc comparisons. Is it possible to add these stats to Table 3?

These stats will not be added to Table 3 as we prefer to keep the table results separate from our statistical analyses. We will clarify in the Methods section that we used an ANOVA followed by post-hoc comparisons.

Line 341: “the other thaw lake types”. Would “the mineral thaw lakes” be more accurate here?

The other thaw lake types include mineral thaw lakes and also lakes with varying combinations of mineral and peatland inputs. It would be inaccurate to say that all the lakes outside of the SAS peatland valley are mineral lakes. We would prefer not to change the wording of this sentence.

Line 339-349: Were there interaction effects for any of these parameters? This might make the interpretation a bit more nuanced. It would be useful to state the presence (or absence) of significant interactions in the analysis, and if significant, provide some interpretation here.

We will add information regarding interaction effects and follow the suggestions provided by the reviewer.

Line 361-364: I’m unclear on the meaning of this sentence – can it be re-worked for clarity?

Yes – this sentence will be broken up for clarity.

Line 422: “A rich potential source” would be more appropriate, given that you haven’t measured water flow across this landscape.

This will be added as per the reviewer suggestion.

Line 430: “SAS waters”: clarify that you’re referring to lake waters here, rather than soil pore waters.

This will be added as per the reviewer suggestion.

Line 439: Change “this element” to N, for clarity

This will be changed as per the reviewer suggestion.

Line 447-448: The relatively high P could also indicate P enrichment in local rocks / till? This is also suggested by the higher P concentrations that you find at depth.

The sentence will be changed to include this possibility as recommended by the reviewer.

Line 459-466: I found that this paragraph could use a bit of extra text to tie these findings from the literature back to your own work.

A sentence will be added to better link this with the results of this study as per the reviewer comment.

Line 497 and onwards: These concerns about particle-based bacteria are certainly well-founded. It's worth considering these caveats when thinking about how to best present the fractionation data (as discussed in the overarching comments). I certainly agree with the assertion, however, that particle attachment is important in these systems.

Please see our comments above. We are pleased that the reviewer concurred with our conclusions about the importance particle attachment.

Line 562 – 563: Lakes in this region would be more similar to your mineral thermokarst lakes, I believe.

The reviewer could well be right, but we feel that we do not have sufficient information to reliably make this comparison.