

The Cryosphere Discuss., author comment AC2
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Reply on RC2

Shawn M. Doyle and Brent C. Christner

Author comment on "Variation in bacterial composition, diversity, and activity across different subglacial basal ice types" by Shawn M. Doyle and Brent C. Christner, The Cryosphere Discuss., <https://doi.org/10.5194/tc-2022-68-AC2>, 2022

Doyle and Christner have produced new data documenting the taxa present and active in basal ice from two different glaciers in spatially distant locations. They supplement the data by integrating it into a meta-analysis of several other studies of microorganisms in ice. This is an important study, with regard to the new data alone, but the review/synthesis of existing data is something the biological glaciology community needs. The analysis is excellent despite the inconvenience of sequencing platform evolution during the 2010's when most the work was conducted. I enjoyed reading the paper and learned a lot from it while reading. I support publication of the manuscript. My comments and requests are focused on extracting more out of the data and for clarity of presentation. Alexander Michaud

General Comments:

Results: I would advocate the authors normalize their results in terms of a volume of ice. This allows for budgets to be made based on volumes of basal ice in a glacier. Normalizing results by grams of sample makes it more difficult to relate results back to the environment and to other studies. Is this frozen weight of ice currently? If you do want to keep it that form, is there a way to incorporate the density of the different basal ice types studied?

In our experience, normalizing by volume (mL) works well for debris-free glacial ice but leads to confusion in ice that contains large amounts of sediment and stones. Normalizing by mL in these samples artificially inflates biomass estimates to account for their reduced water content. We contend that debris-rich ices are more analogous to a frozen soil environment like permafrost, which are typically normalized by mass (g), than an aquatic environment where per volume measurements are typical.

Englacial ice: Do you mean meteoric ice throughout? This becomes important when you are classifying your samples for section 4.5. The glaciological community would likely argue that glacial ice begins at the bubble close off depth (see your line 560). Supraglacial and englacial may be better as "meteoric ice" or "glacial ice" and supraglacial should be distinguished between accumulation and ablation zone (see your line 547). I recognize this is a bit semantic, but some of the discussion dances around these ideas when trying

to explain the results.

Yes, supraglacial and englacial ice would both be considered meteoric ice. However, we did not want to use a single category because, from a microbial perspective, relatively young meteoric ice near the surface is quite different versus older meteoric ice located deep in a glacier. As we describe in our discussion, supraglacial and englacial ice likely represent different sections of vertical gradient through the body of a glacier. We are not sure if relabeling all samples as meteoric and then distinguishing those from the accumulation or ablation zone would reflect this gradient any better than our current approach. This approach would also be difficult to implement as most studies did not report what zone they collected samples from.

Specific Comments:

L29: When saying that basal ice is an important mechanism for dispersing microorganisms in the subglacial aquatic environment, I think the important point that needs to come along with this for the reader (especially the non-glaciologist) is that the freeze on or melting conditions vary spatially at ice sheet and glacier beds at multiple scales and thus represent a mechanism of dispersal.

Agreed. We have added this detail to the text.

L37: When you say overlying englacial ice, do you mean meteoric ice? I think I understand that you are trying to say this is where the englacial microbes are, but stick to consistent terms (see general comment above).

See our response above.

L51: Laufer et al 2021 Nature Communications demonstrates this from Svalbard glaciers.

Thank you. Citation added.

Paragraph starting at L52: This paragraph oscillates between basal ice sediments and subglacial sediments which makes it confusing for the reader. With this said, the point of this paragraph is, I think, that knowing the subglacial conditions is important for understanding basal ice conditions because of the imprint subglacial conditions can have on basal ice. Without this connection, the focus on subglacial conditions leads to confusion. Please revise this paragraph.

We have revised to be more clear.

L91: Please define temperate ice when used first in the introduction.

Done.

L122: The last sentence of this paragraph is confusing. Please rewrite.

Agreed. We have revised the sentence.

L125: Were ATP samples melted at 4C like DNA extraction samples? Please clarify.

Yes. We have added this detail to the text.

L145: Describe your blanks.

Description added to the text.

L214: Perennially freshwater or “perennially frozen, freshwater environments”?

Thank you. Typo corrected.

L282: How were the potential contaminants flagged and removed? I missed this in the methods.

This process is described in the end of the section 2.6.

Table 2: What is your limit of detection for the ATP method?

The limit of detection for ATP was 10pM which comes out to ~0.01 pg/g ice using our approach. Measurements beneath this limit were thus listed as being <0.01 (pg/g) in Table 2.

Figure 3: I am curious to hear your rationale for including the weathering crust of Matanuska Glacier into the englacial ice category. To me, a weathering crust is analogous to the active layer of permafrost environments, and active layer samples were excluded from this analysis. This is a seasonally thawed part of the glacier. Also, should it be englacial, seems supraglacial. Also, based on the Figure, then there would be two large clusters of supraglacial samples. I almost think the microbial communities are helping you to determine the sample cluster definitions rather than forcing a label on them a priori.

“I am curious to hear your rationale for including the weathering crust of Matanuska Glacier...”

We considered this. Only samples from that study that had “glacial ice” as their listed isolation source in the NCBI metadata were included. All samples isolated from the “weathering crust aquifer” were excluded.

“I almost think the microbial communities are helping you to determine the sample cluster definitions rather than forcing a label on them a priori.”

It does appear that in many cases microbial community composition is indicative of ice type, which is exciting. However, we feel labeling the ice samples *a priori* based on their physical descriptions rather than the composition of their microbiomes is more appropriate. Ice type certainly affects microbial community composition, while the vice versa is unlikely.

- Also, I think the WISSARD samples need explanation that they were drill water sampled from the drill, which represents an integrated sample of firn and englacial ice given firn meltwater was used to start drilling.

Agreed. We have added an asterisk to the WISSARD samples and noted this detail in the caption.

- Some of these issues may be resolved by categorizing the samples as accumulation zone samples and ablation zone samples. Consider rerunning the cluster analysis with these categories.

This is an interesting idea, but it would not meaningfully change anything within the analysis. The categories in Figure 3 are added afterwards for visualization; the clustering algorithm only “sees” sequence data and is agnostic to ice type. It would also be difficult to implement this new labeling scheme accurately as most

studies did not report what zone they collected samples from.

Figure 4: Can you clarify what the scale is for the x-axis of the right panels. Some taxa appear twice in the list. Is there something else to be learned here for taxa that appear more than once? Indicate in the legend that the lineage listed on the right list is the lowest and confident taxonomic assignment, which varies for some listed?

The x-axis in the right panel represents the Euclidean distances of the scalar projections from the left panel. We have added this detail to the figure. Following your suggestion, we have also modified the caption about taxonomic assignments.

L437: The sentence about pasteurization needs a citation.

Citation added.

L461: What is meant by "larger number of high-ratio ASVs"?

We have revised the text so that high-ratio is explicitly defined as those ASVs having an 16S rRNA/rDNA abundance log-ratio >1.

L533: By decline, you mean the dormant, strictly aerobic taxa are moving to the pool of dead microbes? Since you invoke that most all cells are already in a dormant state, then for a population to decline in this situation would mean die off, right?

Correct. We have replaced "decline" with "die-off".

L541-543: Given the shift to anaerobic taxa due to loss of oxygen, then there is a maximum CO₂ production that can occur in clean ice until dirty basal ice can provide alternative electron acceptors.

Maybe, but we would have to assume fermentation does not occur.

L572: Or maybe they were transported to the basal ice region from cryoconites above?

That's certainly possible but difficult to say for certain. Given these marginal basal ice samples were exposed to sunlight for quite some time before they were sampled, we feel our explanation is less speculative.

FigureS2: Please insert a scale bar into the image.

Done.