

The Cryosphere Discuss., author comment AC1
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Reply on RC1

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-AC1>, 2022

REVIEWER #1

This is a very well written and presented piece of research, investigating the microbial communities associated with basal ice of different thermal regimes of glacier. The aims are clearly stated, the work rigorous and placing their results within the context of other previously published work in particular a great addition to the literature.

I have some minor comments relating to the wording / writing, and one slightly less minor point about the way in which 16S rRNA sequencing was analysed. In particular, the use of a now out of date SILVA 16S rRNA database. These comments are addressed below in order they arise in the manuscript:

Thank you

Line 8: "providing a habitat"?

Corrected.

Line 15: This sentence could be clearer to better distinguish between the amplification of the 16S rRNA gene from extracted DNA vs RNA (via cDNA). It took me a few reads of the sentence to see the difference in the current way it is written.

Agreed, it was too wordy. We have revised this sentence to "...high 16S rRNA/rDNA amplicon ratios implicated...".

Lines 162-167: If I understand correctly, the coarse and fine sediments were separated to "facilitate filtration of the large sample sizes" but surely this would have led to a loss of biomass associated with coarse sediment? Acknowledgement of this would be pertinent.

Good point. We have added this point to the revision.

Line 193: Missing word between "included" and "at 95°C..."

Thank you. Corrected.

Line 209: One less minor issue I have with this manuscript is the use of SILVA v132 for taxonomic assignment of 16S rRNA gene sequencing data. The more recent version - v138 - is much larger and the associated updated taxonomy has altered lineage assignments to SSU sequencing data, especially at higher taxonomic ranks (e.g. Phylum and Class level). For what reason was v132 chosen over v138? - the former has been available since 2019, and is the norm now for taxonomic assignments. How different would your taxonomic assignments be if you used the newer version?

Using the older SILVA v132 for taxonomic assignment was an oversight and we have reclassified the 16S data using the latest version (v138.1) in our revision. The differences are largely superficial: 90.2% of the ASVs have identical assignments at the genus rank using the newest database. About 35% of the ASVs have new Phylum/Class level assignments, mostly due to minor spelling or suffix changes (*Bacteroidetes* and *Actinobacteria* are now *Bacteroidota* and *Actinobacteriota*, respectively) or modifications to the names of some lineages (e.g. *Betaproteobacteriales* have been renamed to *Burkholderiales*, with no changes at lower taxonomic ranks (e.g. Family/Genus)).

Line 239-243: It is not clear to me what reference database was used for taxonomic assignments of these previously published datasets, please clarify.

We have reclassified the metaanalysis with SILVA 138.1 and added this detail to the text.

Fig 5 caption: You mention black triangles but do you mean black diamonds? I can't see any triangles on the figure

Sorry about that, caption corrected to diamonds.

Line 426: Is this cited reference valid for the whole statement? If so, move to the end of the sentence. Otherwise it seems there are missing references relating to the highly-resistant endospores belonging to Firmicutes and Actinobacteria

This reference was only for the opening statement regarding the physiochemical stresses faced by cells under frozen conditions. In our revision, we have added additional references to the end of the sentence citing the durability of Firmicutes endospores and Actinobacteria spores.

Line 436-7: What data relates to this claim? Link to results if it relates to your study, otherwise cite relevant studies

The citation was left out by mistake and has been added.

Line 592: Please swap "(46)" with a citation in a consistent style to the rest of the manuscript

Corrected.