The Cryosphere Discussions

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# Comment on tc-2021-215

Anonymous Referee #2

Referee comment on "Temporal variation of bacterial community and nutrients in Tibetan glacier snowpack" by Yuying Chen et al., The Cryosphere Discuss., https://doi.org/10.5194/tc-2021-215-RC2, 2021

The authors are presenting a very well-written manuscript that aims to determine the temporal differences in bacterial community structure between the surface and subsurface layers of snow on a Tibetan glacier and to link any temporal changes within each layer to environmental factors and/or intra-community interactions.

It is really nice to see a study that incorporates a temporal aspect, as many studies carried out are of a single time point nature and a 9-day period is often as much as can be done during a field campaign. However, considering the varied and often slow growth rates of bacteria in these environments, it would be useful to include a brief discussion on the expected doubling times for these communities.

My main reservations about this manuscript relate to the very limited size of the study area and the assumption of presence of nitrogen fixers and denitrifiers based on the potential functional profiles as predicted by 16S data.

The lack of proper spatial resolution limits the conclusions that can be drawn from this study and the impact on the usefulness of the results presented in this manuscript is potentially severe. However, the authors do a good job of presenting an interesting data set that, despite its limitations, would be useful to others in this field. I therefore recommend that the manuscript is considered for publication after major revisions.

I am setting out my comments and recommendations below, starting with the main points and then listing minor points as they appear in the manuscript. Where possible, line numbers or figure numbers are used to indicate the location in the text.

Main issues:

L77-79:

1) At each time point, three replicate samples were collected within a very small area of only 5 m by 3 m. The authors do not state clearly how far apart each replicate was, only that they were at least 30 cm apart. This introduces several serious issues:

- a) The size of the ablation zone is not given in the manuscript, but for most glaciers, the ablation zone would be a lot larger than 5x3 m, and by sampling only within a very small portion of that zone, it is open to discussion whether those samples are representative of the whole ablation zone.
- b) Since the three replicates were collected so close to each other, they could be considered technical replicates, rather than true replicates, which would have a knock-on effect on what statistical tests would be suitable.
- c) The snow pits could have been either 30 cm apart or even 4.5 meters apart, that is a huge difference and without a sampling strategy that takes spatial variation into consideration, it is impossible for the reader to know whether the differences seen are due to spatial or temporal variation – for instance, is this perhaps the reason why the communities are very different on some days but very similar on others? Therefore, the spatial distribution of each individual snow pit and distances between pits must be clearly presented in the manuscript.

If the distances between snowpits varied substantially between sampling days, I also suggest that the authors include distance as a factor in their statistical analyses.

2) It is not clear from the Methods, whether snowpits dug on subsequent days were from within the same 5x3 m square or if a new sampling area was selected each time? Regardless, this needs to be clarified in the manuscript, but, if the former, the data ought to be analysed as a time series and not as a series of independent sampling points.

L101: The filtration setup and method for filtering need to be described in more detail, especially with regards to what steps were taken to prevent contamination, how the samples were thawed and the time scales involved.

L149-154: Although functional profiling of taxa identified by 16S sequencing can give some insights into what abilities the community might have, it is not suitable for this kind of investigation. Although shotgun metagenome sequencing may be out of scope, the authors should be able to provide much more accurate and quantitative data for the presence of nitrogen fixers and denitrifiers in their samples by qPCR of relevant genes (e.g. nifH and narG). In its current form, the method used does not provide the data needed to back up the conclusions drawn by the authors.

Minor points:

L19 and elsewhere: When contrasting data for the surface and subsurface layers, it is easier for the reader to follow if data and location is grouped together, e.g. "Nitrate and ammonium concentrations increased in the surface and decreased in the subsurface snow over time, therefore indicating accumulation and consumption processes, respectively." The same goes for the following sentence re nitrogen fixation and denitrification genes.

Introduction: I though the Introduction was particularly well-written and set the scene very well.

L71: Delete the "the" in front of "October".

L74: There is a mismatch between the dates and the number of dates. Day 5 ($28^{th}$ of October) is missing from the list of Dates and the $2^{nd}$ of November ought to be day 10.

L75: What is the age of the snowpack? I.e. when did the snow first start accumulating in this area?

L89: How were the 0.45 um cellulose membrane filters treated before sampling? Was the initial volume of filtrate discarded before collecting the sample for analysis?

Section 2.7 Statistical analysis (and elsewhere): Function and package names need to be consistently highlighted (single and double quotation marks and no highlighting at all are all used with no apparent system to it). Usually, function and package names are italicised, but any consistent form of highlighting would work.

L195-197 and L203-204: These to statements are contradicting each other. Either there was no significant difference in relative abundance between the two layers or there was a significant difference in bacterial community structure between the two layers.

L198-201: It would help to back this up with absolute numbers of ASVs. E.g. did the cyanobacteria and Chloroflexi really grow in numbers in the subsurface layer over time or did their populations stay the same (in actual abundance) while those of the alpha-Proteobacteria and Firmicutes declined, thereby resulting in an apparent increase due to the increase in relative abundance?

L206-210: It would be useful to see how the environmental factors correlate with these axes.

L245-246, 253: It would really help the authors argument here if they presented a theoretical input of nitrogen for each sample. Given a yearly deposition rate of 282 kg N per km2 and based on the seasonal deposition pattern for glaciers in the region, how much nitrogen would they have expected in the volume of snow that they collected for nitrogen analysis?

L255, 5 Conclusion: Since Tot-N is decreasing in the subsurface over time, the nitrogen is clearly not incorporated into biomass. It would be useful to see a brief discussion on how the authors think the nitrogen is leaving the system. The surface community may not be negatively impacted by increased N deposition, but would the subsurface community be able to cope with an increased N input or would it be exported downstream and add to the N load in glacier-fed rivers?

L258: There is no mention of oxygen levels being measured at the of sampling in the manuscript. If the authors believe that the oxygen levels in the subsurface layer of the snow pack can be expected to be sufficiently low to allow for denitrification to occur based on data from the literature, that evidence needs to be presented in the manuscript.

Regarding test for correlation: A weak correlation is still weak, even if the test is highly significant. Also, I am not clear on why the authors are using correlation tests to test for changes in environmental variables over time?

Figure 5: 1) Some labels are missing from markers in Fig 5a, 2) Out of curisosity: In most cases the samples taken on the same day are very closely clustered (e.g. Surface Day 1, 4 and 9; Subsurface Day 9), so what is special about Subsurface Day 3 and some of the others, where the replicate samples are very different from each other?

Figure 6: It is very difficult to identify the taxonomic affiliation of even the largest nodes, due to the selected colour-scheme. In addition, considering how common red-green colour-blindness is in the general population, it would be impossible for a large proportion of readers to distinguish between red and green nodes. I therefore recommend that the colour-scheme is reworked for this figure.