The Cryosphere Discussions

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Comment on tc-2021-215

Anonymous Referee #1

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-RC1, 2021

Chen et al. provide a characterization of the bacterial diversity of a snowpack located on Dunde glacier (NE Tibetan Plateau) over a 9-day period in Oct/Nov, twinned with measurements of aqueous geochemistry. Sampling was performed on a total of 7 days, with the snowpack divided into an upper 15 cm and a lower 'sub-surface' 15 - ~30 cm layer (though no real rationale is provided for this). From each layer, sequencing of a 16S hypervariable region was employed to catalogue bacterial communities (as amplicon sequence variants) with corresponding measurements of organic carbon, nitrogen and major ions. The authors present this work as a temporal series of datapoints over their 9-day study period.

In general, the work is well presented and written with minimal language corrections required throughout. The justification for the work is laid out well in the introduction. The methodology is strong in many places (sequencing and subsequent analytics) but lacks detail in some areas and overall is lacking any justification for the sampling design (i.e. the timing of the sampling in particular and what this informs on in the larger context). I find the results to be relatively poorly presented with questions raised about the accuracy of the nitrogen datasets for example. Much weight is given here to numerous correlations between parameters with limited attempt to make more sophisticated comparisons and often over presentation/interpretation of weak relationships. This continues into the discussion that has a rather narrow focus on the role of nitrogen within the study and no real discussion of the dataset and its placing/importance in the wider context.

I provide detailed comments below on each section, but overall find that in its present

form the presentation of the work undertaken does not correspond well to the size of the actual dataset available. As such, the authors are making rather bold conclusions from a limited dataset. I believe that this can be revised down into a more appropriate manuscript that may be acceptable for publication should the breadth of the dataset be sufficient for the scope of this journal.

Abstract: Is generally well written and clear but needs some tidying up of the language as below:

Line 17 – word 'sub' missing I believe. Same sentence starting 'Our results' mixes past and present tense.

Line 18 – sentence starting 'Nitrate and ammonium'…this first past of this sentence is pretty vague – can you improve this with some data values please (or at least proportional change information).

Line 21 – sentence starting 'The nitrogen limitation'….place into past tense and remove 'the' throughout for clarity… e.g. 'Nitrogen limitation and dominance of denitrification in subsurface snow suggested stronger environmental and biotic filtering processes than in surface snow.'

Line 24: Sentence starting 'Collectively' – place into present tense and change 'revealed' to 'provides insight into nitrogen metabolism….'

Introduction:

Lines 28 – 38:

- There is a mix of snow and glacier related information up front here (lines 28 – 38) – ie. Glaciers melting, and glacier ice being oligotrophic, but then snow related bacterial communities. I think it important to make clear the environment you are working with (presumably supraglacial snowpacks) and try to not mix information between snow and glacier ice itself, which also houses diverse autotrophic and heterotrophic populations that undertake significant biogeochemical cycling of carbon and major nutrients.

Lines 39 - 46:

- again, I think you need to be more explicit here that you are presumably talking about precipitation in the ablation zone of glaciers….i.e. snow fall that ablates in the subsequent melt season as opposed to that which is accumulated and eventually turned into glacial ice.
- The description of dominant groups is limited here – Cyanobacteria were shown by Carey et al. 2016 to dominate the surface (0-15cm) of a sub-Alpine snowpack based on 16S sequencing, but it is widely known that numerous eukaryotic microalgae can dominate surface snow on and off glaciers. Please expand this information to include the suite of microbial communities that are known to dominate snowpacks or alternatively change the wording throughout to focus only on bacterial communities as opposed to 'microbial communities'.
- I find the sentences at the end of this paragraph contradicting. You state that differences in physiochemical conditions shape community structure (with the previous example of cyanos in surface and not in subsurface snow), but then state whether microbes in different snowpack layers show similar responses to environmental selection is still largely unknown. Can you clarify this please?

Lines 56 – 60:

- add "The" before 'Tibetan Plateau' in the first sentence please.

- Line 58 sentence starting 'The glacier melting' – this is a very vague statement with no references…can you please add details and appropriate references to evidence this.
- I would recommend to blend this paragraph with the subsequent paragraph and add in references to sentences where needed (e.g. ablation zone microbes having a greater impact on downstream processes).
- The research questions seem sound but you have a strong emphasis on temporal changes, and are only monitoring here for 9 days – arguably a very small time slice of a snowpack's melt period. How will this limited data be representative of larger temporal patterns?

Methods

Line 71 – remove 'the' before October.

Line 74 – OK, so actually not daily sampling, but spread over nine days. Also, your dates and day numbers don't match – you have 6 dates that you list, and 7 'days' that you list….

Line 75: sampling during October and November presumably means recently deposited snow, i.e. snow from the start of the autumn/winter season. Thus you are sampling relatively fresh snow, rather than e.g. snow just before the start of the next melt season, ie. the snow that would be released into the (downstream) environment. How are these data then reflective of the type of microbial communities and biogeochemistry that are important for downstream ecosystems as asserted earlier in the manuscript? Will the communities and nutrient load not continue to change significantly throughout the whole autumn/winter/spring season until the onset of melt? This links back to my previous question on whether a 9-day sampling period is big enough to address the larger questions being related to here.

Line 80: You have matched the same vertical distribution profile of Carey et al. 2016 (i.e. upper 15 cm and 15 – 30cm) – can you justify why these layer depths were appropriate

for a completely different snowpack with presumably different physiochemical conditions? i.e. do you have any density measurements etc to quantify your layer characteristics?. Snowpacks can have numerous different layer profiles dependent on precipitation and subsequent metamorphism processes.

Line 82/83: just to clarify – a total of n = 3 surface and subsurface snow samples, and n = 3 surface and subsurface samples for aqueous geochemistry were taken on each sampling day?

A nice level of detail throughout for DNA extraction, amplification, sequencing, sequence processing and identification and subsequent analyses.

Results:

Section 3.1.

- Can you clarify the nitrogen data in figure 1 please? It appears that the total nitrogen (presumably inorganic + organic N fractions) is much lower than the corresponding nitrate concentration. It states in the methods that different methods were used for TN, NH4 and NO3 quantification. How do you account for this ~4-fold lower TN concentration than NO3 concentration for example? Unless the TN data reflect just the organic fraction after subtraction of inorganics? This isn't clear. Given the focus on N in the manuscript this needs to be clear.
- Looking at Supp Fig. S2, the standard curve for TN goes up to ~0.4 mg/L but you are presenting data up to ~ 1.25 mg/L in the manuscript, well above this level. Have you calculated TN concentration from peak areas much greater than the standard curve bounds you have performed? Also, please quantify the relationships on Figure S2 with linear regression, not correlation.
- The 'significant increases' in NO3 and NH4 through time are rather small in magnitude

– please include the actual R value in the text to illustrate the effect size of this relationship.

- How can you be certain the concentration of N has changed through time given that you are not repeat sampling in the same locations? The methods detail that new snow pits were dug each sampling day (as would be expected). How can you be certain that the variability 'through time' in your data, does not simply reflect variability in space, i.e. heterogeneity in snow pack N content across your sampling area?

Section 3.2.

- Again, I find the presentation and reliance on correlations between diversity indices / relative abundance and time / Geochem data to be a bit misleading. These correlations are rather weak but the phraseology presents them as 'significant' changes. Can you reword presentation of correlations to reflect their actual strength, e.g. "a negative correlation was apparent between Shannon and Chao1 indices and time in the subsurface layer".
- Can multiple linear regressions be tested here to account for differences in diversity taking into account multiple factors simultaneously rather than simplistic single variable correlations?
- Please move the second paragraph on the actual community composition to the start of this section, which should then be followed by comparisons to other datasets.
- Looking at Figure 4 there appears no real distinction between surface and sub-surface community composition. I wonder whether this relates to an arbitrary 15 cm distinction between surface and subsurface snow, rather than basing this on clearly defined snow layers?
- Same comments as above for the phraseology surrounding the data in figure S6 – there is a very large reliance here on simplistic correlations.

Section 3.3.

- Similar comments to previous sections, in that much is made of relatively weak analysis outcomes (e.g. PCoA axis 1 = 16.31% of variation, and PCoA axis 2 = 11.06%), numerous correlations are employed that whilst they have 'significant' p-values often have very low r values, and nitrogen cycling gene content relative abundance that is

inferred from 16S data rather than being measured on the samples. Given the lack of causative relationships established in these results care needs to be taken on their interpretation regarding larger scale processes.

Discussion:

Generally I find the direction of the discussion immediately and dominantly toward nitrogen dynamics to be concerning given that i) the nitrogen dataset is very modest (triplicate samples on a total of 7 days all within a single 9 day period) and ii) there remain a few concerns that I have detailed above about the validity of this dataset in terms of both analytics and sampling design. Much of the discussion text around the impact of nitrogen on the bacterial community is rather vague and is mainly conjecture. There are inappropriate references to non-snow studies (e.g. sub-glacial works) and there is no attempt to place the findings into the wider context in regard to when/where samples were taken. For example, all samples are taken over a 9-day period in relatively recently deposited snow long before any melt processes are likely to begin – how representative are these data of what is actually happening in the snowpack on timescales longer than a week or so? Or how might they contrast to a melting snowpack? How do they fit with snowpack metamorphism processes?

I believe that the first two sections as they stand could be reduced down into one short discussion paragraph on potential links between the bacterial community and nitrogen dynamics, but that the breadth of the dataset presented does not warrant the drawing of such definite conclusions on the controlling role of nitrogen within the snowpack sampled. The latter third paragraph is better and could form the basis for a new discussion of the dataset. The authors should provide a more general discussion into their datasets if this work is to be accepted for final publication. They should focus on what they can report rather than drawing grand conclusions from a relatively limited study and they should attempt to justify what insight their samples can provide given the timing and location of their sampling regime.