We thank Anonymous Referee #2 for their considered, detailed, and helpful review of our manuscript. Below we present our point-by-point responses.

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:

Question 1. 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.

Response:

We thank reviewer #2 for raising this concern. We did not intend to represent the entire ablation zone, instead, we attempted to focus on the nutrient and bacterial community dynamics immediately following a snowfall event, which will be buried following the next precipitation. Furthermore, we did not sample from the entire ablation zone to avoid the influence of spatial heterogeneity. The introduction, method, and the title were modified to represent the scope of the present in a better way.

Amended manuscript (Title):

Temporal variation of bacterial community and nutrients in Tibetan glacier snowpack

Introduction (Lines 50-63):

Several studies investigated the dynamics of nutrient and bacterial changes in supraglacial snow during the ablation period. Larose et al. (2013a) revealed that the form of nitrogen varied as a function of time in supraglacial snow during a two-month field study at the Svalbard, Norway and fluctuations in microbial community structure have been reported with the relative abundance of fungi and bacteria (such as Bacteroidetes and Proteobacteria) increased and decreased, relatively. Seasonal shifts in snowpack bacterial communities have also been reported in the mountain snow in Japan, rapid microbial growth was observed with increasing snow temperature and

meltwater content (Segawa et al., 2005). However, the results of these studies are likely the consequence of these several precipitation events due to the long period of time. During precipitation, a new snow layer forms above the previous ones, which is responsible for the stratified snowpack structure. These different snow layers have distinct physical and chemical characteristics and their age also differed substantially (Lazzaro et al., 2015). Thus, the microbial process across the aged snowpack could be complex, whereas focusing on supraglacial snow from a single snowfall event could provide unique insights into the bacterial and nutrient dynamics. Hell et al. (2013) reported bacterial community structure changes during the ablation period across five days in the high Arctic, while the bacterial and nutrient dynamics during the snow accumulation period remain elusive.

And (Lines 83-93):

Glacier melting increases the discharge of microorganisms and nutrients in meltwater into downstream aquatic ecosystems (Kohler et al., 2020), which substantially impacts the bacterial community and biogeochemical processes (Liu et al., 2021). Thus, it is crucial to understand the transformation processes of the bacterial community and nutrients in the supraglacial snow. Several studies have investigated the nutrient and bacterial community changes in supraglacial snow across the winter (Brooks et al., 1998; Liu et al., 2006), but the bacterial and nutrient dynamics of freshly fallen snow have been largely overlooked. These short temporal changes will influence the following post-depositional processes after it is buried by the next snowfall, and will ultimately determine the physicochemical properties of the stratified snow in the following year. In the present study, we investigated the bacterial community and snow physiochemical property changes in the surface and subsurface supraglacial snow during a nine-day period after a single snowfall event at the Dunde Glacier on the northeast of the Tibetan Plateau.

Question 2. 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.

Response:

Unfortunately, we do not fully agree that the three samples of the same day are technical replicates. By definition, technical replicates are repeated measurements of the same sample. The three snowpits were dug randomly within the 5 m × 3 m area, and any two snow pits were at least 30 cm apart. This sampling design is to ensure that the sample differences are not caused by heterogeneity from a large geospatial distance, while we are not sampling two adjacent areas on the same day (as the reviewer has suggested) to avoid the results observed to be caused by geospatial pattern.

Question 3. 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.

Response:

We chose a small flat area for sampling to reduce the impact of spatial variation in snowfall. The distance between the three was were mostly similar, generally between 30-50 cm apart. This is mainly due to a footpath that needs to be left between snowpits, and samples were not taken on the area where footprint was left. Given the short distance among the three snowpits and the exact distance between snowpits was not kept, we cannot include this distance in the calculation. In addition, the differences in the bacterial community and physiochemical factors among the three snowpits of the same day are most likely random. We have modified the description in the sampling design to clarify this.

Amended manuscript (Lines 105-124):

No supraglacial snow was observed on the glacier surface on the 10th of October when first arrived at the camp. Snowfall started on the 18th and ended on the 23rd of October.

Sampling was conducted over a nine-day period after the snowfall stopped on a flat 5 m × 3 m small area to reduce the impact of sample heterogeneity due to spatial variations. Snow samples were collected on the 24th, 25th, 26th, 27th, and 29th of October, and the 2nd November (which are referred as day 1, 2, 3, 4, 6, and 9) until the next snowfall started. This enabled us to follow the development of bacterial communities and the chemical environment through time after deposition. The ambient air temperature at the sampling period is averaged -8 °C (data available through the European Centre for Medium-Range Weather Forecasts, Supplementary Fig. S2), no snow melting was observed over the nine days.

On each day, three snow pits were randomly dug within the 5 m × 3 m area and any two snow pits were 30-50 cm apart. Each snow pit was approximately 30 cm deep, then the snow was further divided equally into the surface and subsurface layers (approximately 15 cm deep for each layer) to get enough snow samples to extract DNA, after Carey et al. (2016). For each snow pit, the top 1 cm in contact with the air was removed using a sterile spoon to avoid contamination, and then surface and subsurface snow were collected using a sterilized Teflon shovel into 3 L sterile sampling bags separately. Approximately 100 mL were used for physicochemical analyses, whereas the rest was used for DNA extraction. A total of 36 samples were collected. Tyvek bodysuits and latex gloves were worn during the entire sampling process to minimize the potential for contamination, and gloves were worn during all subsequent handling of samples. Samples were kept frozen during the transportation to the laboratory and stored at -20 °C until analysis.

Question 4. 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.

Response:

We thank the reviewer for this constructive suggestion. The distance between the three snowpits was mostly similar, generally between 30-50 cm apart. As the exact distance between snowpits was not kept, we cannot include the distance between the three

snowpits as a spatial factor in the analysis. Nevertheless, the differences in the bacterial community and physiochemical factors among the three snowpits of the same are most likely random. Therefore, the distance is unlikely to have a substantial impact on the diversity and community patterns, we have modified the description in sampling design to clarify this.

Amended manuscript (Lines 105-124):

No supraglacial snow was observed on the glacier surface on the 10th of October when first arrived at the camp. Snowfall started on the 18th and ended on the 23rd of October. Sampling was conducted over a nine-day period after the snowfall stopped on a flat 5 m × 3 m small area to reduce the impact of sample heterogeneity due to spatial variations. Snow samples were collected on the 24th, 25th, 26th, 27th, and 29th of October, and the 2nd November (which are referred as day 1, 2, 3, 4, 6, and 9) until the next snowfall started. This enabled us to follow the development of bacterial communities and the chemical environment through time after deposition. The ambient air temperature at the sampling period is averaged -8 ℃ (data available through the European Centre for Medium-Range Weather Forecasts, Supplementary Fig. S2), no snow melting was observed over the nine days.

On each day, three snow pits were randomly dug within the 5 m × 3 m area and any two snow pits were 30-50 cm apart. Each snow pit was approximately 30 cm deep, then the snow was further divided equally into the surface and subsurface layers (approximately 15 cm deep for each layer) to get enough snow samples to extract DNA, after Carey et al. (2016). For each snow pit, the top 1 cm in contact with the air was removed using a sterile spoon to avoid contamination, and then surface and subsurface snow were collected using a sterilized Teflon shovel into 3 L sterile sampling bags separately. Approximately 100 mL were used for physicochemical analyses, whereas the rest was used for DNA extraction. A total of 36 samples were collected. Tyvek bodysuits and latex gloves were worn during the entire sampling process to minimize the potential for contamination, and gloves were worn during all subsequent handling of samples. Samples were kept frozen during the transportation to the laboratory and stored at -20 °C until analysis.

Question 5. 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.

Response:

These snow pits were dug within the same 5*3 m square. We agree that time-series analyses would be required. Therefore, we have performed additional linear regression analyses on the changes in bacterial community diversity and geochemical properties.

Amended manuscript (Lines 107-110):

Snow samples were collected on the 24th, 25th, 26th, 27th, and 29th of October, and the 2nd November (which are referred as day 1, 2, 3, 4, 6, and 9) until the next snowfall started.

And (Lines 114-115):

On each day, three snow pits were randomly dug within the 5 m × 3 m area and any two snow pits were 30-50 cm apart.

Question 6. 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.

Response:

We thank for this comment. We have added the filtration setup and procedures into the method section.

Original manuscript:

For assessing the bacterial community composition, each of the melted snow samples (3 L) was filtered onto a 0.22 μm polycarbonate membrane (Millipore, USA) with a vacuum pump (Ntengwe 2005).

Amended manuscript

For physiochemical measurements (Lines 126-131):

The 100 mL snow sample for physicochemical analysis was melted at room temperature for 3 hours before being analysed. For dissolved organic carbon (DOC) and major ions measurements, 100 mL of snow meltwater was syringe-filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter (Macherey–Nagel) into 20-mL glass bottles. The membrane has been pre-treated with 1% HCl, deionized water rinsed, and 450 °C > 3 h combusted to remove any potential carbon and nitrogen on the membrane, and the initial 10 mL of the filtrate was discarded before collecting the sample for analysis to eliminate any residual compound on the membrane.

For bacteria filtering (Lines 142-143):

For assessing the bacterial community composition, snow samples (3 L) were melted at 4 °C overnight and filtered onto a sterile 0.22 μm polycarbonate membrane (Millipore, USA) with a vacuum pump (Ntengwe 2005).

Question 7. 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.

Response:

We totally agree with the reviewer that functional prediction based on 16S rRNA gene data is not fully reliable, despite the original publication of *Tax4Fun2* package revealing a high correlation between the function predicted and those from metagenome data. More accurate and quantitative measurements (including metagenome sequencing and qPCR) are necessary to confirm the results. Unfortunately, the concentration of the sample was quite low and they were barely enough for amplicon sequencing. The functional prediction was used to explain the nitrogen (nitrate and ammonium) changes. Instead to be a definitive explanation, the pattern of changes in these nitrogen cycling-

related genes provides a hint on the potential function changes. We have amended the manuscript so the discussion of functional prediction results was minimized, and it was used to propose an alternative route of nitrogen accumulation and consumption in the surface and subsurface snow.

Amended manuscript (Lines 364-406):

Both ammonium and nitrate concentrations increased in the surface snow (Fig. 1). The increase in ammonium is traditionally explained by biogenic emissions due to local vegetal and animal sources (Filippa et al., 2010), while the increase in nitrate has been largely attributed to atmospheric deposition (Björkman et al., 2014). Nitrogen deposition occurs at a rate of 282 kg N km^{-2} yr^{-1} in the region of our investigation (Lü and Tian, 2007), this equals 0.19 mg N for the 0.5 m × 0.5 m area sampled each day (assuming nitrogen deposition occurred evenly across the year). If further assuming the deposited nitrogen only affects the surface snow (i.e., the top 15 cm as defined in the present study), the daily nitrogen increase is estimated to be 0.084 mg N L^{-1}. This is lower than the slope of total nitrogen increase observed in the surface snow of the present study (0.21 mg N L^{-1} day^{-1}). Thus, either the atmospheric nitrogen deposition has more than doubled, or bacterial nitrogen fixation could be an alternative source of nitrogen input (Telling et al., 2011). The latter is supported by the biosynthesis of nitrogen-containing compounds by bacteria with increased dissolved organic nitrogen reported in the Antarctic surface snow (Antony et al., 2017). The contribution of bacterial nitrogen fixation is further supported by the increase in the relative abundance of Cyanobacteria and the predicted abundance of *nifH* gene in surface snow (Supplementary Fig. S6 and Fig. S12). The exact nitrogen fixation rate was not quantified in the present study, but the results suggest that microbial nitrogen fixation could be an overlooked source of nitrogen in Tibetan glacier snow, further transcriptomic and nitrogen-isotope analyses may provide further evidence on the microbial activity in nitrogen fixation.

In contrast with the surface layer, nitrogen concentrations (nitrate and ammonium) significantly decreased in the subsurface snow with time (Fig. 1). In a snow reactive nitrogen oxides (NO_y) survey in Greenland, NO_y flux was reported to exit snow in 52

out of 112 measurements, and the magnitude cannot be explained by the photolysis of nitrate alone (Dibb et al., 1998). Furthermore, the short sampling period of the present study does not allow rapid photolysis to occur (Larose et al., 2013b), therefore collectively suggesting an alternative source of NO_y emission could exist. The denitrification process could contribute to nitrogen consumption, which is evidenced by the increase of predicted genes associated with denitrification processes (*narG*; Supplementary Fig. S12) (Telling et al., 2011; Zhang et al., 2020). This is consistent with the high relative abundance of denitrification-related genes being detected in the snowpack of Spitsbergen Island of Svalbard, Norway (Larose et al., 2013a). Despite the oxygen level in the subsurface snow was not measured, the occurrence of anaerobic denitrification reactions in subsurface snow has been reported in Arctic snowpacks (Larose et al., 2013a). Furthermore, Poniecka et al. (2018) showed that cryoconite microorganisms can generate an anoxic zone 2 mm below the sediment surface within an hour. Thus, anaerobic pockets in subsurface snow at 15-30 cm deep could exist, which allows denitrification reactions to occur. Further metatranscriptomic analyses targeting the genes associated with nitrogen cycling are required to further confirm the distinct nitrogen transformation processes between the surface and subsurface layers.

Minor points:

Question 8. 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.

Response:

We appreciate the reviewer for this comment. We have rewritten the sentence to make the data and location grouped.

Original manuscript:

Nitrate and ammonium concentration increased and decreased in the surface and

subsurface snow over time, therefore indicating accumulation and consumption processes, respectively. This is also evidenced by the dominance of organisms predicted to carry nitrogen fixation and denitrification genes in the surface and subsurface layers, respectively.

Amended manuscript (Lines 21-26):

Nitrate and ammonium concentrations increased from 0.44 to 1.15 mg/L and 0.18 to 0.24 mg/L in the surface snow and decreased from 3.81 to 1.04 mg/L and 0.53 to 0.25 mg/L in the subsurface snow over time, therefore indicating accumulation and consumption processes, respectively. The nitrate concentration covaried with bacterial diversity, community structure, and the predicted nitrogen fixation and denitrification-related genes, suggesting nitrogen could mediate bacterial community changes.

Question 9. Introduction: I though the Introduction was particularly well-written and set the scene very well.

Response:

We greatly thank reviewer #2 for this encouragement.

Question 10. L71: Delete the "the" in front of "October".

Response:

The grammar mistake has been corrected as indicated by the reviewer.

Original manuscript:

Snow samples were collected from the ablation zone at Dunde glacier (38°06′N, 96°24′E, 5325 m above the sea-level), during the October and November, 2016 (Supplementary Fig S1).

Amended manuscript (Lines 102-103):

Snow samples were collected from the ablation zone at Dunde glacier (38°06′N, 96°24′E, 5325 m above the sea-level), during October and November, 2016 (Supplementary Fig. S1).

Question 11. L74: There is a mismatch between the dates and the number of dates. Day

5 (28th of October) is missing from the list of Dates and the 2nd of November ought to be day 10.

Response:

We apologize for the mistake. The mismatch mistake has been corrected.

Original manuscript:

Sampling was conducted over a nine-day period (on the 24th, 25th, 26th, 27th, and 29th of October, and the 2nd November, which are referred as day 1, 2, 3, 4, 5, 6, and 9 thereafter).

Amended manuscript (Lines 107-110):

Snow samples were collected on the 24th, 25th, 26th, 27th, and 29th of October, and the 2nd November (which are referred as day 1, 2, 3, 4, 6, and 9) until the next snowfall started.

Question 12. L75: What is the age of the snowpack? I.e. when did the snow first start accumulating in this area?

Response:

The snowpack was freshly formed. The surface of the glacier was icy when first arrived. Snowfall started on the 18th of October and stopped on the 23rd of October. Sampling started on the 24th of October. We have amended the method to reflect this.

Amended manuscript (method section) (Lines 105-113):

No supraglacial snow was observed on the glacier surface on the 10th of October when first arrived at the camp. Snowfall started on the 18th and ended on the 23rd of October. Sampling was conducted over a nine-day period after the snowfall stopped on a flat 5 m × 3 m small area to reduce the impact of sample heterogeneity due to spatial variations. Snow samples were collected on the 24th, 25th, 26th, 27th, and 29th of October, and the 2nd November (which are referred as day 1, 2, 3, 4, 6, and 9) until the next snowfall started. This enabled us to follow the development of bacterial communities and the chemical environment through time after deposition. The ambient air temperature at the sampling period is averaged -8 °C (data available through the European Centre for Medium-Range Weather Forecasts, Supplementary Fig. S2), no

snow melting was observed over the nine days.

Question 13. 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?

Response:

The 0.45 μm polytetrafluoroethylene (PTFE) membrane filter was treated with 1% HCl, deionized water rinsed, and then incubated at 450 °C for 3 hours to eliminate carbon or nitrogen contamination from the filters. The first 10 ml of the filtrate was discarded before collecting the sample for analysis. We added the following sentences in the method part.

Amended manuscript (Lines 126-131):

The 100 mL snow sample for physicochemical analysis was melted at room temperature for 3 hours before being analysed. For dissolved organic carbon (DOC) and major ions measurements, 100 mL of snow meltwater was syringe-filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter (Macherey–Nagel) into 20-mL glass bottles. The membrane has been pre-treated with 1% HCl, deionized water rinsed, and 450 °C > 3 h combusted to remove any potential carbon and nitrogen on the membrane, and the initial 10 mL of the filtrate was discarded before collecting the sample for analysis to eliminate any residual compound on the membrane.

Question 14. 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.

Response:

We appreciate the reviewer for this suggestion. We have rewritten the "Statistical analysis" part as below.

Amended manuscript (Lines 189-213):

Shannon-Wiener and Chao1 indices, which were used to estimate the species richness

in the snow community, were calculated using the "*diversity*" function in the R package "*vegan*" (Oksanen et al., 2010). Functional profiling of bacterial taxa was carried out using the package "*Tax4Fun2*" in R (Wemheuer et al., 2020). While the application of functional profiles predicted from 16S rRNA gene-based community composition data is limited by the functional information available in databases, we present these data as one possible interpretation of the patterns we detected, and note that the "*Tax4Fun2*" package performed well compared to older widely used programs (Wemheuer et al., 2020). The pairwise Wilcoxon rank-sum test was used to compare the depth-horizon differences in environmental variables, alpha-diversity, and the relative abundance of taxonomic groups at the phylum level. Linear regression modelling was implemented in R using the "*lm*" function to estimate the trend of changes over time. The bacterial community structure was subjected to principal coordinate analysis (PCoA) carried out using the "*pcoa*" function of the "*ape*" package in R. The significance of dissimilarity of community composition among samples was tested using permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distance metrics with the "*adonis*" function in the R package "*vegan*" (Oksanen et al., 2010). Test results with $P < 0.05$ were considered statistically significant. Mantel test based on Spearman's rank correlations was performed using the bacterial dissimilarity and environmental dissimilarity matrix, calculated based on the Bray-Curtis distance metrics and Euclidean distance metrics in the "*vegan*" R package, respectively. The normalized stochasticity ratio (NST) based on the Bray–Curtis dissimilarity was calculated using the "*NST*" package in R to estimate the determinacy and stochasticity of the bacterial assembly processes with high accuracy and precision (Ning et al., 2019). The NST index used 50% as the boundary point between more deterministic (<50%) and more stochastic (>50%) assembly processes. All environmental variables were normalized before the calculation. All statistical analyses were executed in R version 3.4.3 (R Core Team, 2017).

Question 15. 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.

Response:

We apologize for not stating this clear enough. The non-significant difference was for the community structure at the phylum level (line 195-197), whereas the significant difference was observed at the ASV level (line 203-204). We have rephrased the sentence to clarify this.

Amended manuscript (Lines 274-276):

The bacterial community structure at the ASV level significantly differed in the surface and subsurface snow (PERMANOVA, $F = 2.78$, $P < 0.001$, Fig. 5a), as well as among the different sampling times (PERMANOVA, $F = 3.31$, $P < 0.001$ and $F = 2.17$, $P < 0.001$, respectively).

Question 16. 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?

Response:

We totally agree with the reviewer on this. The increase in relative abundance could be either due to the increased Cyanobacteria abundance or the reduction of non-Cyanobacteria bacteria. However, this is one of the drawbacks of amplicon sequencing that the absolute number reads depends not only on the abundance of organisms but also on the depth of sequencing. Nevertheless, we plotted the ASV number of Cyanobacteria and Chloroflexi, which also increased in the subsurface layer over time.

Original manuscript:

In the surface layer, the relative abundances of Alphaproteobacteria, Gammaproteobacteria, and Firmicutes significantly decreased with time (Supplementary Fig S6), while those of Cyanobacteria and Deinococcus-Thermus significantly increased (all $P < 0.05$). In the subsurface layer, the relative abundance of

Alphaproteobacteria and Firmicutes significantly decreased with time, while Cyanobacteria and Chloroflexi significantly increased (all $P < 0.05$).

Amended manuscript (Lines 243-256):

In the surface layer, negative associations were apparent in the relative abundances and ASV number of Alphaproteobacteria, Gammaproteobacteria, and Firmicutes with time ($F_{1,16} = 6.97$, $P = 0.018$, $R^2 = 0.30$; $F_{1,16} = 23.8$, $P < 0.001$, $R^2 = 0.60$, and $F_{1,16} = 22.28$, $P < 0.001$, $R^2 = 0.58$ in relative abundance; $F_{1,16} = 7.56$, $P = 0.014$, $R^2 = 0.32$; $F_{1,16} = 27.12$, $P < 0.001$, $R^2 = 0.63$, and $F_{1,16} = 16.68$, $P = 0.001$, $R^2 = 0.51$ in ASV number, respectively), while positive associations were apparent in the relative abundances and ASV number of Cyanobacteria and Deinococcus-Thermus with time ($F_{1,16} = 6.94$, $P = 0.018$, $R^2 = 0.30$ and $F_{1,16} = 13.10$, $P = 0.002$, $R^2 = 0.45$ in relative abundance; $F_{1,16} = 3.42$, $P = 0.083$, $R^2 = 0.18$ and $F_{1,16} = 4.07$, $P = 0.061$, $R^2 = 0.20$ in ASV number, respectively; Supplementary Fig. S6). In the subsurface layer, negative associations were apparent in the relative abundance and ASV number of Alphaproteobacteria and Firmicutes with time ($F_{1,16} = 15.17$, $P = 0.001$, $R^2 = 0.49$ and $F_{1,16} = 15.43$, $P = 0.001$, $R^2 = 0.49$ in relative abundance; $F_{1,16} = 18.98$, $P = 0.083$, $R^2 = 0.54$ and $F_{1,16} = 15.17$, $P = 0.001$, $R^2 = 0.53$ in ASV number, respectively, Supplementary Fig. S7), while positive associations were apparent in the relative abundance and ASV number of Cyanobacteria and Chloroflexi with time ($F_{1,16} = 5.62$, $P = 0.031$, $R^2 = 0.26$ and $F_{1,16} = 12.81$, $P = 0.003$, $R^2 = 0.44$ in relative abundance; $F_{1,16} = 5.34$, $P = 0.034$, $R^2 = 0.25$ and $F_{1,16} = 14.49$, $P = 0.002$, $R^2 = 0.47$ in ASV number, respectively).

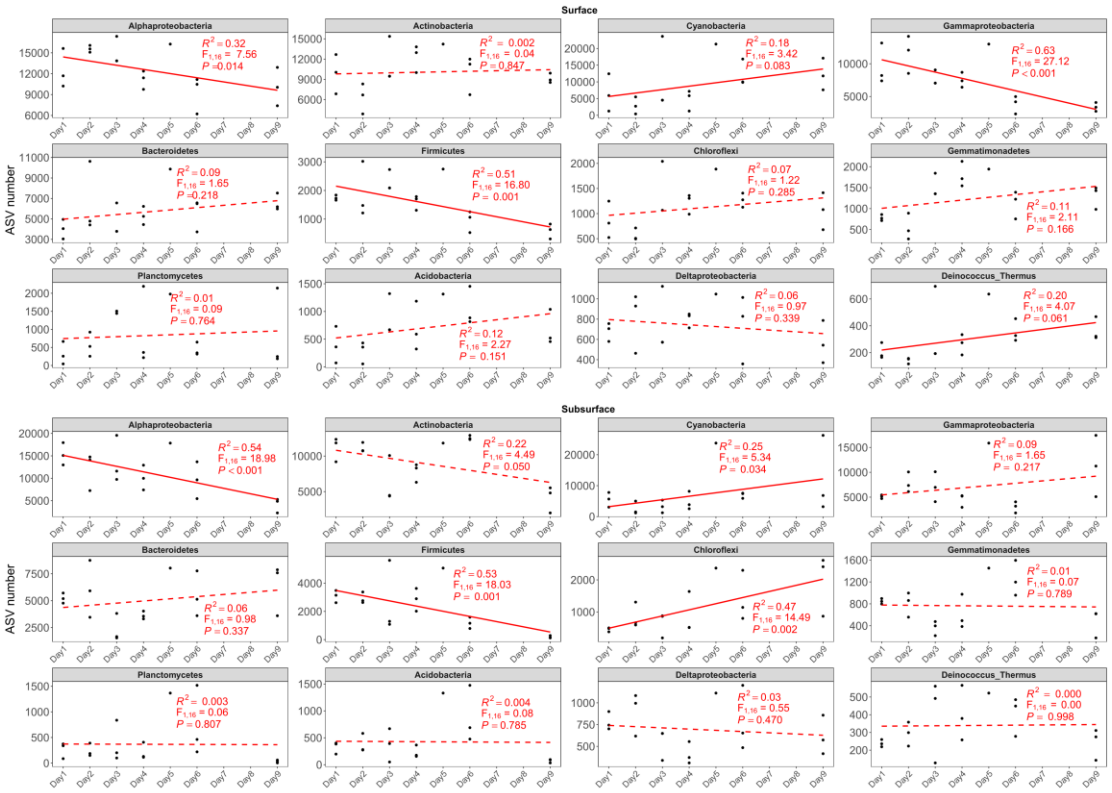


Fig S7 Temporal changes of the ASV number of dominant bacterial phyla in the surface and subsurface snow. Each dot represents an individual sample. The solid and dashed lines indicate significant and nonsignificant changes, respectively. Significance is based on linear regression.

Question 17. L206-210: It would be useful to see how the environmental factors correlate with these axes.

Response:

We performed linear regression for the environmental factors against the PCoA axes. The result showed that the PCOA1 did not correlate with any environmental factors both in the surface and subsurface layers. However, the PCOA2 showed a significant correlation with all environmental factors except Na^+ in the subsurface layer. We have added this result to the manuscript.

Amended manuscript (Lines 278-284):

Specifically, only the second principal coordinate (PCoA2) values of the surface snow significantly varied with time ($F_{1,16}$ = 141.8, P < 0.001, R^2 = 0.89, Fig. 5b), while the PCoA1 values of the surface snow did not. Furthermore, PCoA1 and PCoA2 of the surface snow exhibited no significant correlation with the measured environmental

factors (Supplementary Fig. S9 and S10). In comparison, both PCoA1 and PCoA2 values of the subsurface snow co-varied with time ($F_{1,16}$ = 6.35, P = 0.023, R^2 = 0.28 and $F_{1,16}$ = 8.38, P = 0.011, R^2 = 0.34, respectively, Fig. 5b), while the PCoA2 also demonstrated significant association with nitrate, ammonium, potassium, sulfate, and DOC concentrations (Supplementary Fig. S10).

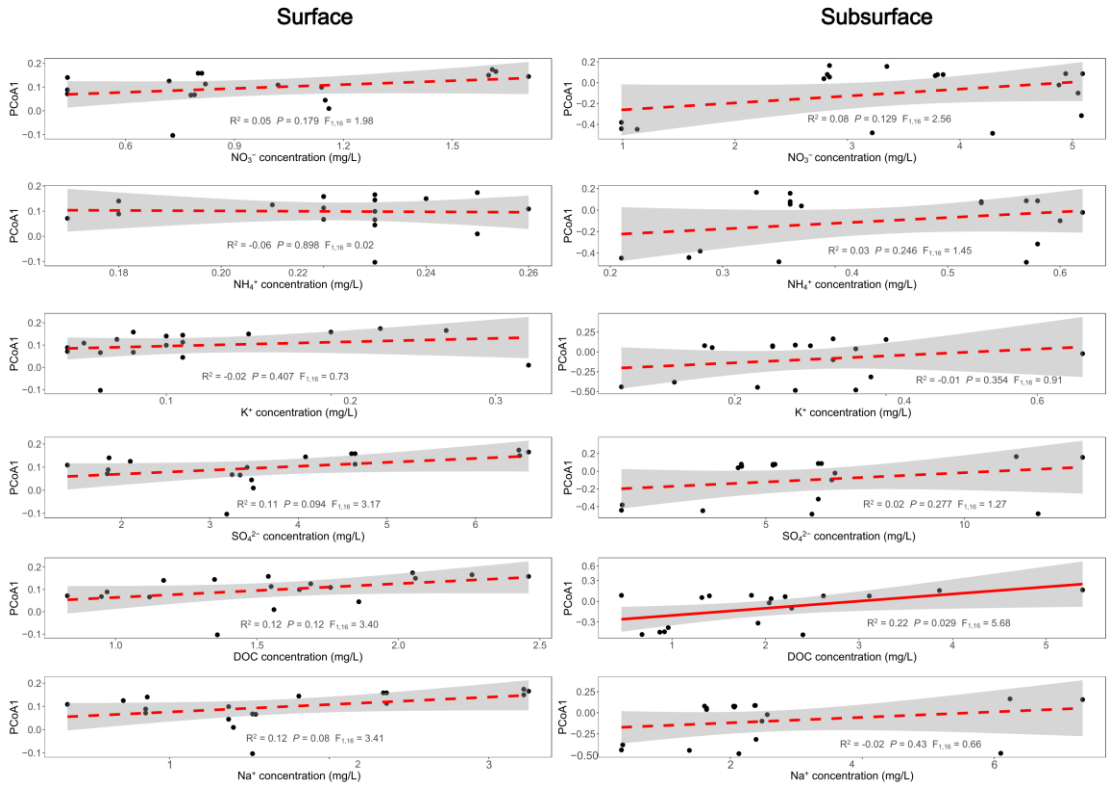


Fig S9: Pairwise regression analyses between PCoA1 scores and environmental factors. The solid and dashed lines indicate significant and nonsignificant changes (based on linear regression at $P <$ 0.05), respectively. PCoA1 exhibits no significant relationship with the measured environmental factors in the surface snow, while in the subsurface layer, the PCoA1 is significantly associated with DOC concentrations.

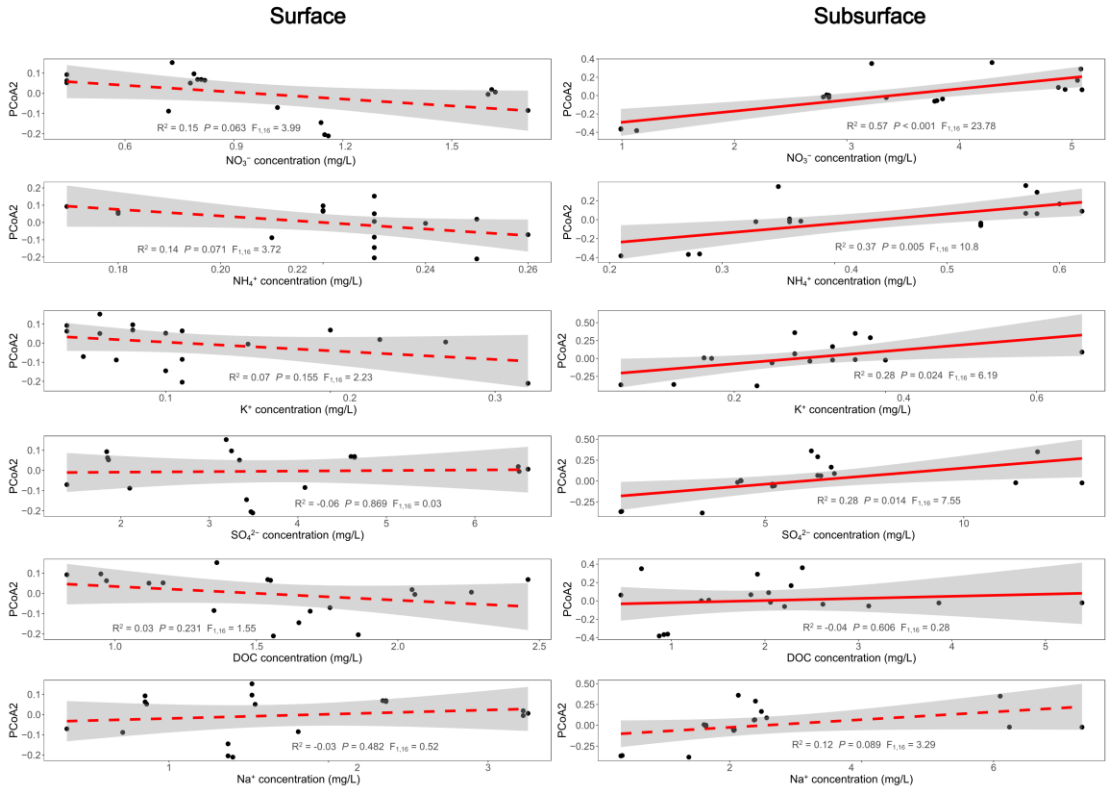


Fig S10 Pairwise regression analyses between PCoA2 scores and environmental factors. The solid and dashed lines indicate significant and nonsignificant changes (based on linear regression at $P < 0.05$), respectively. PCoA2 exhibits no significant relationship with the measured environmental factors in the surface layer, while in the subsurface layer, the PCoA2 is significantly associated with nitrate, ammonium, potassium, sulfate, and DOC concentrations.

Question 18. 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?

Response:

We appreciate the reviewer for this constructive suggestion. We have calculated the theoretical input of nitrogen for our samples, and this information was used to demonstrate the existence of a nitrogen input gap in the present study. We then proposed nitrogen fixation could be responsible.

Amended manuscript (Lines 368-386):

Nitrogen deposition occurs at a rate of 282 kg N km^{-2} yr^{-1} in the region of our investigation (Lü and Tian, 2007), this equals 0.19 mg N for the 0.5 m × 0.5 m area sampled each day (assuming nitrogen deposition occurred evenly across the year). If further assuming the deposited nitrogen only affects the surface snow (i.e., the top 15 cm as defined in the present study), the daily nitrogen increase is estimated to be 0.084 mg N L^{-1}. This is lower than the slope of total nitrogen increase observed in the surface snow of the present study (0.21 mg N L^{-1} day^{-1}). Thus, either the atmospheric nitrogen deposition has more than doubled, or bacterial nitrogen fixation could be an alternative source of nitrogen input (Telling et al., 2011). The latter is supported by the biosynthesis of nitrogen-containing compounds by bacteria with increased dissolved organic nitrogen reported in the Antarctic surface snow (Antony et al., 2017). The contribution of bacterial nitrogen fixation is further supported by the increase in the relative abundance of Cyanobacteria and the predicted abundance of *nifH* gene in surface snow (Supplementary Fig. S6 and Fig. S12). The exact nitrogen fixation rate was not quantified in the present study, but the results suggest that microbial nitrogen fixation could be an overlooked source of nitrogen in Tibetan glacier snow, further transcriptomic and nitrogen-isotope analyses may provide further evidence on the microbial activity in nitrogen fixation.

Question 19. 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?

Response:

We appreciate the reviewer for this constructive suggestion. We have added a brief discussion in the conclusion section on the impact of enhanced nitrogen deposition on the subglacial ecosystem and downstream ecosystems.

Amended manuscript (Lines 458-466):

Due to atmospheric nitrogen deposition and bacterial nitrogen fixation activities, nitrogen limitation is unlikely to occur in the surface snow, thus additional nitrogen deposition due to global climate change is unlikely to substantially impact the bacterial community in surface snow. In contrast, nitrogen consumption was inferred in the subsurface snow. Nitrogen is traditionally recognized to be released from supraglacial environmental due to photolysis, whereas the present study hints that bacterial denitrification process could be an alternative route. Therefore, the increased nitrogen deposition due to anthropogenic activities may enhance the denitrification process in the subsurface snow. The enhanced nitrogen emission could reduce the impact of increased nitrogen deposition on downstream glacier-fed rivers, but may feedback global warming positively.

Question 20. 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?

Response:

We appreciate the reviewer for this comment. We have added relevant references to the manuscript to evidence that denitrification could occur in the subsurface snow (i.e., 15-30 cm). We have also added linear regression analysis to support the temporal pattern of the bacterial diversity and environmental factors changes across the nine days.

Amended manuscript (Lines 397-406):

Despite the oxygen level in the subsurface snow was not measured, the occurrence of anaerobic denitrification reactions in subsurface snow has been reported in Arctic snowpacks (Larose et al., 2013a). Furthermore, Poniecka et al. (2018) showed that cryoconite microorganisms can generate an anoxic zone 2 mm below the sediment surface within an hour. Thus, anaerobic pockets in subsurface snow at 15-30 cm deep

could exist, which allows denitrification reactions to occur. Further metatranscriptomic analyses targeting the genes associated with nitrogen cycling are required to further confirm the distinct nitrogen transformation processes between the surface and subsurface layers.

Question 21. 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?

Response:

We apologize for the missing labels. We revised the figure as below. The large community variation in Days 3 and 6 could be due to the larger variation of nitrate in the snow sampled. This is likely to be random, such as a large chunk of dust could be in one of the samples collected on these days.

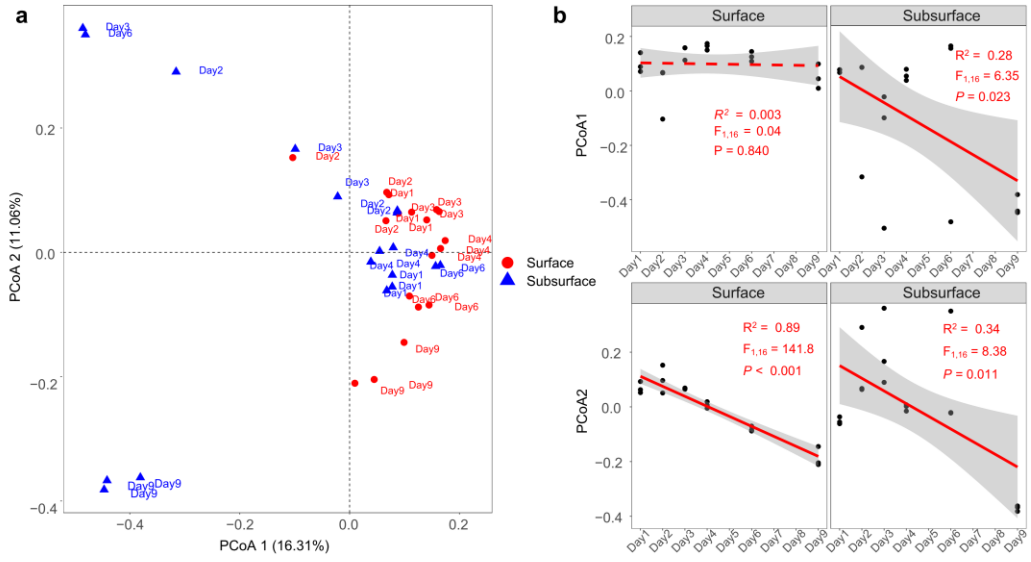
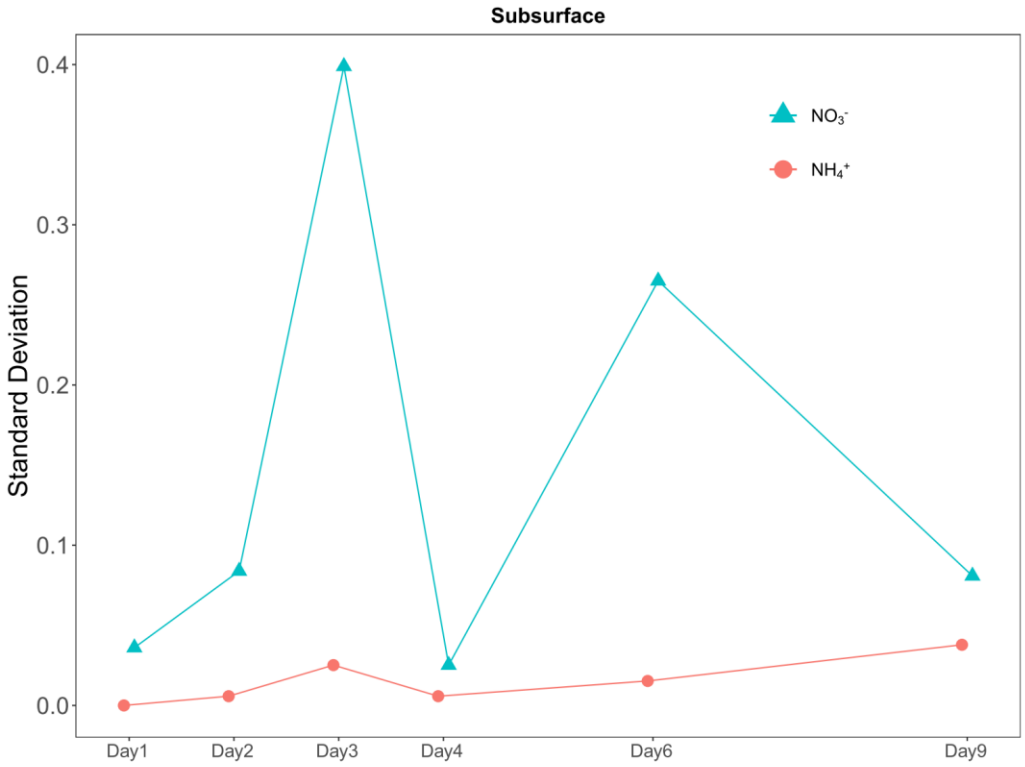


Fig. 5 Principal coordinate analysis (PCoA) of microbial communities in the surface and subsurface snow.

(a) Bray-Curtis distance-based PCoA ordination plot. The microbial community structures of the surface and subsurface snows are significantly different (PERMANOVA, $P < 0.001$). (b) Pairwise regression analysis between PCoA scores and sampling time. The solid and dashed lines indicate significant and insignificant changes (based on linear regression), respectively. The PCoA1

scores for the bacterial community in surface layer exhibit no significant correlation with time, while the PCoA2 scores significantly correlated with time. The PCoA1 and PCoA2 are both significantly correlated with time in the subsurface layer.



Question 22. 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.

Response:

We are sorry for the color usage. We have used a better colour scheme to enhance visibility.

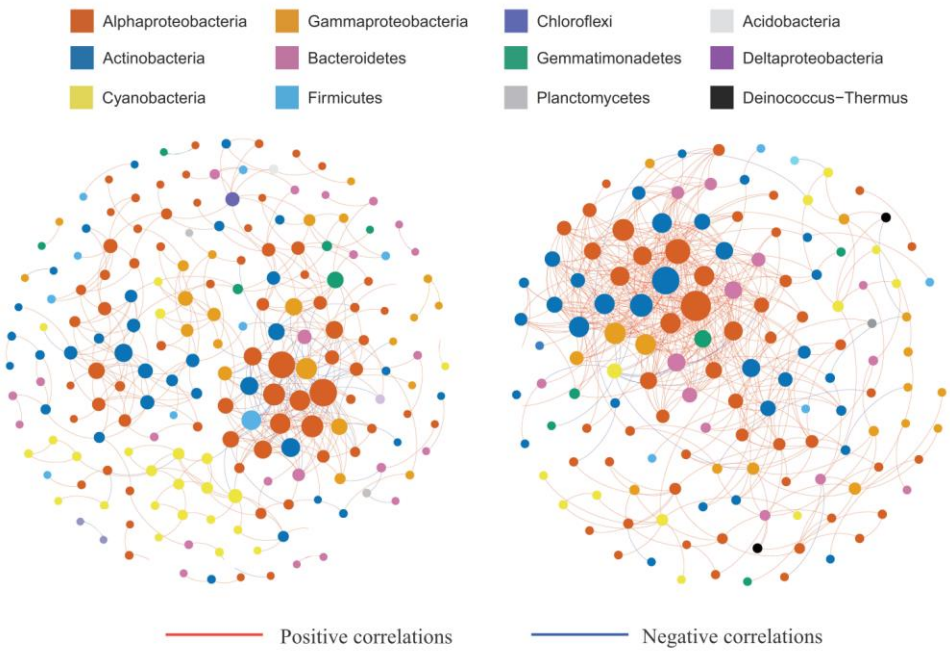


Figure 6: Bacterial Co-occurrence networks for the surface and subsurface layers communities. Each node represents a bacterial amplicon sequence variant (ASV). The red solid lines represent positive correlations, and the blue solid lines represent negative correlations. Nodes are colored by taxonomy at the phylum level. The subsurface community networks are more complex with a higher positive-to-total correlation ratio.