Anonymous Referee #1

The authors have made some good revisions to the paper. I think there are still some that require change before accepting for publication as detailed below.

Question 1: Overall, the English needs a quick check throughout - there are numerous errors that should be caught quite easily.

Response:

We thank for this comment. We did a throughout check on the manuscript.

Intro:

Question 2: I can buy the new rationale of examining processes in recently deposited snow. This is stronger than the previous manuscript. I still don't like reference to only cyanobacteria dominating surface snow. Though you have chosen to only look at bacteria, many eukaryotes are more likely to dominate surface snow. Whilst you have catalogued bacterial diversity here relative to snow chemistry, what if there are eukaryotes present using up/producing the chemical species you are measuring?

Response:

We thank for the comment. We tried to clarify this better in the revised version. We did not intend to mean that Cyanobacteria are the dominant microbial group in the surface snow, but Cyanobacteria are more abundant in the surface snow than in subsurface snow. Thus, we have amended the introduction to clarify this and included other taxa that are more abundant in the surface than in the subsurface snow. We have also included the potential impact of algae on nitrogen assimilation in the discussion (algae cannot perform nitrogen fixation).

Introduction part:

Original manuscript:

Surface and subsurface typically have distinct bacterial community structures due to the environmental filtering from the vertical profile of temperature, solar radiation intensity, and nutrients (Xiang et al., 2009; Møller et al., 2013; Carey et al., 2016). For example, Cyanobacteria tend to dominate upper snow layers (0-15 cm) (Carey et al., 2016), while their relative abundance is greatly reduced in the deeper snow layer (Xiang et al., 2009). This is likely due to the lower light intensity in the deeper snow, which favors heterotrophic bacteria such as the Actinobacteria and Firmicutes.

Amended manuscript (Lines 55-65):

Surface and subsurface snow typically harbour distinct bacterial community structures (Xiang et al., 2009; Møller et al., 2013; Carey et al., 2016). For example, algae (chloroplasts), Proteobacteria, Bacteroidetes, and Cyanobacteria were more abundant in surface snow, while Firmicutes and Fusobacteria were more abundant in the deeper snow layer (Møller et al., 2013). A previous study had proposed that nitrogen availability could also be a driver of microbial community structure and function in snow (Larose et al., 2013b), where the NO_3^- and NH_4^+ concentrations drove the community composition in Ny-Ålesund snowpack. A dissolved inorganic nitrogen addition experiment also showed a clear community response with the bacterial abundance elevated and genera richness declined in the final time point compared to the initial time point, suggesting potential specialization of heterotrophic communities (Holland et al., 2020).

Discussion part:

Amended manuscript (Lines 364-368):

Alternatively, microorganisms may carry out assimilatory nitrate reduction, which is used to incorporate nitrogen into biomolecules (Larose et al., 2013a; Richardson and Watmough, 1999). The assimilatory process is performed by a range of microorganisms including bacteria, algae, yeasts, and fungi (Huth and Liebs, 1988). Thus, further studies on eukaryotes, including algae, may provide a full understanding of the nitrogen consumption mechanisms in subsurface snow.

Methods:

Question 3: The authors have now removed their TN data, but did not really provide an explanation as to why it was so contradictory to their inorganic nutrient species datasets, i.e. are we happy that the inorganic datasets are reliable, whilst apparently the total nitrogen datasets were not?

Response:

We appreciate the reviewer for raising this concern. We have carefully examined standard methods for TN determination and proposed the following possible explanations for the underestimation of TN:

1. Total nitrogen, nitrate, and ammonium were from independent measurements using different methods. The lower total nitrogen than the sum of nitrate and ammonium could be due to the accumulation of measurement variance. i.e., the measurement variance of TN, nitrate, and ammonium (dissolved inorganic nitrogen, DIN) exceeds the concentration of organic nitrogen. Lee et al. (2005) gave several examples that total dissolved nitrogen could be less than dissolved inorganic nitrogen. A similar conclusion was reported by Sharp et al. (2002) that measurement variance greatly impacts the dissolved organic nitrogen measurement (i.e., the difference between TN and DIN). This is particularly vital for low biomass ecosystems such as surface waters and drinking waters.
2. The accuracy of measurement may depend on the standards (organic and different forms of inorganic compounds, such as ammonia and nitrate) (Pathak et al., 2015). In the present study, nitrate was used as the reference to calculate total nitrogen, which may underestimate ammonium and organic nitrogen fractions.
3. The range of the standard curve for total nitrogen quantification was much smaller than the total nitrogen value obtained, and this is most likely to cause the underestimation of total nitrogen and greatly increase the measurement variance.

Based on these reasons, we have decided to remove the total nitrogen results from the manuscript.

Lee, W., Westerhoff, P.J.E.S., and Technology (2005). Dissolved organic nitrogen measurement using dialysis pretreatment. 39(3), 879-884.

Sharp, J.H., Rinker, K.R., Savidge, K.B., Abell, J., Benaim, J.Y., Bronk, D., et al. (2002). A preliminary methods comparison for measurement of dissolved organic nitrogen in seawater. 78(4), 171-184.

Pathak, B., Al-Omari, A., Wadhawan, T., Higgins, M., and Murthy, S.J.P.o.t.W.E.F. (2015). Analytical errors in the measurement of dissolved organic nitrogen in wastewater effluent. Proceedings of the Water Environment Federation 2015(10), 4214-4225.

Question 4: No details on random forest analysis that is now implemented, ie. On what data, and to what end?

Response:

We have replaced the random forest analysis with multiple linear regression, please see response below in Q6.

Results:

Question 5: Whilst the authors have now switched from correlations to mainly regressions, they are still interpreting the outputs incorrectly in my opinion, i.e. line ~225 'The concentration of NO3 and NH4 … increased with time' quotes R2 values of 0.27 and 0.35, respectively. Thus ~ 70% of the variability in NO3 and NH4 was NOT related to the time of sampling….I would not interpret this as a strong indication that NO3 and NH4 increased with time (a quick look at the associated plots supports my conclusions here). The trends are stronger in the subsurface snow but similar issues are found throughout the rest of the results section e.g. info on bacterial community composition through time. I would not recommend publication until this has been amended. Just because you get a 'significant' p-value on a regression does not mean the relationship is 'significant' or even important. You need to look at e.g. R2 values and think about the effect size.

Response:

We thank the reviewer for this constructive comment. We have revised the interpretation of the low R^2 results in the Results and Discussion sections and clarify that the associations, although significant, were weak.

Original manuscript:

The concentration of NO_3^- and NH_4^+ ions in the surface snow increased with time ($F_{1,16} = 5.97$, $P = 0.027$, $R^2 = 0.27$ and $F_{1,16} = 8.58$, $P = 0.010$, $R^2 = 0.35$, respectively, Fig. 1b). In comparison, they decreased with time in the subsurface snow ($F_{1,16} = 40.66$, $P < 0.001$, $R^2 = 0.72$ and $F_{1,16} = 50.74$, $P < 0.001$, $R^2 = 0.76$, respectively).

Amended manuscript (Lines 203-207):

The concentration of NO_3^- and NH_4^+ ions in the surface snow exhibited a weak, but significantly positive association with time ($F_{1,16} = 5.97$, $P = 0.027$, $R^2 = 0.27$ and $F_{1,16} = 8.58$, $P = 0.010$, $R^2 = 0.35$, respectively, Fig. 1b). On the other hand, stronger negative associations were found between inorganic nitrogen and time in the subsurface snow ($F_{1,16} = 40.66$, $P < 0.001$, $R^2 = 0.72$ and $F_{1,16} = 50.74$, $P < 0.001$, $R^2 = 0.76$, respectively).

Original manuscript:

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).

Amended manuscript (Lines 214-228):

In the surface layer, weak, but significant negative trends were observed between the relative abundances and ASV number of Alphaproteobacteria, Gammaproteobacteria and Firmicutes, and 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 weak positive correlations were observed between the relative abundances and ASV number of Cyanobacteria and Deinococcus-Thermus, and 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). Relative to the surface snow, the subsurface layer had stronger negative correlation between the relative abundance and ASV number of Alphaproteobacteria and Firmicutes, and 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 weak correlations were observed between the relative abundance and ASV number of Cyanobacteria and Chloroflexi, and 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).

Original manuscript:

In the surface snow, the Shannon and Chao1 indices were similar across the nine days

($F_{1,16} = 0.37$, $P = 0.553$, $R^2 = 0.02$ and $F_{1,16} = 0.01$, $P = 0.939$, $R^2 = 0.001$, respectively; Fig. 3b). In comparison, negative associations were observed in both Shannon and Chao1 indices with time in the subsurface snow ($F_{1,16} = 12.33$, $P = 0.003$, $R^2 = 0.44$ and $F_{1,16} = 8.73$, $P = 0.009$, $R^2 = 0.35$, respectively). In the surface layer, the positive correlations of Shannon and Chao1 indices with the DOC and sodium ions were apparent ($F_{1,16} = 4.90$, $P = 0.042$, $R^2 = 0.23$ and $F_{1,16} = 4.91$, $P = 0.042$, $R^2 = 0.24$, respectively; Fig. 4a,b). In the subsurface snow, the positive correlations of Shannon and Chao1 indices with the concentrations of NO_3^- and NH_4^+ were apparent (Shannon diversity: $F_{1,16} = 9.13$, $P = 0.008$, $R^2 = 0.36$ and $F_{1,16} = 5.17$, $P = 0.037$, $R^2 = 0.24$, respectively; Chao1 index: $F_{1,16} = 8.60$, $P = 0.009$, $R^2 = 0.36$ and $F_{1,16} = 5.32$, $P = 0.035$, $R^2 = 0.25$, respectively; Fig. 4cd).

Amended manuscript (Lines 231-241):

In the surface snow, the Shannon and Chao1 indices were similar across the nine days ($F_{1,16} = 0.37$, $P = 0.553$, $R^2 = 0.02$ and $F_{1,16} = 0.01$, $P = 0.939$, $R^2 = 0.001$, respectively; Fig. 3b). Beside, weak positive associations of Shannon and Chao1 indices with the DOC and sodium ions were detected ($F_{1,16} = 4.90$, $P = 0.042$, $R^2 = 0.23$ and $F_{1,16} = 4.91$, $P = 0.042$, $R^2 = 0.24$, respectively; Fig. 4a,b). In contrast, although weak, significant negative correlations were observed in both Shannon and Chao1 indices with time in the subsurface snow ($F_{1,16} = 12.33$, $P = 0.003$, $R^2 = 0.44$ and $F_{1,16} = 8.73$, $P = 0.009$, $R^2 = 0.35$, respectively). Weak, but significant positive associations of Shannon and Chao1 indices with the concentrations of NO_3^- and NH_4^+ were detected (Shannon diversity: $F_{1,16} = 9.13$, $P = 0.008$, $R^2 = 0.36$ and $F_{1,16} = 5.17$, $P = 0.037$, $R^2 = 0.24$, respectively; Chao1 index: $F_{1,16} = 8.60$, $P = 0.009$, $R^2 = 0.36$ and $F_{1,16} = 5.32$, $P = 0.035$, $R^2 = 0.25$, respectively; Fig. 4cd).

Original manuscript:

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).

Amended manuscript (Lines 249-255):

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 ($F_{1,16} = 0.04$, $P = 0.840$, $R^2 = 0.003$, Fig. 5b). Furthermore, PCoA1 and PCoA2 of the surface snow exhibited no significant correlation with the measured environmental factors (all $P > 0.05$, Supplementary Fig. S8 and S9). In comparison, both PCoA1 and PCoA2 values of the subsurface, albeit weakly, 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 (all $P < 0.05$, Supplementary Fig. S9).

Question 6: Why has random forest analysis been applied here rather than suggested multiple linear regression? Perhaps because MLR does not give good predictive capability on the datasets? This does not seem to provide an explainable test of the relative importance of different factors in reproducing the data. No metrics on the goodness of fit of the RF model are provided in the text.

Response:

We appreciate the reviewer for raising this comment. We have performed multiple linear regression to determine the contribution and significance of the environmental characteristics to the alpha diversity. The stepwise AIC method was also performed to select the best model. This result is consistent with the linear regression results, which identified the concentration of nitrate and ammonium as the significant determinants of bacterial diversity in the subsurface snow layer. We have added this result in the Result section and supplementary as Table S2.

Original manuscript:

This is consistent with the random forest analysis results, which identified the concentrations of NO_3^- and NH_4^+ as the significant determinants of bacterial Shannon diversity in the subsurface layer (Supplementary Fig. S8).

Amended manuscript (Lines 241-243):

This is consistent with the multiple linear regression results, which consistently identified the concentrations of NO_3^- and NH_4^+ as the significant determinants of bacterial Shannon diversity in the subsurface layer (Supplementary Table S2).

Table S2 Results of multiple linear regression using Akaike's information criterion (AIC), correlating community alpha diversity with environmental variables. Only significant variables were displayed. Best models are in bold.

	Diversity index	Formula	AIC	R^2	*P*	Explanatory variables
Surface	Shannon	Shannon ~ NO_3^- + NH_4^+ + K^+ + SO_4^{2-} + DOC + Na^+	-34.69	0.28	*0.14*	NH_4^+ (-2.36)*
		Shannon ~ NH_4^+ + K^+ + SO_4^{2-} + DOC + Na^+	-36.58	0.33	*0.07*	NH_4^+ (-2.46)*
		Shannon ~ NH_4^+ + SO_4^{2-} + DOC + Na^+	-37.53	0.35	*0.05*	NH_4^+ (-2.43)*
		Shannon ~ NH_4^+ + SO_4^{2-} + DOC	-38.18	0.35	*0.03*	NH_4^+ (-2.28)*
		Shannon ~ NH_4^+ + DOC	**-38.44**	**0.33**	***0.02***	**DOC (3.20)****
	Chao1	Chao1 ~ NO_3^- + NH_4^+ + K^+ + SO_4^{2-} + DOC + Na^+	194.21	0.07	*0.36*	
		Chao1 ~ NO_3^- + NH_4^+ + K^+ + SO_4^{2-} + DOC	192.22	0.15	*0.23*	

		Chao1 ~ NO_3^- + NH_4^+ + SO_4^{2-} + DOC	190.59	0.20	*0.15*	
		Chao1 ~ NO_3^- + SO_4^{2-} + DOC	189.54	0.22	*0.1*	
		Chao1 ~ NO_3^- + SO_4^{2-}	**188.72**	**0.22**	***0.06***	**SO_4^2 (2,54)***
Subsurface	Shannon	Shannon ~ NO_3^- + NH_4^+ + K^+ + SO_4^{2-} + DOC + Na^+	-25.59	0.61	*0.008*	NO_3^- (3.79)**, NH_4^+ (-2.54)*
		Shannon ~ NO_3^- + NH_4^+ + SO_4^{2-} + DOC + Na^+	**-26.91**	**0.63**	***0.003***	**NO_3^- (3.98)**, NH_4^+ (-2.76)*, SO_4^2 (-2.20)***
	Chao1	Chao1 ~ NO_3^- + NH_4^+ + K^+ + SO_4^{2-} + DOC + Na^+	183.77	0.73	*0.001*	NO_3^- (5.02)***, SO_4^{2-} (-4.52)***, Na^+ (4.34)**
		Chao1 ~ NO_3^- + NH_4^+ + K^+ + SO_4^{2-} + Na^+	181.77	0.76	*<0.001*	NO_3^- (5.25)***, NH_4^+ (-2.41)*, SO_4^2 (-5.20)***, Na^+ (5.22)***
		Chao1 ~ NO_3^- + NH_4^+ + SO_4^{2-} + Na^+	**181.25**	**0.75**	***<0.001***	**NO_3^- (5.40)***, NH_4^+ (-2,67)*, SO_4^2 (-5.48)***, Na^+ (5.40)*****

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Discussion:

Question 7: The same caveats as listed for presentation of the results section apply to the degree of interpretation apparent in the discussion section.

Response:

We thank for the suggestion. We have changed the interpretation of nitrogen changes in the surface snow as unchanged or slightly increased, which indicates the microbiome is not subjected to nitrogen-limitation, rather than an accumulation process.

We amended the abstract, discussion, and conclusion.

Abstract part:

Amended manuscript (Lines 20-22):

Therefore, we suggest that the surface snow is not nitrogen-limited, while the subsurface snow is associated with nitrogen consumption processes and nitrogen limited.

Discussion part:

Amended manuscript:

Lines 292-294:

Bacterial richness and diversity exhibited little change throughout the nine days in the surface snow layer, while they exhibited a reduction trend in the subsurface snow layer (Fig. 3b).

Lines 306-308:

In comparison, the surface layer is unlikely to be subjected to nitrogen-limitation and the nitrogen in the surface snow slightly increased.

Lines 311-313:

The bacterial community structure also exhibited temporal changes in the subsurface layer. Furthermore, associations between nitrogen and the microbial community structure were observed to a certain degree (Table 1 and Fig. 5), again indicating some level of environmental filtering (Kim et al., 2016).

Lines 322-324:

Our results suggest that both bacteria and snow physiochemical properties experience changes across the nine days during the snow deposition period in the Tibetan glacier investigated here, and those changes were more stronger in the subsurface layer than in the surface layer.

Lines 335-337:

Both ammonium and nitrate concentrations showed a weak increasing trend in the surface snow (Fig. 1). The weak increase in ammonium could be explained by biogenic emissions due to local plant and animal sources (Filippa et al., 2010)

Conclusion part:

Amended manuscript (Lines 411-412):

Inorganic nitrogen was unchanged or slightly increased in the surface snow, while it decreased in subsurface snow.

Question 8: Back of the envelope calculations of potential N deposition are obviously likely to be highly inaccurate, so using this to justify N-fixation is tenuous at best. Better to just outline the possible causes of N increase (if indeed there was N increase) of deposition versus biological processes. You are inferring a lot here - as was one of the main criticisms of the previous discussion.

Response:

We thank for the constructive suggestion. We have deleted the inferring part about the potential N deposition and revised this part to only discuss the possible nitrogen source in the surface snow layer.

Amended manuscript (Lines 335-356):

Both ammonium and nitrate concentrations showed a weak increasing trend in the surface snow (Fig. 1). The weak increase in ammonium could be explained by biogenic emissions due to local plant 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), which equals to 0.19 mg N for the 0.5 m × 0.5 m area sampled each day (assuming nitrogen deposition occurred evenly across the year). Another potential source of nitrogen input could be nitrogen fixation process (Telling et al., 2011). Bacteria are the only microorganisms that are capable of fixing atmospheric nitrogen (Bernhard, 2010). Potential nitrogen input from microbial processes is supported by the increase in the nitrogen-fixing Cyanobacteria (Supplementary Fig. S6) and *nifH* gene (Supplementary Fig. S11). Cyanobacteria are known as free-living phototrophs capable of nitrogen fixation, especially in extreme environments (Chrismas et al., 2018; Makhalanyane et al., 2015; Levy-Booth et al., 2014). For example, Cyanobacteria were found as the main group of potential nitrogen fixers determined by quantitative PCR with three sets of specific *nifH* primers on the surface of the Greenland Ice Sheet (Telling et al., 2012). The nitrogen fixation rate was not quantified in the present study, but the present study suggests that microbial nitrogen fixation could be an overlooked source of nitrogen in Tibetan glacier snow. Further transcriptomic and nitrogen-isotope analyses may provide additional evidence on the microbial activity in nitrogen fixation.

Question 9: Nitrogen use in sub-surface snow; again, I would simply outline potential pathways of N use rather than trying to make a definitive conclusion here based on inferred datasets. You can’t really claim with certainty the denitrification story pushed here.

Response:

We thank for the comment. We have rewritten this part to discuss the potential pathways of N use in the subsurface layer.

Amended manuscript (Lines 356-380):

In contrast with the surface layer, nitrogen concentrations (nitrate and ammonium) significantly decreased in the subsurface snow with time (Fig. 1). A possible explanation for this might be the microbial utilization and photochemical degradation of nitrogen compounds (Björkman et al., 2014). The microbial processes, i.e. nitrate reduction and denitrification process, are evidenced by the increase of *narG* gene (Supplementary Fig. S11) (Telling et al., 2011; Zhang et al., 2020). Alternatively, microorganisms may carry out assimilatory nitrate reduction, which is used to incorporate nitrogen into biomolecules (Larose et al., 2013a; Richardson and Watmough, 1999). The assimilatory process is performed by a range of microorganisms including bacteria, algae, yeasts, and fungi (Huth and Liebs, 1988). Thus, further studies on eukaryotes, including algae, may provide a full understanding of the nitrogen consumption mechanisms in subsurface snow. The denitrification process converts nitrate to N_2 and generates nitrite, nitric oxide (NO), and nitrous oxide (N_2O) intermediates (Kuypers et al., 2018). A previous study detected microbial specific phylogenetic probes that targeted genera whose members are able to carry out denitrification reactions such as Roseomonas in a snowpack of Spitsbergen Island of Svalbard, Norway (Larose et al., 2013a). Amoroso et al. (2010) also proposed that denitrification can explain the microbial isotopic signature observed in winter snow at Ny-Alesund. Although 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). Lastly, photochemical degradation of nitrogen compounds is the most well-known nitrogen degradation pathway, and the release of both NO and NO_x by NO_3^- photolysis on natural snow has been reported in European High Arctic snowpack (Amoroso et al., 2010; Beine et al., 2003). 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 (Dibb et al., 1998).

Question 10: I would amend the conclusion as well based on comments above.

Response:

We thank the reviewer for the constructive suggestion. We rewrite the conclusion section based on the above modifications.

Amended manuscript (Lines 410-422):

Our results showed the dynamics of nitrogen and bacterial community in supraglacial snow over nine days. Inorganic nitrogen was unchanged or slightly increased in the surface snow, while it decreased in subsurface snow. Due to atmospheric nitrogen deposition and potentially bacterial nitrogen fixation activities, nitrogen limitation is unlikely to occur in the surface snow. In contrast, nitrogen consumption was inferred in the subsurface snow. Nitrogen is traditionally recognized to be released from the supraglacial environment due to photolysis, whereas the present study hints that nitrogen assimilation and denitrification could be alternative routes. Therefore, the increased nitrogen deposition due to anthropogenic activities may enhance the nitrogen consumption in the subsurface snow, which reduces the impact of increased nitrogen discharge on downstream glacier-fed rivers. In summary, our results provide a new perspective of the nutrients and bacterial community dynamics in supraglacial snow of the Tibetan Plateau. Further studies based on metagenome and metatranscriptome can enhance the understanding of bacterial functions.

Figures

Question 11: Eyeballing the revised figures again shows there is no real increase in NO3 of NH4 through time above the variability evident in the data. 95% CIs should be shown on regressions.

Response:

We thank for the comment. We added the 95% CIs on regressions. Please see Figures and Supplementary.

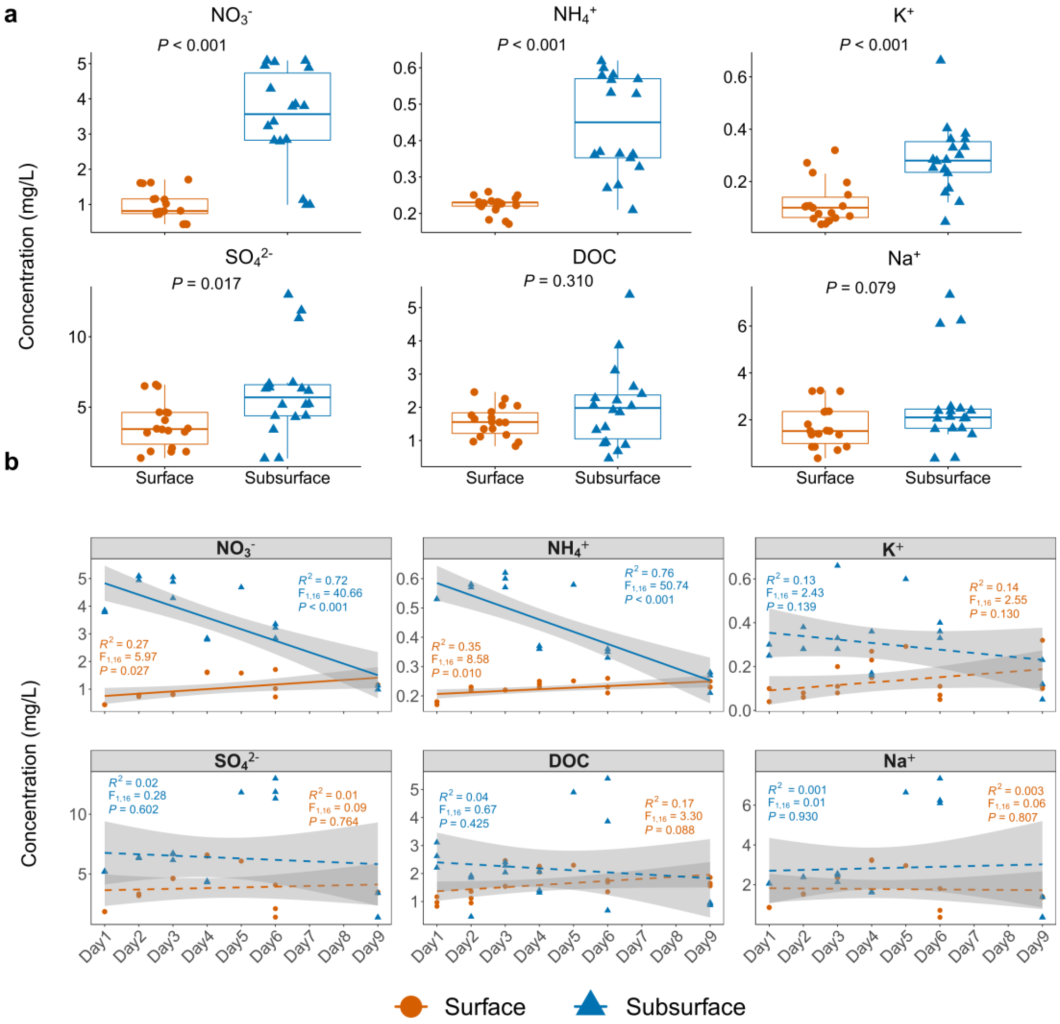

Fig. 1 The pattern of environmental factors changes in the surface and subsurface snow layers. (a) Environmental factor comparisons in the surface and subsurface snow layers. Each dot represents an individual sample. Significantly higher concentrations of NO_3^-, NH_4^+, K^+, and SO_4^{2-} were observed in the subsurface layer based on Wilcoxon rank-sum test. (b) Temporal changes of environmental factors in the surface and subsurface layers. The solid and dashed lines indicate significant and non-significant temporal changes, respectively. The concentration of NO_3^- and NH_4^+ in the surface layer significantly increased with time while the concentration of NO_3^-, and NH_4^+, in the subsurface layer, significantly decreased with time. Significance is based on linear regression. Grey shading indicates the 95% confidence interval of regression.

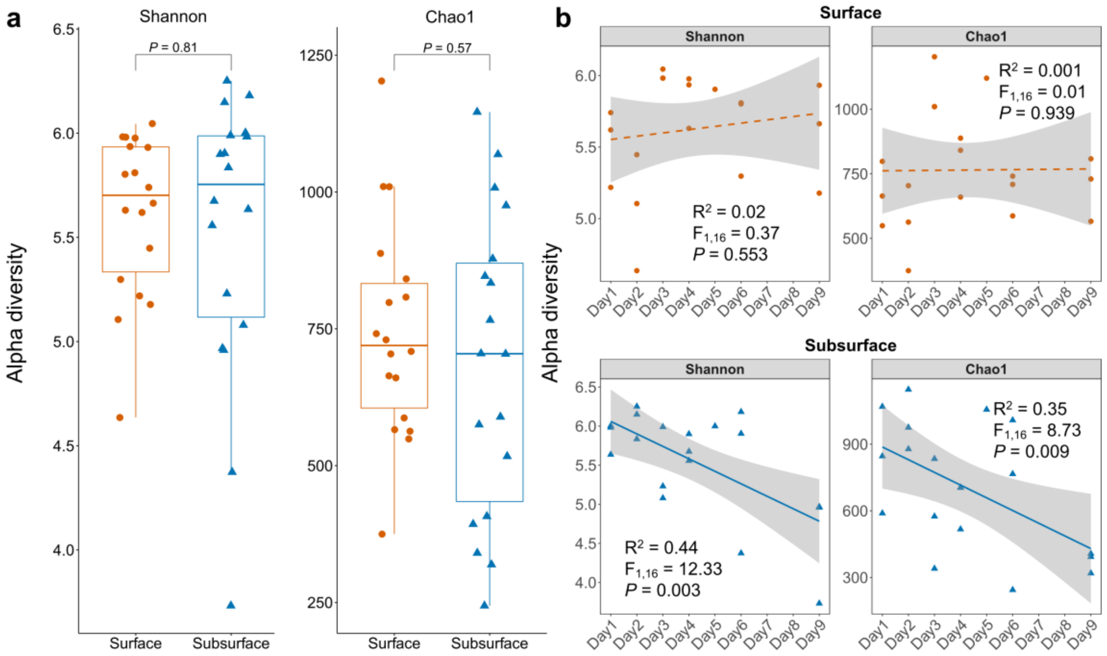

Fig. 3 Bacterial alpha diversity in snow layers. (a) Bacterial alpha diversity comparison between the surface and subsurface layers. Each dot represents an individual sample. For both Shannon and Chao1 indices, no significant difference was observed between the surface and subsurface snow layers. Comparison is based on Wilcoxon rank-sum test. (b) Temporal changes of the alpha diversity indices in the surface and subsurface snow layers. For the surface layer, no significant correlation was observed, while both Shannon and Chao1 showed a significantly reduction with time in the subsurface layer. Significance is based on linear regression. Grey shading indicates the 95% confidence interval of regression.

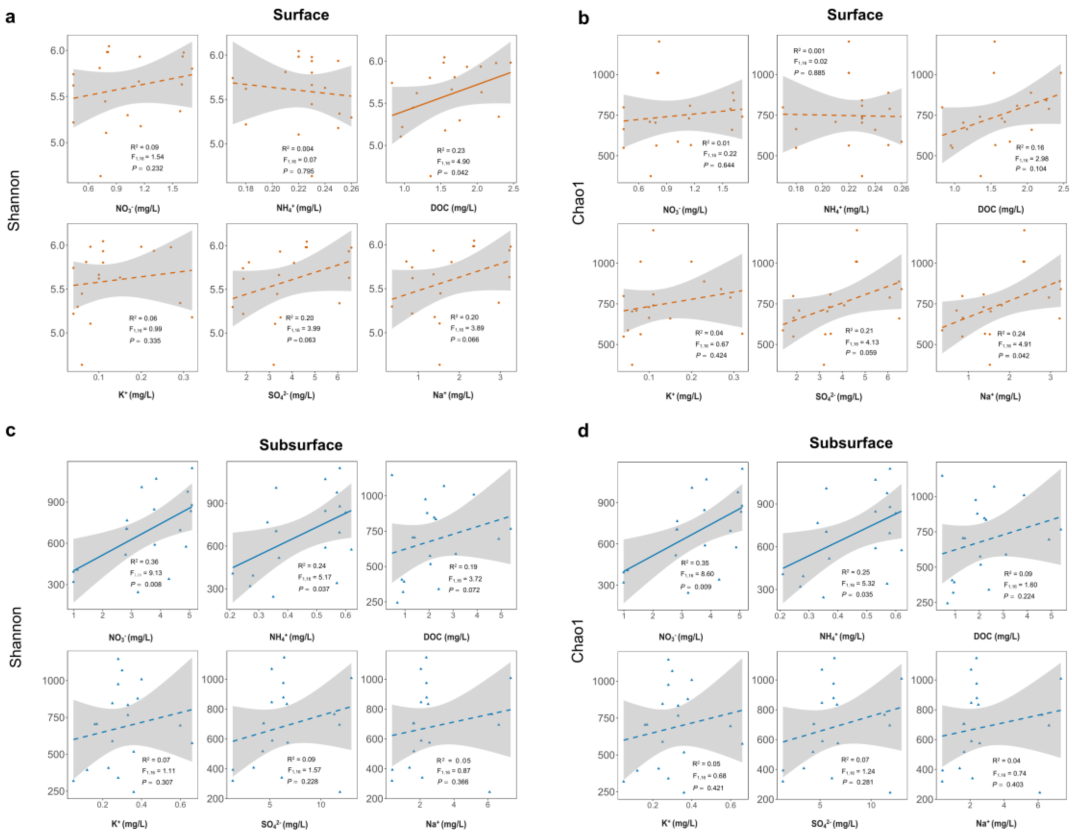

Fig. 4 The influence of environmental factors on bacterial diversity. Correlations of Shannon (a, c) and Chao1 (b, d) diversity indices with environmental factors in the surface and subsurface layers. Each dot represents an individual sample. The solid and dashed lines indicate significant and nonsignificant changes respectively. Significance is based on linear regression. Grey shading indicates the 95% confidence interval of regression.

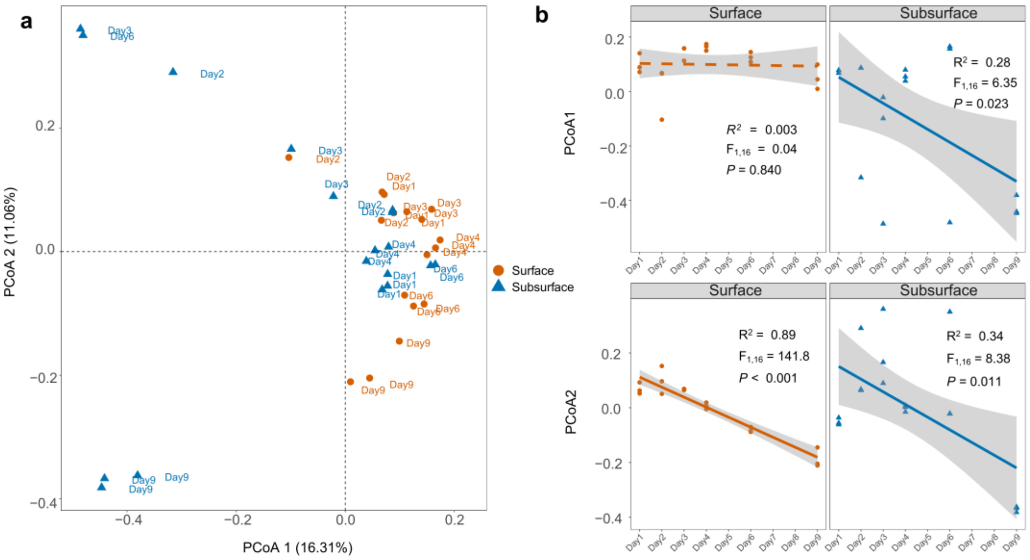

Fig. 5 Principal coordinate analysis (PCoA) of microbial communities in the surface and subsurface snow. (a) Bray-Curtis distance-based PCoA ordination plot. The microbial community structures of the surface and subsurface snows are significantly different (PERMANOVA, $P < 0.001$). (b) Pairwise regression 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 the 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. Grey shading indicates the 95% confidence interval of regression.