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The Cryosphere Discussions

Interactive comment on "Permafrost thawing exhibits a greater influence on bacterial richness and community structure than permafrost age in Arctic permafrost soils" *by* Mukan Ji et al.

Mukan Ji et al.

wdkong@itpcas.ac.cn

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The authors would like to thank the reviewer for the constructive feedback, and the thorough assessment of the manuscript. Below we provide a point-to-point response to each comment, reviewer comments are given in black, responses are given in blue. Additionally, we have included details of how we intend to address these changes in a revised submission.

This manuscript reports significant influences of both permafrost thawing and age on bacterial richness and community structure. It also documented that permafrost thawing increased the contribution of determinism to bacterial community assembly,

but didn't lead to community convergence. The study then showed that permafrost thawing had a greater influence on bacterial community than permafrost age. They extrapolate their findings to highlight that permafrost thawing in different ages can lead to distinct bacterial community compositions and different soil organic carbon degradation processes. The manuscript is well organized and figures are well prepared. I have several major concerns about this manuscript:

Q1. I am not an expert in permafrost. But I noticed that the samples used in this study should be the same samples reported in Kao-Kniffinet al. 2015. In this study, the permafrost age of different samples had been measured by Kao-Kniffin et al. 2015. However, basin age is used in that study instead of permafrost age. Does it mean that basin age is equal to permafrost age? If so, Why did this study reported different soil total organic carbon and total nitrogen from Kao-Kniffin et al. 2015?

Response:
The "basin age" used by Kao-Kniffin et al. 2015 was obtained from Hinkel et al., 2003. This age is calculated using radiocarbon dating on sample at the interface of the lacustrine sediment and the in situ peat, which represents the point in time of lake drainage and revegetation of the basin surface. The basin drainage is associated with vegetation establishment, organic matter accumulation, and ice-wedge growth below the drained lake basin (Hinkel et al., 2003). Therefore, we used the basin age as a proxy for the formation of permafrost (permafrost age), and explored the influence of permafrost age on the response of bacteria to permafrost thawing. We did not use the term basin age, as we feel that permafrost age is more meaningful than basin age in this case. We apologize for not stating this clearly, and following sentences will be added to clarify this.

Amended manuscript:

Approximately 20% of the Arctic coastal plains of northern Alaska contain thaw lakes drained at various stages since the mid-Holocene, which were then developed into ice-rich permafrost (Hinkel et al., 2003). These drained lake basins contain soils ranging from freshly developed organic layers on sediments to fully developed ancient permafrost soils (Mueller et al., 2015). By using the drained thaw lake basin age as a proxy for the time of permafrost formation, it provides an opportunity to investigate the influence of permafrost age on the microbial community during permafrost degradation.

Regarding the inconsistent values of environment factors with Kao-Kniffinet al. 2015, these environmental factors (SOC and TN) were independently measured in the present study. They are not substantially different from those reported in the Kao-Kniffinet al. 2015 (please see Supplement table 1 attached), and the differences could be related to sample storage and the equipment used.

Q2. It seems that Kao-Kniffin et al. 2015 also used amplicon sequencing of 16S rRNA gene to analyze bacterial communities in different permafrost ages and thawing status. They found that community composition appeared to converge in the active layer, however, the authors in this study didn't observe the community convergence due to permafrost thawing. Can you explain why you reanalyzed bacterial communities of these samples? At least, please compare your study with the results of Kao-Kniffin et al. 2015 and provide more discussion.

Response:
We appreciate the reviewer for this comment. In this manuscript, we focused on the bacterial community only, whereas Kao-Kniffin et al. 2015 investigated the community structure of the entire prokaryotes (Both bacteria and archaea). For bacteria, Kao-Kniffin presented the taxonomic composition, community phylogenetic distance, and biomass. Thus, the interactive influence of permafrost age and thawing on bacterial

diversity, community structure, and assembly processes still remain unexplored. Therefore, we believe further investigation is necessary and could provide essential knowledge on how permafrost age interact with permafrost thawing.

To address the reviewer's comments, we will add the following sentences to clarify our aims and distinguish our work from Kao–Kniffin et al.:
An earlier study has revealed a high abundance of Candidatus Methanoflorens archaeon in the community (Kao–Kniffin et al., 2015), but how the bacteria in the permafrost of various ages would respond to thawing remains undiscussed. Thus, we take this opportunity to re-analyze these samples to investigate the interactive influence of permafrost thawing and age on the permafrost soil bacterial community.

Following sentences will be added to discuss the inconsistency between our work and Kao–Kniffin et al on community convergence:
Our results demonstrated that the bacterial community structure did not converge due to permafrost thawing, as reflected by the non-significant difference in sample heterogeneity among the various permafrost layers (Supplementary Fig. 3, Supplementary Table 11). This contradicts previous studies (Deng et al., 2015; Yuan et al., 2018) in the Arctic, but was consistent with Mackelprang (2011). Our results also contradict to Kao–Kniffin et al. (2015), which reported a lower prokaryotic community differences in the active layer than in the transition and permanently frozen permafrost. Several reasons could cause this inconsistency. Firstly, different microbial communities were targeted. Kao–Kniffin et al. (2015) focused on the Archaeal community, and a single archaeon OTU accounted for over 30% of the community (Fig. 3 in Kao-Kniffin et al., 2015). This may drive the convergence of the prokaryotic community. In comparison, only bacterial community were targeted in the present study, and an early study has reveal distinct community structure of bacteria and archaea with archeal demonstrating lower variation across soil depth (Frank-Fahle et al., 2014). Furthremore,

the inconsistency may also related to the different community dissimilarity metrics used. Kao–Kniffin et al. (2015) used unweighted UniFrac, which only account for the phylogenetic closeness of the OTUs, and the relative abundance was not considered. This is distinctively different from the Bray-Curtis dissimilarity used in this study, and it has been reported that unweighted and weighted community metrics examine different features of the community (Lozupone et al., 2011).

Q3. The thickness of active, transition and permafrost layers should be different in young, medium, old and ancient permafrost. Please provide more information about the soil profile of different layers in four kinds of permafrost. More variables should be taken into account to undermining the mechanism of bacterial response to permafrost thawing in different permafrost age. I'm not sure that structural equation modelling is a good method to quantify the relative importance of permafrost thawing status and age on bacterial community without any other environmental variables. Please incorporate more variables in structural equation modelling to show how permafrost thawing status and age influenced bacterial community directly or indirectly.

Response:
We appreciate the reviewer for this comment. We agree with reviewer that soil profile characteristics are important in determining the response of bacteria to permafrost thawing. Unfortunately, we have only obtained a small quantity of soil samples, and this does not allow us to measure soil properties in great detail. Therefore, we decided to focus on TOC and TN, which are known to be substantially different in the permafrost of different ages and thawing status (Mueller et al., 2015). We will amend the manuscript to acknowledge the limitation of our work and propose further works that are required to identify the environmental factors that shape bacterial response to permafrost thawing.

Amended manuscript (method section)
Due to sample quantity limitation, only two of the most important soil physicochemical properties: total organic carbon (TOC) and total nitrogen (TN) were measured using dry combustion (Vario MAX CNS Analyzer, Elementar, Hanau, Germany). These factors have been reported to substantially vary in samles with different permafrost age and thawing status (Mueller et al., 2015). For other soil properties and soil profile descriptions of the different layers please see Kao-Kniffin et al (2005).

To address the reviewer's concern and emphasize the importance of environmental heterogeneity in the different permafrost layers, we will add the following paragraph to the manuscript:

Amended manuscript (discussion section)
Bacterial community structure in the active layer is more similar to the transition layer than to the permanently frozen layer (Fig. 3). This is consistent with those observed in other Arctic permafrost (Monteux et al, 2018, Deng et al., 2015), confirming that thawing can homogenize bacterial community structure of different soil depths. However, significant differences in the bacterial community were still observed between the active and transition layers (Supplementary Table 8), instead of being identical (Monteux et al, 2018). This could be due to physiochemical heterogeneity between the soils of different permafrost layers (Fig. 1, Kao-Kniffin, et al., 2015, Mueller et al. 2015). Thus, physicochemical properties (such as the total nitrogen observed here) differences also contribute to the bacterial community heterogeneity and led to the significantly different bacterial communities observed.

Amended manuscript (Conclusion section)
Further studies are required to identify the environmental and historical factors that lead to the distinct response of bacteria in the permafrost of different ages.

Using ranked soil age in SEM to investigate the influence of pedogenic development has been used by Laliberté et al., 2017. Here, we use this idea to investigate the influence of permafrost age on the response of bacteria to permafrost thawing. We repeated the SEM analysis incorporating the TOC and TN as suggested by the reviewer, and a large proportion of the richness and community structure were still explained by the permafrost age and thawing status. Therefore, the permafrost age and thawing status can influence richness and community structure via other unmeasured environmental factors or historical effects. The present study does not attempt to identify these factors, but instead, we tried to raise the awareness that different aged permafrost may response to climate change induced permafrost thawing differently. To address this comment, we have incorporated TOC and TN into the SEM (attached as supplementary Fig 1), performed Random Forest analysis to identify the importance of permafrost age and thawing status (attached as supplementary Fig 2), and also amended the results and conclusion section to address the requirement of identifying environmental/historical factors that directly cause the different responses of bacteria in the permafrost of various ages.

Amended manuscript (Results section)
Structural equation modelling (SEM) revealed that both permafrost thawing status and age significantly contributed to bacterial richness. Permafrost thawing status exhibited a higher contribution than age (standard regression weight of 0.51 and -0.30, respectively, both $P < 0.05$) to bacterial richness (Fig. 4a). However, the influence of TOC and TN on bacterial richness was not observed. This is consistent with the Random Forest analysis results, which only identified the permafrost thawing and age as the significant determinants of bacterial richness (Supplementary Fig. 5). For community structure, permafrost thawing exhibited an indirect influence on NMDS1 via TN (standard regression weight of 0.58 and -0.63, both $P < 0.001$, Fig. 4b). In comparison, both permafrost age and thawing status significantly contributed to the NMDS2 (standard regression weight of -0.34 and 0.59, respectively, both $P < 0.01$),

while TN also exhibited an significant influence on NMDS2 (-0.49, $P = 0.002$). The significant contribution of TN, permafrost thawing and age were consistently identified using Random Forest approach (Supplementary Fig. 5).

Amended manuscript (Conclusion section)
Further studies are required to identify the environmental and historical factors that lead to the distinct response of bacterial in the permafrost of different ages.

Minor comments:
Q4. Fig.2 Can you provide information about bacterial phylogenetic diversity of bacteria in different permafrost age and thawing status?

Response:

We appreciate the reviewer for this comment. The results of Phylogenetic diversity and Shannon diversity will be added to the results and discussion sections.

Amended manuscript (Results section)

In comparison, the influence of permafrost age and thawing on bacteria Shannon diversity were non-significant (Two-way ANOVA, $P = 0.058$ and 0.53, respectively, Supplementary Fig. 2). This contrastively different from those observed on phylogenetic diversity, where significant influence was observed for thawing ($P = 0.015$ and 0.001, respectively).

Amended manuscript (Discussion section)

Furthermore, the phylogenetic diversity exhibited greater sensitivity to permafrost thawing than to the Shannon diversity. As phylogenetically close-related microorganisms have similar habitat associations, phylogeny-based community metrics could infer potential community functional change (Stegen et al., 2012). Hence, this suggests that community function could be more sensitive to permafrost thawing than the community composition.

Q5. Fig.4 the path coefficients in structural equation modelling can indicate the positive or negative correlations between two variables. Therefore, the raw value should be shown here, instead of the absolute value.

Response:
We appreciate the reviewer for this comment. We will includ the signs of path coefficient in the figure (attached as supplment figure 1) and amend the manuscript accordingly.

Amended manuscript
Structural equation modelling (SEM) revealed that both permafrost thawing status and age significantly contributed to bacterial richness. Permafrost thawing status exhibited a higher contribution than age (standard regression weight of 0.51 and -0.30, respectively, both $P < 0.05$) to bacterial richness (Fig. 4a). However, the influence of TOC and TN on bacterial richness was not observed. This is consistent with the Random Forest analysis results, which only identified the permafrost thawing and age as the significant determinants of bacterial richness (Supplementary Fig. 5). For community structure, permafrost thawing exhibited an indirect influence on NMDS1 via TN (standard regression weight of 0.58 and -0.63, both $P < 0.001$, Fig. 4b). In comparison, both permafrost age and thawing status significantly contributed to the NMDS2 (standard regression weight of -0.34 and 0.59, respectively, both $P < 0.01$), while TN also exhibited an significant influence on NMDS2 (-0.49, $P = 0.002$). The significant contribution of TN, permafrost thawing and age were consistently identified

using Random Forest approach (Supplementary Fig. 5).

Q6. Line 95 vary -> varies

The mis-spelling is now corrected, and the amended manuscript is:

The surface organic layer thickness varies with permafrost age, which was < 5, 10–15, 15–30, and 40–50 cm for the young, medium, old, and ancient–aged permafrost soils (Kao–Kniffin et al., 2015)

Q7. Line272 Please rewrite this sentence.

The sentence is rephrased as:

Deltaproteobacteria has strong catabolic potentials to decompose recalcitrate aromatic compounds and other plant detritus (Jansson and Tas, 2014), which may explain their enhanced richness in the surface active layer of permafrost soil.

Q8. 280 results is -> results are

The mis-spelling is now corrected, and the amended manuscript is:

Our results are consistent with Mondav et al.(2017), who found that permafrost activity better separated the community structure than soil depth in peatland permafrost soil in Sweden.

Table 1 Comparison of soil organic carbon (SOC) and total nitrogen (TN) using in the present study and Kao-Kniffin et al.

	SOC (%)		TN (%)	
	The present study	Kao-Kniffin et al. 2015.	The present study	Kao-Kniffin et al. 2015.
Basin age				
Young	8.8 (2.0)	6.9 (1.9)	0.43 (0.09)	0.43 (0.11)
Medium	9.8 (2.5)	8.4 (1.6)	0.45 (0.11)	0.49 (0.09)
Old	12.4 (2.2)	13.4 (1.6)	0.57 (0.10)	0.77 (0.09)
Ancient	13.3 (3.2)	10.7 (2.5)	0.60 (0.13)	0.62 (0.14)
Soil depth layer				
Active	16.9 (2.1)	14.1 (2.1)	0.73 (0.09)	0.78 (0.11)
Transition	10.4 (2.1)	9.6 (1.8)	0.50 (0.09)	0.57 (0.11)
Permafrost	5.6 (1.0)	5.8 (1.4)	0.29 (0.05)	0.36 (0.09)

The values indicate means and standard error (in parentheses)

Fig. 1. Comparison of TOC and TN between the present study and Kao-Kniffin et al. 2015

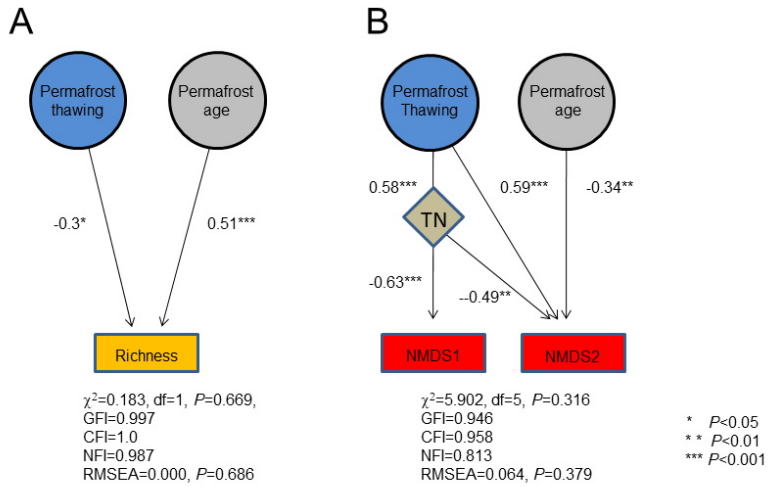

Fig. 2. The relative importance of permafrost thawing status and age on bacterial richness (a) and community structure (b)based on structural equation modelling

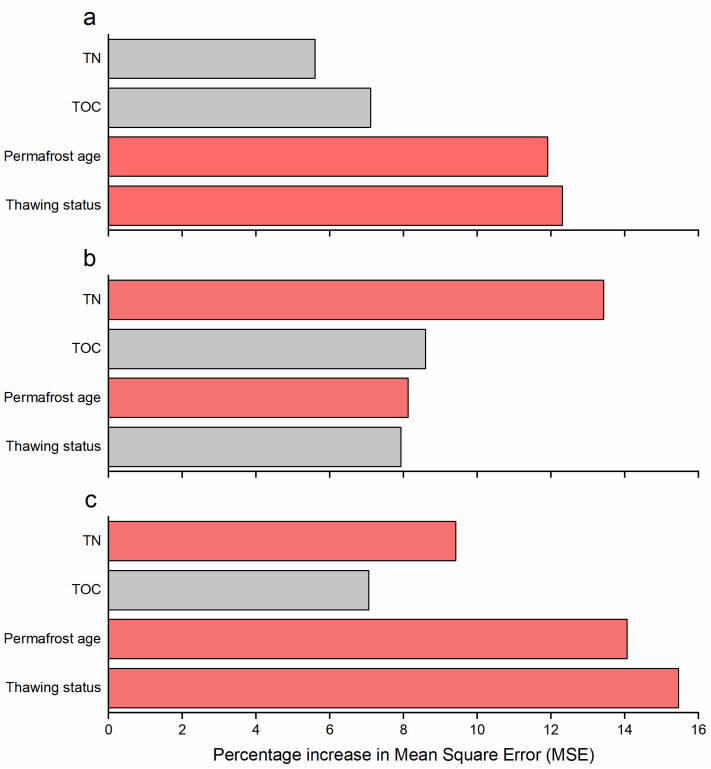

Fig. 3. Random Forest analysis results showing the contribution of permafrost age, thawing status, TOC and TN on the bacterial richness (a), nmds1 (b), and nmds2 (c))