

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 is a technically correct manuscript on a currently relevant topic in the context of climate change and biogeochemical cycles - the response of microbes to permafrost thawing. The study shows changes in bacterial community structure and richness of

C1

drained lake basins with permafrost soil age and permafrost thawing status (active, transition and permanently frozen soil layer). In addition, there is data on soil carbon and nitrogen. The results are presented clearly and the figures are well prepared.

Major concerns:

Q1. Are the samples in this study from the same soil cores as those in Kao-Kniffin et al. 2015, which is cited in the section on sampling? Kao-Kniffin et al. 2015 also describe bacterial communities with permafrost soil age and thawing status. If the soil cores are the same, please make it clear in the aims why a second analysis of bacterial communities in these samples is needed and explain what new this study adds. In any case, please take the results of Kao-Kniffin et al. 2015 into account in the discussion, especially as their conclusion (communities in active layers converge) seems to be the opposite from this manuscript (no convergence of active layer communities).

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 would influence the bacterial community interactively 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.:

C2

An earlier study has revealed a high abundance *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 are to 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). Furthermore, 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

C3

features of the community (Lozupone et al., 2011).

Q2. I am concerned that the connection of soil layers to thawing status is too simplified and does not take into account variation in the soil profile. Was the soil structure/chemical composition of the profiles homogeneous with depth? The description of organic layer on l. 94-96, Fig. 1 and Kao-Kniffin et al. 2015 and Mueller et al. 2015 cited in the manuscript suggest they were not. In this case, the differences in bacterial communities between soil layers cannot be directly interpreted as a thawing response (l.37., l.318), because the state of the system before thawing is not known and the differences between the layers can be due differences in other soil properties (for example organic vs. mineral layer). It is possible to compare the active, transition and frozen layers with permafrost age but that seems to have already been done by Kao-Kniffin et al. 2015? In any case, the issue of other differences between the soil layers than thawing status should be better taken into account in the manuscript. Do soil carbon and nitrogen explain the community changes?

We appreciate the reviewer to raise this concern. As the reviewer has pointed out, the physicochemical properties of the soils in different permafrost depths are not homogenous. Hence, the response of microbial community to thawing could be the collective effects of both thawing and the environmental factors difference. However, microbial transformation (as a result of permafrost thawing) would substantially change the quantity and composition of organic compounds (Mueller et al. 2015). Thus, soil physicochemical properties and bacterial community structure are interactive, and we have to admit that the individual influence would be very difficult to disentangle. Nevertheless, Mondav et al. (2017) reported that permafrost thawing has a stronger influence on microbial community structure than soil depth. 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:

C4

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 in different permafrost layers (Fig. 1, Kao-Kniffin, et al., 2015, Mueller et al. 2015). Thus, other unmeasured physicochemical properties (such as the total nitrogen) in the different permafrost layers also contributed to the bacterial community heterogeneity and led to the significantly different bacterial communities.

We will also add the following sentence to the conclusion section, which demands further investigation to identify the factors (both environmental and historical) that caused the distinct microbial response to thawing.

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:

Q3. 82-91 Please indicate where your replicate samples come from and how many there are. Here it is mentioned that there are four age classes and one soil core per age class, but the figures show a lot more data points (over 40?). Table S6: I am confused how mean relative abundance can be over 100

We thank the reviewer for this comment, the following sentence will be added to clarify

C5

the number of replicates used.

In brief, 16 soil cores were collected along a chronosequence of drained lake basin, spanning in age from young (< 50 yr old), medium (< 300 yr old), old (< 3,000 yr old), to ancient (3,000–5,000 yr old) in April 2010.

And

For each permafrost age-layer combination, there were four sample replicates, except for the young frozen permafrost, which had only two.

For Table S6, we apologize for the mistake. The number presented is the bacterial richness (i.e., number of OTUs observed), but not a percentage number, the amended table is attached in the supplementary file. The spelling of S.D. is corrected throughout the manuscript.

Minor comments on spelling and grammar:

Q4. The spelling and grammar mistakes have been corrected as indicated by the reviewer.

I. 30 Deltaproteobacterai -> Deltaproteobacteria

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

The bacterial richness was significantly higher in the young and thawed permafrost, and the richness increase was mainly observed in Firmicutes, Actinobacteria, Chloroflexi, Deltaproteobacteria, and Alphaproteobacteria.

C6

I. 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)

I. 253 early -> earlier

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

Furthermore, an earlier study on the freshwater ecosystem also confirmed that organic carbon composition determined bacterial richness and community structure

I. 270 Alphaproteobacterai -> Alphaproteobacteria

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

One possible explanation is that the surface active layer may be the major location for root exudates, which favours Alphaproteobacteria

I. 272 Please check language. What enhances their richness?

The sentence is now rephrased as:

C7

Deltaproteobacteria has been reported to have a strong catabolic potential on the degradation of recalcitrant aromatic and other plant detritus (Jansson and Tas, 2014), which enhances their richness in the surface active layer of permafrost soil.

I. 292 have -> has

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

Collectively, this suggests that permafrost thawing has a stronger influence on the bacterial community structure than permafrost age.

I. 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.

Interactive comment on The Cryosphere Discuss., <https://doi.org/10.5194/tc-2020-39>, 2020.

C8

		Mean±S.D.	Active	Transition	Frozen
<i>Firmicutes</i>	Active	87±15	-	-	-
	Transition	47±15	0.013	-	-
	Frozen	49±8	0.042	0.986	-
<i>Actinobacteria</i>	Active	128±14	-	-	-
	Transition	106±10	0.062	-	-
	Frozen	71±2	0.002	0.021	-
<i>Chloroflexi</i>	Active	36±4	-	-	-
	Transition	29±3	0.095	-	-
	Frozen	16±6	0.002	0.02	-
<i>Alphaproteobacteria</i>	Active	35±6	-	-	-
	Transition	19±7	0.016	-	-
	Frozen	16±4	0.016	0.774	-
<i>Deltaproteobacteria</i>	Active	25±8	-	-	-
	Transition	12±2	0.028	-	-
	Frozen	11±2	0.049	0.976	-

Fig. 1. The richness of bacteria phyla by permafrost thawing status