https://doi.org/10.5194/tc-2020-39
Preprint. Discussion started: 4 March 2020

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Permafrost thawing exhibits a greater influence on bacterial richness and community structure than permafrost age in Arctic permafrost soils

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Running title: Relative influences of permafrost thawing and age on soil bacteria

https://doi.org/10.5194/tc-2020-39
Preprint. Discussion started: 4 March 2020

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Abstract

Global warming accelerates permafrost thawing and changes permafrost microbial community structure, but little is known about how microorganisms in permafrost with different ages respond to thawing. Herein, we disentangled the relative importance of permafrost age (young, medium, old, and ancient) spanning from 50 to 5,000 yr and thawing status (active, transitional, and permanently frozen) in shaping bacterial community structure using Hiseq sequencing of the 16S rRNA gene. Our results revealed significant influences of both permafrost thawing and age on bacterial richness. The bacterial richness was significantly higher in the young and thawed permafrost, and the richness increase was mainly observed in *Firmicutes*, *Actinobacteria*, *Chloroflexi*, *Deltaproteobacterai*, and *Alphaproteobacteria*. Permafrost thawing led to a gradual change in bacterial community structure and increased the contribution of determinism to shape the bacterial community assembly. Permutational analysis of variance demonstrated that thawing significantly changed bacterial community structure at all soil ages, but the community convergence due to permafrost thawing was not observed. Structural equation modeling revealed that permafrost thawing exhibited a greater influence on both bacterial richness and community structure than permafrost age. Our results indicate that microorganisms in permafrost with different ages respond differently to thawing, which eventually leads to distinct bacterial community compositions and different soil organic carbon degradation processes during permafrost thawing.

Keywords: Permafrost thawing; permafrost age; bacterial community; richness; Arctic

https://doi.org/10.5194/tc-2020-39
Preprint. Discussion started: 4 March 2020


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1 Introduction

Global warming accelerates permafrost thawing, and 200 billion tons of carbon is estimated to be released into the atmosphere from global permafrost over the next 300 years (Turetsky et al., 2019). The degradation of soil organic carbon (SOC) is predominately driven by microorganisms (Frank–Fahle et al., 2014), and the quality and quantity of SOC also control the abundance and community structure of microbial community (Chen et al., 2016). It has been reported that permafrost of different thawing status and ages exhibits distinct labile and recalcitrant carbon quantities, with a higher carbohydrates in relation to aliphatic carbon in older than in younger permafrost (Chen et al., 2016; Mueller et al., 2015; Yang et al., 2009). Thus, the distinct SOC composition may subsequently impact the microbial community structure in permafrost soil, and the distinct microbial community structure may respond differently to permafrost thawing. However, the impacts of permafrost age and its interaction with thawing on microbial community remain largely elusive.

In addition to permafrost age, contrasting structure of labile and recalcitrant carbons was also reported in the thawed and frozen permafrost. This was proposed to be due to the distinct microbial transformation process in the different permafrost thawing status (Mueller et al., 2015). The microbes in the frozen permafrost are predominately in a state with reduced metabolism rate (Gilichinskii, 1995), thus labile carbon is protected from microbial degradation (Hobbie et al., 2000). In contrast, permafrost thawing substantially activates a diverse range of oligotrophic and copiotrophic bacteria, and enriches carbohydrate transporter and metabolism–related genes (Schostag et al., 2019). This leads to an increased bacterial richness and converged community metabolic functions, and the soil carbon being dominated by aliphatic carbon resulted from microbial transformation (Deng et al., 2015; Mackelprang et al., 2011; Monteux et al., 2018; Schostag et al., 2019).

Soil development leads to changes in bacterial community structure, predominately due to nutrient accumulation and vegetation colonization (Bardgett and Walker, 2004; Park et al., 2011). Distinct bacterial community structure has been reported in soils of different ages. For example, young soils in the deglaciation chronosequence exhibit significantly lower bacterial richness than aged soils, and autotrophs play a major role in the accumulation of nutrients (Kazemi et al., 2016; Kim et al., 2017; Liu et al., 2016). In contrast, aged soils with vegetations are dominated by heterotrophs, such as *Acidobacteria* and *Actinobacteria* (Kwon et al., 2015). However, little is known about the influence of

https://doi.org/10.5194/tc-2020-39
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permafrost age on soil microbial community.

To explore the effects of permafrost age on the response of bacteria to permafrost thawing, soil bacterial community in Arctic permafrost was characterized using the Illumina sequencing targeting the 16S rRNA gene. Given the continuously changed bacterial community with increasing soil age (Kazemi et al., 2016; Kim et al., 2017), we hypothesized that bacterial richness and community structure would also significantly differ in the permafrost of various ages and response differently to permafrost thawing. Permafrost in northern Alaska varies in age (Hinkel et al., 2003), and thus provides a perfect opportunity to investigate the influence of permafrost thawing status and age on the permafrost soil bacterial community.

2 Materials and methods

2.1 Site description

The permafrost was sampled in the Barrow Peninsula between 71°20′to 71°27′N latitude and between 156°4′and 156°7′W longitude (Kao–Kniffin et al., 2015). Barrow Peninsula is located at the northernmost coast of Alaska, and is part of the Arctic Coastal Plain with continuous permafrost. The mean annual temperature is -12°C, and the mean annual precipitation is 104 mm (Mueller et al., 2015). In brief, 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. The chronosequence was determined by the degree of plant community succession and ^{14}C carbon dating (Hinkel et al., 2003). At each lake basin, a soil core was collected using a SIPRE corer measuring 80 to 150 cm long and 7.5 cm diameter attached to a Big Beaver earth drill apparatus (Litter Beaver, Inc., Livingstone, TX, USA) mounted on a sledge. Each soil core contained three layers: active, transition, and permanently frozen. The active layer represents the surface soil layer that thaws and refreezes on an annual basis; the transition layer remains frozen, but occasionally thaws during warmer summers; the permanently frozen layer remains annually frozen (Kao–Kniffin et al., 2015). The surface organic layer thickness vary 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). The frozen soil cores were cut with a chop–saw into sections of corresponding soils horizons in a cold room in Barrow, and soils were homogenized, stored, and transported at -20 °C until processed (Mueller et al., 2015).

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Soil total organic carbon (TOC) and total nitrogen (TN) were measured using dry combustion (Vario MAX CNS Analyzer, Elementar, Hanau, Germany) (Mueller et al., 2015).

2.2 DNA extraction and sequencing

Total DNA was extracted using the MO BIO Power Soil DNA extraction kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Universal primer set 515F (5'–GTGCCAGCMGCCGCGGTAA–3') and 806r (5'–GGACTACHVGGGTWTCTAAT–3') with 12–nt unique barcodes was used to amplify the V4 hyper–variable region of the 16S rRNA gene (Caporaso et al., 2012). The PCR mixture (25 µl) contained 1x PCR buffer, 1.5 mM of $MgCl_2$, 0.4 µM each of deoxynucleoside triphosphate bases, 1.0 µM of each primer, 0.5 U of Ex Taq (TaKaRa, Dalian, China) and 20 ng of DNA template. The PCR amplification program included an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR products were pooled in equal molar amounts, and then used for pair–end sequencing (2 x 250 bp) on the Illumina HiSeq 2500 sequencer at the Magigene (Guangzhou, China).

2.3 Data processing

Three samples generated very low reads, to avoid artefact from different sequencing batches, these three samples were removed from down steam analysis. Raw sequence data were processed using the MOTHUR v. 1.34.3 (Schloss et al., 2009). Paired–end reads were merged and quality screened with the following settings: as the amplicon size was approximately 300 bp, sequences with length < 250 or > 350, more than 1 mismatch in the primer region, average quality < 30, ambiguous bases > 0 and homopolymer length > 9 were removed from the subsequent downstream analysis. The sequences were then aligned against the Silva reference alignment (release 128), which was trimmed to include only the same region amplified, and those sequences that did not align were removed. Chimeric sequences were identified using the UCHIME (Edgar et al., 2011) and removed. The remaining sequences were classified using the Bayesian classifier against the Silva database (release 128), with a minimum confidence score of 80 % (Wang et al., 2007), and all Eukaryota, chloroplasts, mitochondria and unknown sequences were removed. Archaeal sequences were also removed to concentrate the study on the bacterial community. Finally, sequences were classified into operational taxonomic units (OTUs) at

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the 97 % identity, and singletons were then removed. The dataset was sub–sampled to an equal depth of 16,144, which was the smallest sample size across the entire dataset. Bacterial richness (OTU recovered) was calculated using the summary.single command in the Mothur program (Schloss et al., 2009).

2.4 Statistical analysis

Significant differences in bacterial richness, total organic carbon and total nitrogen across permafrost age and thawing status were tested using the two–way ANOVA, and the pairwise differences were assessed by the Tukey's HSD test using SPSS 23 (SPSS Inc., Armonk, NY, USA). The Levene's test was used to ensure the homogeneity of variances for the dependent variables (bacterial richness, TOC, and TN) for each combination of the independent variables (Brown and Forsythe, 1974). One–way ANOVA was used to examine the significance of the differences among the permafrost soils of different thawing status with the same permafrost age.

Non–metric Multidimensional Scaling (NMDS) was generated from the Hellinger–transformed bacterial community dataset based on the Bray–Curtis dissimilarity matrix using Primer 6 (Clarke and Warwick, 2006). The contributions of carbon, nitrogen, C:N ratio, permafrost age, and thawing status to the community structure were quantified using the distance–based linear model (DistLM) after normalisation. Permutational analysis of variance (PERMANOVA) was used to examine the influence of permafrost thawing and age on bacterial community structure (Anderson, 2001) using Primer 6. We compared the multivariate dispersion homogeneity to assess the bacterial community convergence by permafrost thawing status, using permutational analysis of multivariate dispersions (PERMDISP) (Anderson, 2006).

2.5 Quantifying the contribution of stochasticity

Bray–Curtis dissimilarity based normalised stochasticity ratio (NST) index was calculated using the 'NST' package in R (http//www.r-project.org) to represent the contribution of stochasticity to community assembly (Ning et al., 2019). The NST index values range from 0 % to 100 %, a 0 % indicates zero contribution of stochasticity, whereas 100 % indicates the community assembly being completely stochasticity–driven.

2.6 Structural equation modelling (SEM) analysis

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We conducted SEM using AMOS 21 software (IBM SPSS Inc., Chicago, IL, USA) to assess the relative importance of permafrost thawing status and age in shaping bacterial richness and community structure. The permafrost age was ranked from 1 to 4 for the youngest to the oldest permafrost soils, whereas the thawing status was ranked from 1 to 3 for the active to frozen permafrost. The standardised regression weights were calculated for the bacterial richness and the 1st and 2nd axis coordinates of the NMDS ordination plot. The goodness of fit for the model was judged by the following measures (Guo et al., 2015): (1) comparative fit index (CFI, the model has a good fit when $0.97 \leq CFI \leq 1.00$, and an acceptable fit when $0.95 \leq CFI < 0.97$); (2) goodness–of–fit index (GFI, the model has a good fit when $0.95 \leq GFI \leq 1.00$, and acceptable fit when $0.90 \leq GFI < 0.95$); (3) normed fit index (NFI, the model has a good fit when $0.95 \leq NFI \leq 1.00$ and an acceptable fit when $0.90 \leq NFI < 0.95$); (4) χ^2 test; the model has a good fit when $0 \leq \chi^2 / d.f. \leq 2$ and $0.05 < P \leq 1.00$, and an acceptable fit when $2 < \chi^2 / d.f. \leq 3$ and $0.01 \leq P \leq 0.05$); and (5) the root mean square error of approximation (RMSEA, the model has a good fit when $0 \leq RMSEA \leq 0.05$ and $0.10 < P \leq 1.00$, and an acceptable fit when $0.05 < RMSEA \leq 0.08$ and $0.05 \leq P \leq 0.10$).

3 Results

3.1 The influence of permafrost age and thawing status on soil organic carbon and nitrogen

Across all samples, soil total organic carbon (TOC) ranged from 0.5 % to 35.6 %, and exhibited significant differences by permafrost thawing status (Two–way ANOVA, $P < 0.01$, Fig. 1a), but not by permafrost age ($P = 0.343$, Fig. 1b). The active layer soil exhibited the highest TOC (16.7 %), and was significantly higher than the permanently frozen layer soil (5.6 %, Tukey's HSD $P < 0.001$). Soil total nitrogen (TN) ranged from 0.1 % to 1.5 %, and significant differences were only detected by permafrost thawing status ($P = 0.007$, Fig. 1c), but by permafrost age ($P = 0.446$, Fig. 1d). The active layer soil exhibited the highest TN (0.73 %), and was significantly higher than the permanently frozen layer soil (0.29 %, Tukey's HSD, $P = 0.004$).

3.2 The influence of permafrost age and thawing status on bacteria richness

A total of 1,679,607 bacterial sequences were retained, with an average sequence length of 292 bp. There were 2,659 OTUs identified at the 97 % nucleic acid sequence identity. After rarefying to an

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equal depth, 2,415 bacterial OTUs were retained, and the community was dominated by *Firmicutes* (42 %), *Actinobacteria* (28.9 %), and *Proteobacteria* (10.6 %, Supplementary Fig. 1).

Our results exhibited substantial differences in the bacterial richness among the permafrost soils of different thawing status (Two–way ANOVA, $P < 0.001$; Fig. 2a, Supplementary Table 1) and ages ($P = 0.013$; Fig. 2b). A significantly higher bacterial richness was observed in the active layer soil (358 OTUs) than the transition (287 OTUs; Pairwise Tukey's HSD tests, $P = 0.011$) and the frozen layer soils (248 OTUs, $P < 0.001$, Supplementary Table 2). Young permafrost soil (380 OTUs) exhibited a significantly higher bacterial richness than the medium (265 OTUs, $P = 0.001$), old (287 287, $P = 0.002$), and ancient soils (271 OTUs, $P = 0.009$, Supplementary Table 3).

Within each age category, the significant influence of permafrost thawing was only observed in the young permafrost soil (one–way ANOVA, $P < 0.001$, Fig. 2b, Supplementary Table 4), whereas those in the medium, old, and ancient soils were non–significant ($P = 0.445$, 0.48, and 0.35, respectively). In the young permafrost soil, permafrost thawing significantly increased OTU number from 248 in the frozen layer soil to 471 in the active layer soil (Supplementary Table 5). The increased bacterial richness was mainly attributed to the significantly increase detected in *Firmicutes* (ANOVA, $P = 0.011$), *Actinobacteria* ($P = 0.002$), *Chloroflexi* ($P = 0.002$), *Deltaproteobacteria* ($P = 0.02$), and *Alphaproteobacteria* ($P = 0.008$; Supplementary Table 6).

3.3 The influence of permafrost thawing status and age on bacterial community structure

Bray–Curtis distance based NMDS ordination plot revealed a clear separation of the bacterial community structure by permafrost thawing status (Fig. 3a), while the separation by permafrost age was less obvious (Fig. 3b). The results of DistLM analyses revealed that the measured soil factors, thawing status, and age explained a total of 10.7 % of the bacterial community structure. TN was the most important factor by explaining 7.2 % of the community structure ($P = 0.001$). This was followed by C:N ratio, TOC, soil age and thawing status, which explained additional 3.5 % ($P = 0.028$), 3 % ($P = 0.083$), 2.9 % ($P = 0.105$), and 2.8 % ($P = 0.111$), respectively.

PERMANOVA indicated that significantly different community structure was observed among the various permafrost thawing status and ages (both $P < 0.001$, Supplementary Table 7), and an interactive effect of the two existed ($P < 0.001$). Post–hoc analysis indicated that the community

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structure differences were significantly different among the community structure in soils of different permafrost thawing status (all $P < 0.1$, Supplementary Table 8). In contrast, significant differences were only detected between the young– and older–aged permafrost soils (all $P < 0.05$, Supplementary Table 9), and between the medium– and ancient–aged soils ($P = 0.024$). PERMDISP analysis indicated that the community homogeneity was not significantly different across the different permafrost thawing status ($F(2, 42) = 0.193$, $P = 0.831$). Gradual transition of bacterial community structure due to permafrost thawing was observed in each permafrost age category (Figs. 3c–f). Significantly different soil bacterial community structure across the various thawing status was detected in the young, medium, and ancient–aged permafrost (PERMANOVA, $P = 0.002$, 0.027, and 0.016, respectively, Supplementary Table 10), but not in the old permafrost ($P = 0.124$). Similarly, significantly different soil bacterial structure was also detected among the permafrost of different ages with the same thawing status (Supplementary Table 11, Supplementary Fig. 2).

3.4 The influence of permafrost thawing status and age on the community assembly of bacteria

The average contribution of the stochasticity was 68 %, 74 %, and 86 % in the active, transition, and frozen layers of the permafrost, and significant differences were detected between the active and frozen, and between the transition and frozen layers (both $P < 0.05$, Supplementary Fig. 3a), but not between the active and transition layers ($P = 0.15$). In contrast, the average contribution of stochasticity was 65 %, 76 %, 68 %, and 76 % for the young–, medium–, old–, and ancient–aged permafrost, with no significant difference among the different aged permafrost being detected (all $P > 0.05$, Supplementary Fig. 3b).

3.5 Quantifying the influence of permafrost thawing status and age on bacterial richness and community structure variation

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). In contrast, only permafrost thawing status exhibited a significant contribution to the NMDS1 of the bacterial community structure (standard regression weight of 0.49, $P < 0.001$, Fig. 4b), while permafrost age and thawing status both significantly contributed to the NMDS2 (standard regression

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238 weight of 0.45 and 0.33, respectively, both $P < 0.01$).

239 **4 Discussion**

240 The bacteria richness was significantly higher in the active layer soil (Fig. 2a), and this is consistent
241 with the previous findings that permafrost thawing significantly increases bacterial richness in soil in
242 the Tibetan Plateau and the high Arctic (Chen et al., 2017; Schostag et al., 2019; Wu et al., 2018).
243 Permafrost thawing leads to an accelerated microbial degradation of soil organic carbon that can
244 generate a wide variety of metabolic products (Mueller et al., 2015). The increased metabolic product
245 diversity would lead an increased nutrient diversity and provide additional ecological niches for
246 bacteria (Hernández and Hobbie, 2010). This would explain the increased bacterial richness observed
247 in our study.

248 The soil bacteria in the young permafrost exhibited a stronger response to thawing than those in older
249 permafrost soils (Fig. 2b). The young permafrost soil demonstrated a higher relative abundance of
250 aliphatic carbon but lower carbohydrates than older permafrost soils (Mueller et al., 2015). Thus,
251 bacterial richness could be driven by carbon quality, but not quantity. It has been reported that the
252 degradation of complex carbon molecules requires extensive microbial collaboration, thus leads to a
253 more diverse microbial community in forest soil (Ding et al., 2015). Furthermore, an early study on the
254 freshwater ecosystem also confirmed that organic carbon composition determined bacterial richness
255 and community structure (Docherty et al., 2006). This is in agreement with the higher bacterial richness
256 detected in the active layer of the young permafrost soil (Fig. 2b).

257 The increased bacterial richness due to permafrost thawing was mainly attributed to *Firmicutes*,
258 *Actinobacteria*, *Chloroflexi*, *Deltaproteobacteria*, and *Alphaproteobacteria* in the young permafrost
259 soil (Supplementary Table 6). Increased transcriptional response of *Chlrofolexi* has been reported
260 during permafrost thawing (Coolen and Orsi, 2015), and may be attributed to their recalcitrate organic
261 matter degradation capacity (Colatriano et al., 2018). *Firmicutes* and *Actinobacteria* have been reported
262 to be more abundant in the frozen layer than in the active layer of permafrost soil due to their capacities
263 in maintaining metabolic activity and DNA repair mechanisms at low temperature (Johnson et al., 2007;
264 Tuorto et al., 2014). However, our results showed that their diversity may increase during permafrost
265 thawing, despite their reduction in relative abundance. *Alpha–* and *Delta–proteobacteria* were both

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abundant in the upper permafrost soil in the Tibetan Plateau, and their relative abundance negatively correlated with soil depth (Wu et al., 2017). *Alphaproteobacteria* was identified to be more abundant in the active layer of the permafrost soil in Norway (Mueller et al., 2018). One possible explanation is that the surface active layer may be the major location for root exudates, which favours *Alphaprroteobacterai* (Morgalev et al., 2017). *Deltaproteobacteria* has been reported to have a strong catabolic potential on the degradation of recalcitrate aromatic and other plant detritus (Jansson and Tas, 2014), thus also enhances their richness in the surface active layer of permafrost soil.

PERMANOVA and SEM both demonstrated statistically significant contributions of permafrost thawing and age to soil bacterial community structure (Fig. 4b). However, bacterial communities were better separated by thawing status than by age on NMDS plots (Figs. 3a and 3b). Furthermore, a significantly higher contribution of determinism (lower stochasticity) was observed in the thawed permafrost soils (active and transition layers) than in the permafrost layer, but not between the permafrost soils with different ages (Supplementary Fig. 3). Collectively, this suggests that permafrost thawing have a stronger influence on the bacterial community structure than permafrost age. Our results is 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.

Permafrost thawing significantly increased determinism in bacterial community structure (Supplementary Fig. 3). Increased determinism are frequently attributed to the enhanced environmental filtering (Stegen et al., 2012). Our results demonstrated that nitrogen and the C:N ratio explained a greater proportion of the bacterial community structure than TOC. This is consistent with the previous findings that nitrogen availability strongly regulates microbial community structure and function in the permafrost soils of Arctic and Tibetan Plateau (Chen et al., 2018; Chen et al., 2017; Yergeau et al., 2010). Significantly different soil carbon and nitrogen were observed among the various permafrost thawing statuses, but not among the different permafrost ages (Figs. 1a and 1c). Thus the changed nutrients may explain the significant influence of thawing status on the community structure and assembly processes. The community structure change due to permafrost thawing has also been proposed to be due to the colonization of microorganisms in active layer (Monteux et al., 2018), which coincides with the increased bacterial richness observed here (Fig. 2a).

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294 The influence of permafrost age on bacterial community structure was weaker (Fig. 3b), with only
295 significantly different community structure being observed in the young– and medium–aged permafrost
296 soils (Supplementary Table 8). Substantial influence of permafrost age on community structure has
297 been reported previously (Mackelprang et al., 2017). Investigation on the podegenesis following
298 deglaciation also revealed distinct microbial community structure along the chronosequence (Freedman
299 and Zak, 2015). However, the community structure differences observed were much weaker than that
300 expected particularly between the old and ancient permafrost soils (Supplementary Table 9). This is
301 likely due to the strong influence of permafrost thawing, as thawing enhances environmental filtering
302 (Supplementary Fig. 3) and homogenizes community structure in soils with different ages. This is
303 confirmed by the significantly different bacterial community structure in permafrost soils of the same
304 age along the thawing gradient (except the old permafrost soil, Figs 3c–3f, Supplementary Table 10).

305 Our results also demonstrated that the soil community structure did not converge due to thawing
306 (Supplementary Fig. 2, Supplementary Table 11). This contradicts to previous studies (Deng et al.,
307 2015; Yuan et al., 2018) in the Arctic, but was consistent with Mackelprang (2011). The distinct
308 bacterial community structure in the various aged permafrost soils, yet under the same thawing status,
309 confirms the historic effects of permafrost age on the community structure during permafrost thawing.
310 The distinct bacterial community structure is likely to result in different metabolic functions (Brown
311 and Forsythe, 1974), thus the significantly different bacterial structure under the same thawing status
312 may lead to different organic carbon degradation capacities. Furthermore, older permafrosts enriches
313 pathways involved in the degradation of recalcitrant biomass, while decreases pathways associated
314 with starch and sucrose metabolism comparing with younger soils (Mackelprang et al., 2017). Thus, the
315 thawing of permafrost soils of different ages may also lead to distinct soil carbon degradation schemes.

316 **5 Conclusion**

317 Our results demonstrated that permafrost thawing consistently exhibited greater influence on bacterial
318 richness and community structure than permafrost age. However, permafrost age alters the response of
319 permafrost soil bacteria to thawing, with a stronger response to thawing observed in the young than
320 older permafrost soils. The different community structure during permafrost thawing may present
321 distinct metabolic potentials for soil organic carbon cycling, and may ultimately alter the carbon
322 emission scheme.

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323 **Data availability**

324 Sequence data generated in the present study have been deposited to the National Center for
325 Biotechnology Information (NCBI) Sequence Read Archive under the ID PRJNA554442.

326 **Author contributions**

327 WK conceived the study and developed the idea with MJ, TZ and HZ performed DNA extraction, MJ
328 conducted the data statistical analysis. MJ and WK wrote the first draft of the manuscript, CL and XD
329 revised the manuscript substantially. All authors read and approved the final manuscript.

330 **Competing interests**

331 The authors declare that they have no conflict of interest.

332 **Acknowledgements**

333 This project was financially supported by Chinese Academy of Sciences [grant numbers
334 XDA19070304, QYZDB-SSW-DQC033 and XDA20050101], and National Natural Science
335 Foundation of China [grant number 41771303]. We greatly thank Dr. J Kao – Kniffin for kindly
336 providing the permafrost soil samples for this study.

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Figure legends

Fig. 1 Total organic carbon (a and b) and total nitrogen (c and d) with the permafrost age (young, medium, old, and ancient) and permafrost thawing status (active, transition and permanently frozen).

Fig. 2 Bacterial richness with the permafrost thawing status (a) and age (b). The richness is indicated byoperational taxonomic unit (OTU) number. Different letters indicate significant difference at P <0.05. Young, medium, old, and ancient are permafrost soil ages, active, transition, and permanently frozen are permafrost thawing status.

Fig. 3 NMDS plots showing the bacterial community structure of different thawing status (a) and permafrost age (b). The bacterial community structure of different thawing status in the young, medium, old, and ancient permafrost soils are shown in (c)–(f). Active, transition, and permanently frozen are permafrost thawing status.

Fig. 4 The relative importance of permafrost thawing status and age on bacterial richness (a) and community structure (b) based on structural equation modelling. The community structure variation was assessed by the 1st and 2nd axis coordinates of the NMDS plot (NMDS1 and NMDS2). Numbers adjacent to arrows are the absolute value of the path coefficients, indicative of the standardized effect size of the relationship.*: P <0.05, **: P <0.01 and ***: P <0.001. The arrow thickness represents the strength of the relationship.

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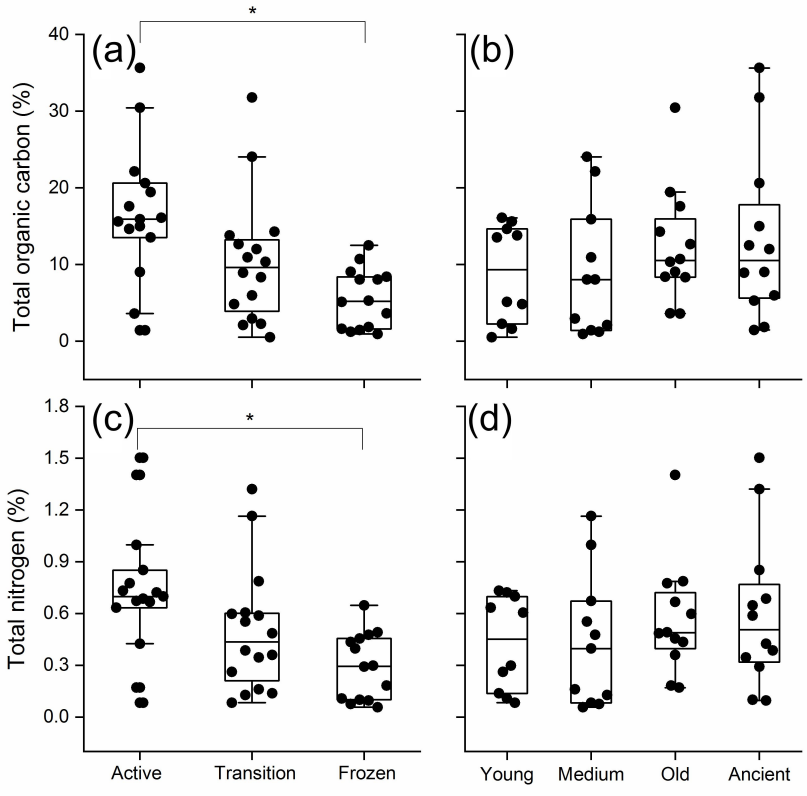
493 Fig. 1



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496 Fig. 2
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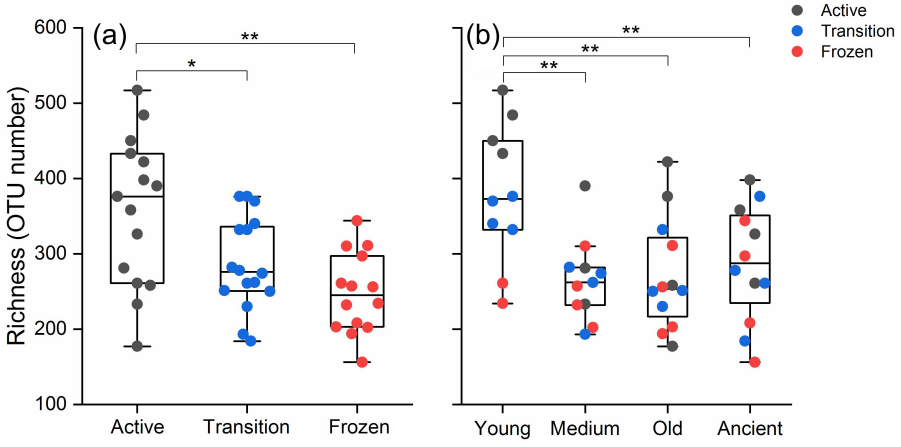

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500 Fig. 3
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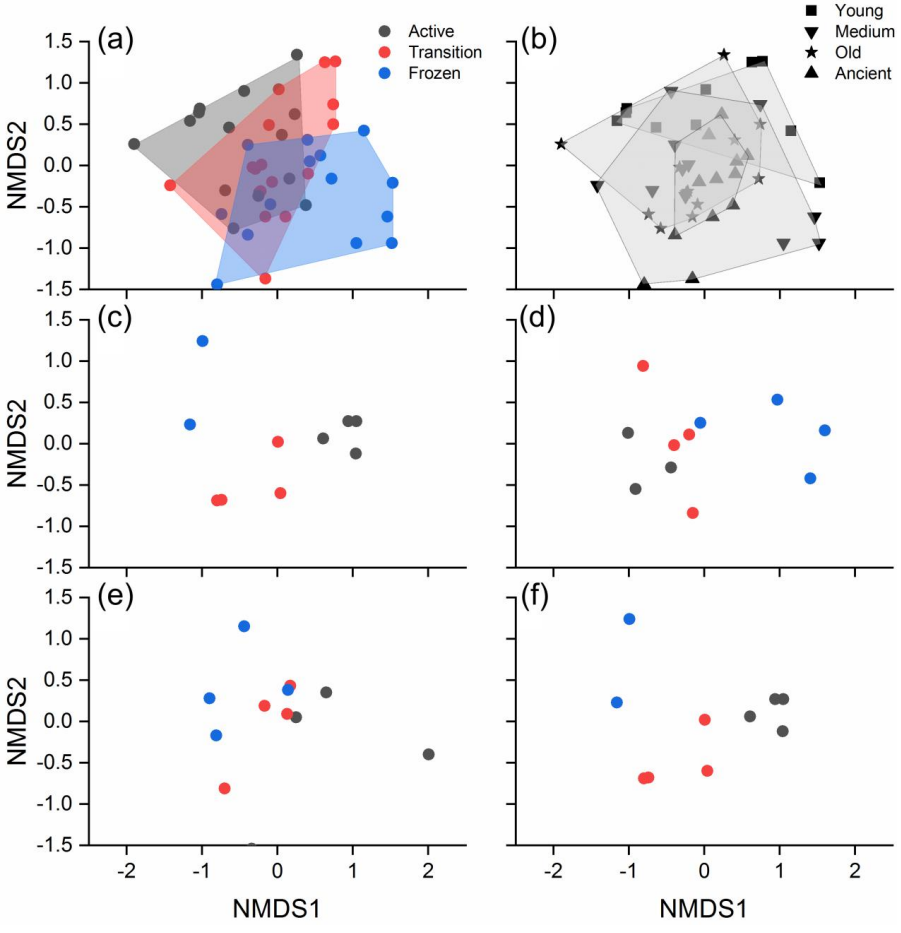

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504 Fig. 4
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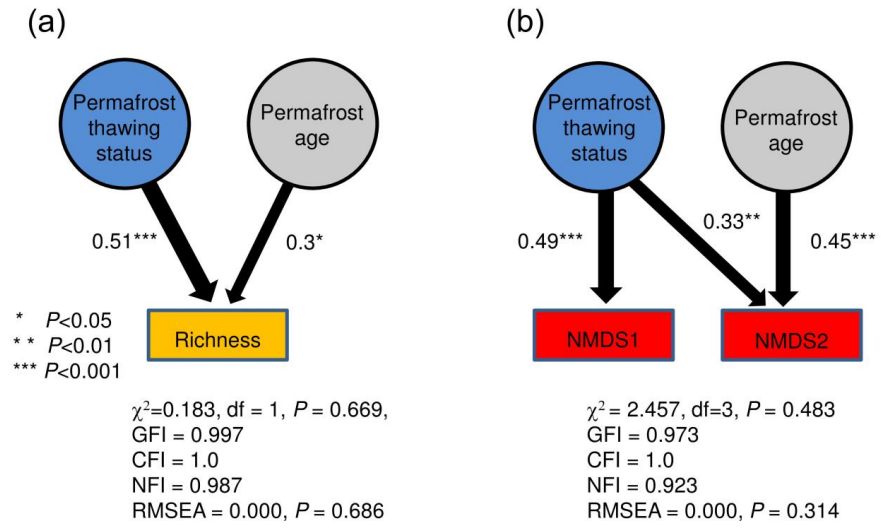

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