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Shifts of tundra bacterial and archaeal communities along a permafrost thaw gradient in Alaska

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Abstract

Understanding the response of permafrost microbial communities to climate warming is crucial for evaluating ecosystem feedbacks to global change. This study investigated soil bacterial and archaeal communities by Illumina MiSeq sequencing of 16S rRNA gene amplicons across a permafrost thaw gradient at different depths in Alaska with thaw progression for over three decades. Over 4.6 million passing 16S rRNA gene sequences were obtained from a total of 97 samples, corresponding to 61 known classes and 470 genera. Soil depth and the associated soil physical–chemical properties had predominant impacts on the diversity and composition of the microbial communities. Both richness and evenness of the microbial communities decreased with soil depth. Acidobacteria, Verrucomicrobia, Alpha- and Gamma-Proteobacteria dominated the microbial communities in the upper horizon, whereas abundances of Bacteroidetes, Delta-Proteobacteria and Firmicutes increased towards deeper soils. Effects of thaw progression were absent in microbial communities in the near-surface organic soil, probably due to greater temperature variation. Thaw progression decreased the abundances of the majority of the associated taxa in the lower organic soil, but increased the abundances of those in the mineral soil, including groups potentially involved in recalcitrant C degradation (Actinomycetales, *Chitinophaga*, etc.). The changes in microbial communities may be related to altered soil C sources by thaw progression. Collectively, this study revealed different impacts of thaw in the organic and mineral horizons and suggests the importance of studying both the upper and deeper soils while evaluating microbial responses to permafrost thaw.

Keywords: 16S rRNA, Illumina MiSeq sequencing, permafrost thaw, soil bacterial and archaeal communities

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Introduction

Permafrost underlies an estimate of 25% of the terrestrial area on Earth. Approximately 1673 Pg of organic carbon (C) was contained in the northern permafrost regions, accounting for 50% of the reported global belowground organic C pool (Tarnocai *et al.* 2009). Meanwhile, the Arctic environments are exposed to greater impacts from global climate warming than any other regions on Earth (Anisimov *et al.* 2007; Romanovsky *et al.* 2010), and an increase of 5–6 °C in average surface air temperature of the Arctic by the end of this century was predicted by the recent International Panel

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on Climate Change (IPCC) report (Seneviratne *et al.* 2012). Such transition will result in a larger thaw area and increased seasonally thawed active layer depth, which in turn will expose the previously frozen permafrost C pool to microbial decomposition. Therefore, C stored in northern permafrost could potentially contribute to the most significant feedback to the warming climate (Schuur *et al.* 2008). Anisimov *et al.* (1999) estimated 25% loss of permafrost C over this century due to warming induced thaw, in the form of released greenhouse gases CO_2 and CH_4 through microbially mediated soil organic C respiration.

In the past decades, a number of studies have monitored a natural permafrost thaw gradient resulted from regional climate warming and showed significant impacts of thaw progression on the landscape, hydrology, plant diversity and ecosystem functioning (Osterkamp 2007; Schuur *et al.* 2007; Vogel *et al.* 2009). The thaw gradient was located on an upland slope in the Eight Mile Lake (EML) watershed near Healy, Alaska. Three sites representing minimal (Mi), moderate (Mo) and extensive (Ex) thaw have been selected for detailed characterization of the aboveground and belowground ecosystem processes (Schuur *et al.* 2007; Vogel *et al.* 2009). The extent of thaw in the three sites was determined based on historical factors and observations from soil temperature, thaw depth, ground subsidence and thermokarst development (Osterkamp & Romanovsky 1999; Osterkamp 2007). The Mi site was characterized by the least permafrost degradation and relatively undisturbed tussock tundra. The Mo site was documented of thaw since 1985 and exhibited some ground subsidence and deeper thawed layer. The Ex site had thawed for more than three decades prior to Mo, had the deepest thawed layers and was characterized of shrub-dominated tundra vegetation. A recent study revealed not only increased ecosystem respiration, but also increased old C loss towards Ex based on radiocarbon ($\Delta^{14}C$) measurements (Schuur *et al.* 2009). Warming and permafrost thaw promoted plant growth, which appeared to have offset the respiration of old C during the initial decades of thaw. However, higher plant C uptake cannot fully balance continued increase in old C loss, and extrapolation of current trend led to an estimated net loss of 4.4–6.0 kg C/m^2 of old C in Ex by the end of the century (Schuur *et al.* 2009). Yet, little was known about the microbial communities who played major roles in C decomposition in these sites, and how they responded to permafrost thaw progression.

This study aimed to characterize the bacterial and archaeal communities in soils sampled from Mi, Mo and Ex sites and reveal their changes in relation to permafrost thaw progression. A total of 97 samples were taken from 18 soil cores in the three sites, including organic and mineral soils in the active layer, as well as soils below the permafrost interface. The profiles of the bacterial and archaeal communities were determined by sequencing the 16S rRNA gene amplicons through the Illumina MiSeq platform. The greater sequencing depth achieved by this technology allows capture of the less abundant taxa and will supply a more thorough characterization of permafrost microbial diversity. We hypothesize that soil chemical–physical properties would have substantial influences on the diversity and composition of the bacterial and archaeal communities. Meanwhile, due to stratified soil abiotic conditions, the changes in bacterial and archaeal communities with thaw progression may be different with respect to soil horizons. Characterization of such changes will reveal responses to thaw progression by different taxonomic groups and within different soil horizons, and facilitate proposal of potential key lineages associated with soil organic matter decomposition.

Materials and methods

Site description and sampling

The sites were located within the discontinuous permafrost zone from the EML watershed in Healy, Alaska (63°52′42.1″N, 149°15′12″W, 700 m elevation) (Schuur *et al.* 2009; Vogel *et al.* 2009; Lee *et al.* 2010; Trucco *et al.* 2012). See Supporting information for detailed site description. A total of 97 soil samples collected from six soil cores at each site, with five to eight samples taken along each core sliced down to a maximum of 116 cm, were used for microbial community characterization and soil chemistry analysis. Soil pH was measured on soil slurries using a standard pH metre. Soil moisture was measured using a wet/dry soil conversion with a soil subsample dried at 105 °C for 12 h. Bulk density was calculated with soil moisture and the known volume for each soil sample. Soil subsamples were dried at 70 °C and measured for C and N content using a Costech CN Analyzer (Valencia, CA, USA) and reported as per cent of dry soil. Soil temperature was monitored in three different boreholes adjacent to sampled soil cores within each site. Soil temperatures were recorded at four depths, 10, 20, 30 and 40 cm, and were recorded every 2 h throughout the growing season.

DNA extraction, amplification and sequencing

Soil DNA was extracted using a PowerMax Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). DNA concentration was measured by Pico Green using a FLUOstar OPTIMA fluorescence plate reader (BMG LABTECH, Jena, Germany). The V4 region of the

16S rRNA genes was amplified with the primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) combined with Illumina adapter sequences, a pad and a linker of two bases, as well as barcodes on the reverse primers (Caporaso *et al.* 2012). Sample libraries were generated from purified PCR products. The Miseq 300 cycles kit was used for 2 × 150 bp paired-ends sequencing on Miseq machine (Illumina, San Diego, CA, USA). Refer to Supporting information for detailed procedures of PCR amplification, purification and library preparation.

Sequence data preprocessing and statistical analysis

Raw sequences with perfect matches to barcodes were split to sample libraries and were trimmed using BTRIM with threshold of QC higher than 20 over 5 bp window size and the minimum length of 100 bp (Kong 2011). Forward and reverse reads with at least 10 bp overlap and lower than 5% mismatches were joined using FLASH (Magoč & Salzberg 2011). After trimming of ambiguous bases (i.e. N), joined sequences with lengths between 240 and 260 bp were subjected to chimera removal by U-Chime (Edgar *et al.* 2011). OTU clustering was through UCLUST at 97% similarity level (Edgar 2010), and taxonomic assignment was through RDP classifier (Wang *et al.* 2007) with a minimal 50% confidence estimate. The above steps were performed through the Galaxy pipeline (http://zhoulab5.rccc.ou.edu/) developed by Y. Qin (unpublished). Subsequent analyses were performed in R (R Core Team 2014). Singletons were removed for downstream analyses. Samples were rarefied at 20 000 sequences per sample. Dissimilarity tests were based on Bray–Curtis dissimilarity index using analysis of similarities (ANOSIM) (Clarke 1993) under the package VEGAN (v.2.0-3) (Oksanen *et al.* 2012). To make principle coordinate analysis (PCoA) of UniFrac distances, OTU representative sequences related to archaea and chloroplast were first removed. The leftover sequences were aligned against GreenGenes public 16s rRNA database (http://greengenes.lbl.gov) using PYNAST (Caporaso *et al.* 2010), followed by tree computation with FASTTREE (Price *et al.* 2009). The PCoA analysis was based on the weighted UniFrac distance matrix using the classical multidimensional scaling method (Coxon 1997). Differences in abundances across soil layers and across sites were determined by ANOVA analysis followed by Least Significant Difference (LSD) test (Calinski 1981).

Results

Soil physical and chemical characterization

A summary of active layer thickness in Mi, Mo and Ex sites, and the thaw depth in each soil core was described in (Table 1). At the time of sampling, the thawed layer was within the upper organic horizon, and the thaw depths were significantly deeper and with higher variation in Ex compared to Mo and Mi. Based on the stratigraphic profile of the soil transacts, we divided the soil cores into four layers (Fig. 1b): the first layer (L1) refers to the thawed soil at the time of sampling within the organic horizon; the second layer (L2) contains the frozen soil within the organic horizon; the third layer (L3) contains soil within the seasonally thawed mineral horizon, that is above the permafrost interface; the fourth layer (L4) refers to soil at or below the permafrost interface and includes only seven soil samples. The purposes of defining these soil layers were (i) to reveal how the categorical soil properties, that is thawed vs. frozen, organic vs. mineral prominence, active vs. permafrost layer, affect the structure and diversity of microbial communities and (ii) whether microbial communities residing at different soil layers respond differently to the thaw progression.

The soil pH, water content, total C, total N and bulk density were all strongly correlated with soil depth (one way ANOVA, $P < 0.01$) (Fig. S1, Table S1, Supporting information), whereas no association with site was observed. C content decreased along soil depth, from around 40% in L1 to 20% in L4. N content was the highest at L2. Soil moisture decreased along depth, dropping from 60% to 80% in organic layer to 20% to 60% in mineral layer. The surface soil was the most acidic, with pH around 4.0, which increased to nearly 6.0 in deep soils. Soil temperature was the highest at 10 cm and decreased to comparable levels at 30 and

Table 1 Site characteristics of Mi, Mo and Ex

Site	Soil temperature* (°C, 10 cm)	Active layer thickness/cm	Thaw depth/cm	Number of samples (L1/L2/L3/L4)
Ex	9.1 ± 0.2	78 ± 5	9, 13, 20, 25, 34, 39	8/16/5/4
Mo	8.8 ± 0.1	70 ± 2	15, 15, 16, 16.5, 17, 19	6/14/13/2
Mi	6.8 ± 0.2	69 ± 2	13, 14, 14, 15, 18, 18	6/12/10/1

*Soil temperature refers to mean (±SE) growth season soil temperature at the depth of 10 cm (Schuur *et al.* 2009).

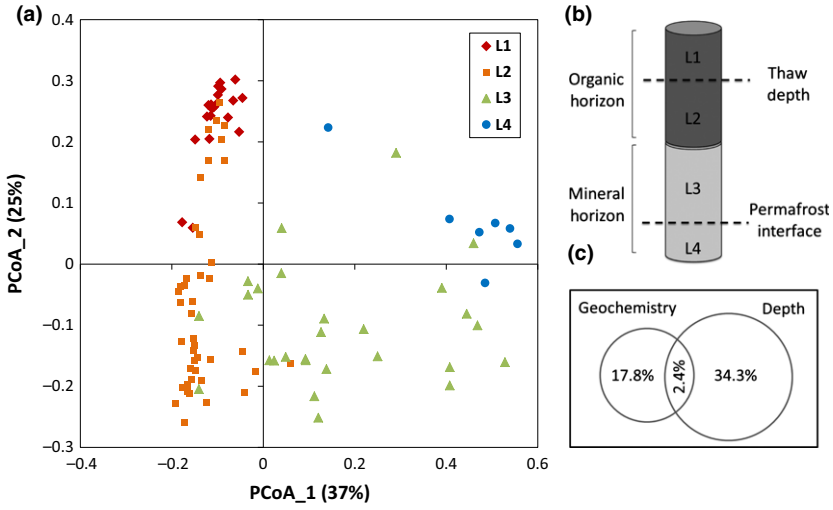

Fig. 1 (a) PCoA plot of bacterial communities from the four soil layers. Each point corresponds to a sample. Dissimilarities among communities were calculated based on weighted UniFrac distances. (b) Categorization of soil into four layers based on thaw depth, soil organic vs. mineral prominence and active layer depth. (c) Explained proportions of variance by soil geochemistry and depth.

40 cm. Ex had the highest daily maximum and minimum temperatures at all depths, whereas the temperature difference between Mi and Mo was not significant (Fig. S2, Supporting information). In all three sites, the soil at 10 cm had the largest variation of temperature compared to deeper soils (Fig. S2, Supporting information). Such variation of soil properties was expected to influence the diversity and composition of microbial communities inhabiting soils at different depths.

Structure of permafrost bacterial and archaeal communities

Over 4.6 million passing sequences were obtained from the total 97 samples. After OTU clustering at 97% sequence identity, removal of singletons and rarefaction at 20 000 sequences per sample, 34 305 OTUs remained, out of which 18 590 OTUs were mapped to 470 known genera (Wang *et al.* 2007). Archaea accounted for 0.31% (59 OTUs) of total population, of which 52% were putative methanogens. Within the bacterial domain, Acidobacteria were the most abundant phylum (31.6% in relative abundance), followed by Verrucomicrobia (17.7%), Proteobacteria (16.1%) and Actinobacteria (15.2%). At the genus level, 32.3% sequences were not assigned to any known genus. OTUs belonging to the *Gp1*, *Gp2* of Acidobacteria (23.7% and 2.3% in relative abundance), as well as a *Subdivision 3 genera incertae sedis* (12.0%), *Spartobacteria genera incertae sedis* (4.7%), *Conexibacter* (3.3%), *Pseudolabrys* (2.3%) and *Steroidobacter* (2.2%) together accounted for 50.5% of total abundance.

Effects of soil physical–chemical on microbial community diversity and composition

Soil depth greatly impacted the α-diversity of microbial communities, yet there was no association with the degree of thaw progression. Both OTU richness [the observed number of OTUs and Chao1 diversity estimator (Chao 1984; Colwell & Coddington 1994)] and evenness decreased as a function of soil depth ($P < 0.001$) (Fig. 2). However, there was no significant difference in richness or evenness among sites with all samples or samples within any soil layer. The principle coordinates analysis (PCoA) based on weighted UniFrac distances showed a combined effect of soil physical–chemical properties on the β-diversity of the bacterial communities (Fig. 1). The two dimensions together explained 61.9% of the observed variation. PCoA_1 was significantly correlated with all measured soil physical–chemical indexes and was best related to soil type ($r^2 = 0.604$, $P < 0.001$) and pH ($r^2 = 0.513$, $P < 0.001$). The ordination in PCoA_2 was primarily driven by differences among communities above and below thaw depth ($r^2 = 0.489$, $P < 0.001$). Neither PCoA_1 nor PCoA_2 was significantly related to the thaw gradient. Using partitioning analysis, soil depth and the combined effect of soil physical–chemical as well as their interactions explained 54.5% of the observed variation. Community compositions in the four soil layers were significantly different from each other (Table S2, Supporting information), which was also reflected on the ordination plot.

Changes of composition of bacterial and archaeal communities along soil depth

Abundance patterns of different taxonomic groups along soil transects were analysed both in relation to soil depth and soil layer. Based on Pearson's correlation with soil depth, the abundances of 15 classes, including Spartobacteria, Sphingobacteria, Alphaproteobacteria, Gammaproteobacteria, and Acidobacteria Gp 2 and 3 were negatively correlated with depth, accounting for

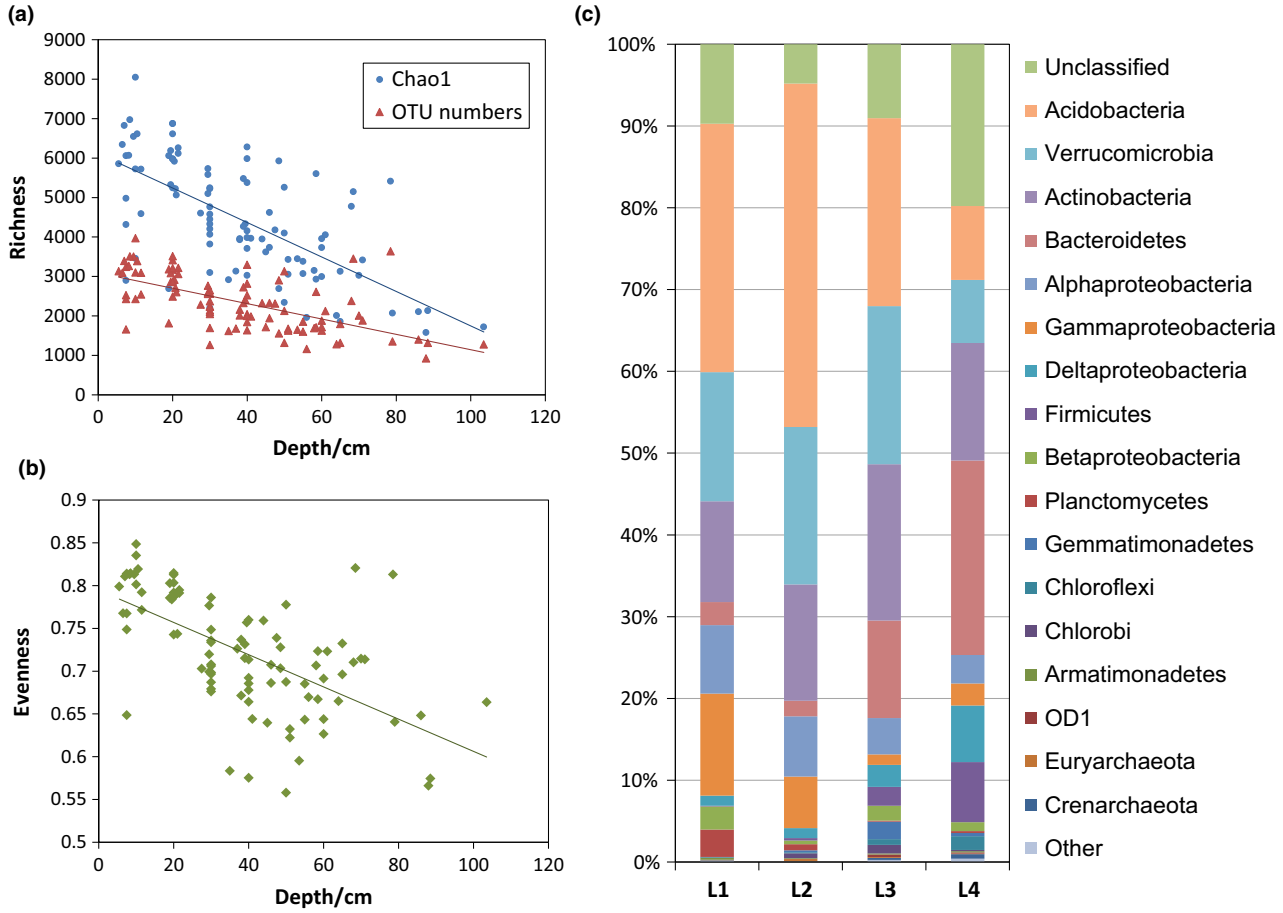

Fig. 2 Relationships between microbial richness (a) and evenness (b) with soil depth. Chao1 estimator was used to infer the true species diversity. Chao1 richness, observed OTU numbers and community evenness were all negatively correlated with soil depth ($P < 0.001$). (c) Community composition in four soil layers at the phylum level. The phylum Proteobacteria was represented by Alpha-, Beta-, Gamma- and Delta-divisions.

48.9% of the total population (Fig. S3, Supporting information). On the contrary, abundances of 29 classes (22.4% of total population), including Actinobacteria, Deltaproteobacteria, Clostridia and some groups of Acidobacteria (Gp 6 and 16), increased with depth. At the genus level, abundances of 50 genera (relative abundance >0.01%) decreased with depth, whereas abundances increased with depth in another 57 genera (Fig. S4, Supporting information). Yet, some genera did not exhibit a linear correlation with depth, but were still distinctive among layers. For instance, the genus *Geothrix*, as well as *Acidobacteria Gp1* had the highest abundances particularly in L2; *Subdivision 3 genera incertae sedis* and *Pseudolabrys* had higher abundances in both L2 and L3. We also observed increased fractions of unclassified taxa with depth (Fig. S5, Supporting information). Particularly in L4, 3205 OTUs (43.3% OTUs, representing 61.5% of total abundance in L4) were not classified at the genus level, and 1367 OTUs (18.5% OTUs, 41.6% of total abundance in L4) were not classified at the class level, indicating a dearth of knowledge on the microbial diversity of the permafrost. The changes in community structure and the distribution of phylogenetic lineages along soil depth were primarily driven by the gradients of soil abiotic conditions (Figs S3 and S4, Supporting information). Correlations with C and N were found in 82 and 18 genera (relative abundance >0.01%, Pearson correlation $P < 0.05$), respectively. Correlations with pH and moisture were found in 107 genera, identical to those correlated with soil depth ($P < 0.05$). Significant changes of microbial community compositions with regard to organic or mineral (O/M) prominence, above or below thaw depth (ATD/BTD), as well as active or inactive layer (Ac/InAc) were also revealed in 93, 47 and 28 genera (ANOVA $P < 0.05$), respectively. Overall, these associations of taxa abundances with soil physical–chemical properties were consistent with their associations with soil depth.

Methane cycling populations in the tundra soils

In wet tundra lands, methane cycling is an important ecosystem process mediated by methanogens and methanotrophs, while methane release is the direct net result of methanogenesis and methane oxidation processes. In total, we identified 3134 sequences from 16 OTUs associated with known methanogens, and 9070 sequences from 202 OTUs associated with methanotrophs and methylotrophs. Methanobacteria and Methanomicrobia were the two classes of methanogens found, with the former accounting for 93.6% of the methanogen population. Particularly, two OTUs (OTU_163, OTU_194), both categorized as *Methanobacterium*, accounted for 92.6% of all methanogens, with OTU_163 alone accounting for 82.1%. Another OTU (OTU_455), categorized as *Methanosarcina* under Methanomicrobia, represented 4.2% of all methanogens. The abundance of Methanomicrobia did not vary significantly among soil layers, whereas *Methanobacterium* showed the highest abundance in L2 (Fig. S6, Supporting information).

A total of 150 OTUs (8438 sequences) from eight genera were associated with potential methanotrophs, out of which 84 OTUs (61.7% in abundance) were Type II methanotrophs belonging to four genera (*Methylocapsa*, *Methylocella*, *Methylocystis*, *Methylosinus*) under Alphaproteobacteria, and 66 OTUs belonged to the Type I methanotrophs within the family of Methylococcaceae under Gammaproteobacteria. Two genera, *Methylocystis* (Type II) and *Methylobacter* (Type I), consisted 90.5% of identified methanotrophs. *Methylobacter* was almost exclusively found in the lower two soil layers (mineral soil), and peaked in L4, whereas abundances of *Methylocystis* did not differ among soil layers (LSD test, $P > 0.05$). Interestingly, 80% of sequences from *Methylobacter* were from one single OTU_3355, and 72.8% of sequences from *Methylocystis* were from OTU_27833. An additional 52 OTUs, despite their lower abundances (631 sequences in total), were associated with methylotrophs, including members from *Methylovirgula*, *Hyphomicrobium*, *Methylobacterium*, *Hansschlegelia*, *Methylopila* in Alphaproteobacteria, as well as *Methylophilus* and *Methylotenera* from Betaproteobacteria. These methylotrophs, though not capable of directly consuming methane, can make use of oxidized products of methane such as methanol and formaldehyde and were therefore also considered important in methane removal processes in natural environments (Chistoserdova *et al.* 2009). In addition, there were another 50 OTUs (1068 sequences) uncategorized at the genus level, yet all within the family Methylocystaceae, which contains mostly methanotrophs or methylotrophs. The abundance of these unknown Methylocystaceae was the highest in L1 and was nearly undetectable in L3 or L4.

Significant effects of thaw in lower organic and mineral horizons

The effect of thaw on microbial communities was tested in L1, L2, as well as combined L3 and L4 (L3–L4) due to limited number of samples in L4 alone. Significant differences in the community structure among sites were only observed in L2 and L3–L4 (Table 2). As both richness and evenness of the microbial communities were at similar levels among sites at any soil layer ($P > 0.05$ by ANOVA), the differences among sites should be driven by compositional changes among the microbial communities. Within L2, the trend of decreasing abundances from Mi to Ex was observed in Sphingobacteria, Gammaproteobacteria and Spartobacteria (Figs 3 and S7, Supporting information). At the genus level, abundances of 14 genera (with relative abundances >0.01%) were significantly lower in Ex compared to the other two sites, out of which two genera, *Conexibacter* and *Spartobacteria genera incertae sedis*, contributed to 89.6% of the associated sequences. *Dehalogenimonas* and *Rhodoblastus* were the only genera with higher abundances towards Ex.

In L3–L4, Gammaproteobacteria, Acidobacteria Gp 4 and 6, as well as Thermoprotei of Crenarchaeota showed the highest abundance in Ex (Figs 3 and S7, Supporting information). At the genus level, 39 genera (relative abundance >0.01%) showed different abundances across sites, all of which except *Anaerovorax* of the Clostridia class were of higher abundances in Ex. Sixteen of these genera belonged to the Actinomycetales order, representing 18.7% of the associated sequences. Also included were four genera of the Rhizobiales order (6.4%), including *Devosia*, *Rhizobium*, *Hyphomicrobium* and *Rhodoplanes*, as well as four genera belonging to Chitinophagaceae of the Sphingobacteriales order (5.7%), including *Chitinophaga*, *Ferruginibacter*, *Segetibacter* and *Terrimonas*. The order Rhizobiales was known to contain symbiotic N-fixing bacteria; Members of the Chitinophagaceae family have also been reported of chitinolytic activities (Sangkhobol & Skerman 1981). Therefore, the changes in abundances of these populations

Table 2 Test of community dissimilarity among sites within each soil horizon by ADONIS

Pr	L1	L2	L3–L4
All sites	0.233	**0.005**	**0.031**
Ex vs. Mo	0.181	0.071	0.117
Ex vs. Mi	0.181	**0.003**	**0.006**
Mo vs. Mi	0.507	0.069	0.128

Pr values of <0.05 are highlighted in bold.

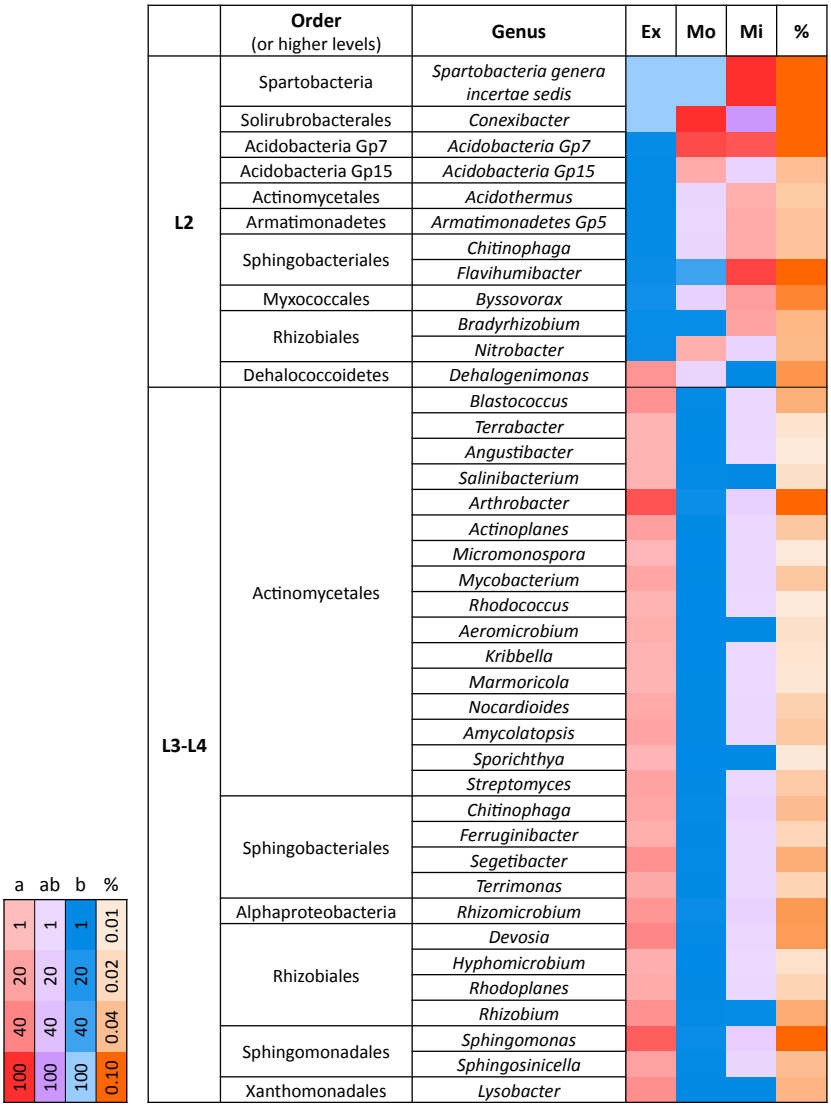

Fig. 3 Selected genera with different abundance levels among sites within the L2 and L3–L4 soil layers. Refer to Fig. S7 (Supporting information) for the complete list. The colour panel under 'Ex', 'Mo' and 'Mi' reflects both the absolute abundance (sequence count) of the corresponding genus and the relative abundance level at each site by LSD test. Red toned cells (a) represent the highest abundance level; Blue toned cells (b) represent significant lower abundance levels compared to (a); Purple toned cells (ab) represent intermediate abundance levels between (a) and (b). The absolute abundances were reflected by the colour gradient within the red, blue and purple tones. The colour panel under '%' reflects the relative abundance of the corresponding genus.

could indicate a shift in ecosystem functions performed by these micro-organisms.

Discussion

Vertical distribution of phylogenetic groups was driven by soil physical–chemical conditions

Due to stratified soil conditions owing to different compositions of organic/mineral materials, seasonal freeze–thaw cycles restricted in the active soil layer, as well as vertical gradients of C, N contents, pH and other conditions, the permafrost underlain tundra soil provides stratifically heterogeneous habitats to microbial communities. Here, soil depth and its associated physical–chemical properties drove the variation in the diversity and composition of bacterial and archaeal communities. Vertical distribution of microbial communities in Arctic soils has been previously reported (Steven *et al.* 2008; Wagner *et al.* 2009; Yergeau *et al.* 2010; Wilhelm *et al.* 2011). However, these studies typically included only one or a few soil cores from each location. In this study, we surveyed a total of 18 soil cores from three sites with different above-ground vegetation. Hence, the observed abundance patterns along soil depth were less affected by the heterogeneity of the tundra soil ecosystem itself and were statistically more confident.

The near-surface layer L1 was the first to have thawed during the growing season, had the highest C content and was the major location for root exudates, which typically favoured the growth of copiotrophic micro-organisms (Fierer *et al.* 2007). L1 soil harboured the highest relative abundances of Gamma- and Alpha-subdivisions of Proteobacteria, primarily represented by obligate aerobes of the Xanthomonadales order, heterotrophic and photosynthetic members from

Rhodospirillales, and the Rhizobiales order that is commonly associated with N-fixing, microsymbiotic bacteria. Compared to L1, deeper soil layers were characterized of lower temperature and lower C content (Figs S1 and S2, Supporting information). Longer periods of frozen state also pose greater stress to microorganisms by lowering water activity and nutrient exchange (Morozova & Wagner 2007). In particular, L4 included only samples reaching below the permafrost interface and represented a habitat that is frozen throughout the years. Redox condition of this horizon was most likely constantly negative owing to permanent water saturation (Fiedler *et al.* 2004), which further impedes aerobic C decomposition. Accordingly, we observed increased abundances of fermentative members of Chloroflexi (Anaerolineae), Bacteroidetes (Bacteroidia) as well as the spore-forming Firmicutes (Clostridia) with soil depth, indicating the potential for anaerobic degradation in deeper soil horizons. The abundances of some groups of Acidobacteria (Gp 6 and 16) also increased with depth, although their ecological relevance was poorly understood. Actinobacteria had similar abundances in all four layers, consistent with previous findings that this group persisted over seasons in a tussock tundra soil (McMahon *et al.* 2011). Actinomycetales of Actinobacteria was reported to have adapted to environments with low C availability (Fierer *et al.* 2003) and showed the highest abundance in L3, which contained relatively lower C. Survival of *Arthrobacter* was found in ancient permafrost soil samples, which was probably associated with their active DNA repair activities under frozen conditions (Johnson *et al.* 2007). Consistently, *Arthrobacter* was of the highest abundance in L4 soil. In summary, the observed abundance patterns suggested a close association between the physiology of the corresponding lineages and the abiotic soil conditions. The depth of sequencing in this study also furthered our understanding of these patterns at finer taxanomic resolutions.

A few OTUs dominated the methanogen and methanotroph populations

Permafrost underlain ecosystems on the northern hemisphere comprise the largest natural source of methane on Earth and are responsible for 25% of total methane released into the atmosphere from natural environments (Fung *et al.* 1991; Chasar *et al.* 2000). Methanogenesis performed by archaeal methanogens is an important terminal step of anaerobic C degradation, while methane oxidation by bacterial methanotrophs is a sink before it releases to the atmosphere (Le Mer & Roger 2001). The permafrost underlain tundra soil of the studied sites hosted both acetoclastic (Methanomicrobia) and hydrogenotrophic (Methanobacteria) members of the methanogen community. The hydrogenotrophic Methanobacteria accounted for 93.6% of total methanogens and was primarily detected in the active soil layers (L1–L3), whereas the acetoclastic Methanomicrobia, though with much lower abundance, increased towards L4. Our results indicated hydrogenotrophic methanogenesis was most likely the dominant pathway of methanogenesis in the active layers at the studied sites. The distribution of Methanobacteria also agreed with previous findings that they were more commonly found in upper soils (Conrad *et al.* 1987; Thauer 1998) and under low pH (Kotsyurbenko *et al.* 2007). BLAST analysis of the representative sequence of OTU_163, which alone contributed to 82.1% of total methanogens, revealed 99% sequence identity to *Methanobacterium lacus*—a species with a type strain isolated from the sediment of a deep, meromictic freshwater lake (Borrel *et al.* 2012). Past studies have presented evidences of consistent patterns between methanogenesis and methanogen abundance (Waldrop *et al.* 2010; Lipson *et al.* 2013). Therefore, the predominant proportion of *M. lacus* indicated its potential as the keystone species in methanogenesis in this ecosystem.

In addition, we identified two OTUs representing 68.3% of the total methanotroph population, with OTU_3355 dominating the sequences from *Methylobacter* (80%), and OTU_27833 dominating those of *Methylocystis* (72.8%). The representative sequence of OTU_3355 shared 99% identity with the *Methylobacter psychrophilus* type strain Z-0021, which was the first psychrophilic methanotroph isolated from a tundra soil (Trotsenko & Khmelenina 2005). Strain Z-0021 had optimal growth temperature ranged from 3.5 °C to 10 °C, which may explain the relative higher abundance of OTU_3355 in L3 and L4, where the temperature was significantly lower than the upper layers. The representative sequence of OTU_27833 shared 98% sequence identity with *Methylocystis rosea* SV97 (Wartiainen *et al.* 2006), a strain isolated from Arctic wetland soil. SV97 also possesses the *nifH* gene, indicating its potential of N fixation. The OTUs identified in this study, due to their higher relative abundances, were probably potential key players in the methane cycling of this environment. Further studies on the isolation and characterization of those micro-organisms will facilitate our understanding of their physiological functions and ecological importance.

Changes of microbial communities in L2 and L3–L4 suggested different responses to permafrost thaw progression

Despite the importance of microbial communities to the stability of permafrost C storage, a sound understanding

of their responses to permafrost thaw is still lacking. Recent advances in investigating permafrost microbial communities and their influences on soil organic C decomposition have revealed a diversity of responses to climate warming and associated permafrost thaw. Studies have mostly focused on permafrost thaw through short-term laboratory incubation and long-term field warming experiments. On one hand, short-term incubation studies using active layer and permafrost soils at above-zero temperatures revealed rapid changes of bacterial and fungal community composition, exoenzyme activities, and abundances of genes involved in soil C and N cycling pathways (Coolen *et al.* 2011; Mackelprang *et al.* 2011). Coolen *et al.* reported different responses of bacterial and fungal groups to thaw in the incubated active layer and permafrost soils. Mackelprang *et al.* showed that the composition of permafrost microbial communities quickly converged towards those in the active layer soil upon thaw. These studies provided important insights into immediate changes of microbial activities and composition upon permafrost thaw. However, they lacked the predicting power of long-term dynamics of the microbial communities and their ecosystem functions. On the other hand, investigations of long-term experimental warming of tundra soils have reached a diverse range of conclusions about ecosystem and microbial responses. Deslippe *et al.* investigated soil bacterial and fungal community compositions from plots warmed by greenhouses for 18 years at the US Long Term Ecological Research site and found the strongest warming effect in the organic horizon (Deslippe *et al.* 2012), highlighted by lowered bacterial and elevated fungal community evenness based on automated ribosomal intergenic spacer analysis. However, Sistla *et al.* (2013) demonstrated stronger effects in the mineral horizon at the same site through characterization of microbial biomass, activity and extractable nutrient pools. The authors proposed that warming had stimulated soil decomposer activity in the mineral horizon, which probably resulted from greater C input into deep soil due to increased root biomass. In addition, investigations targeting several functional genes have revealed distinct responses by microbial communities carrying these genes (Deslippe *et al.* 2005; Walker *et al.* 2008; Lamb *et al.* 2011), suggesting nonmonotonic responses by different microbial populations. The observations from these studies indicated substantial impacts of warming on the composition and structure of tundra soil microbial communities and highlighted the importance of analysing the changes of the microbial communities at finer taxonomic resolutions. Although some of these studies did not specifically address the ecosystem responses to permafrost thaw, long-term warming can result in aggravated permafrost thaw although increasing both the soil temperature and the thickness of seasonally thawed layer (Natali *et al.* 2011), which could pose indirect impacts on microbial communities and ecosystem functions.

This study used the 16S rRNA gene as the molecular marker to profile the archaeal and bacterial communities in three sites along a natural permafrost thaw gradient of over three decades and revealed significant effects of thaw progression on community composition and structure in the lower organic horizon (L2) and the mineral horizon (L3–L4). The absence of thaw effects in L1 may be due to greater temperature variation in near-surface soils (Fig. S2, Supporting information). The soil at 10 cm depth experienced daily ΔT of up to 7 °C during the month of sampling, while in soil at or below 20 cm, the daily ΔTs were significantly smaller (<4 °C) in all sites. As most L1 samples were from soils of the upper 15 cm in depth, microbial communities in L1 were apparently exposed to greater stress from daily temperature variation, which might have overwhelmed the effects from the permafrost thaw progression.

Interestingly, we observed different responses of the microbial communities to the permafrost thaw gradient in L2 and L3–L4 soils. In L2, the differences among sites were dominated by decreased abundances of associated taxa in Ex. Specifically, 14 of the 16 genera (with relative abundances >0.01%) that showed varied abundances across sites were of lower abundances in Ex. On the contrary, in L3–L4, majority of the associated taxa (38 of 39 genera) increased their abundances in Ex. Although the causes of such observation is not yet clear, the fact that the impacted taxa included those previously found to be involved in C and N cycling in natural environments suggested the likelihood of altered ecosystem processes in these permafrost underlain soils.

Conexibacter and *Spartobacteria genera incertae sedis* were the two dominant genera with decreased abundances at Ex in L2. *Conexibacter* is a deep-branching genus in the class Actinobacteria, yet understanding of its ecological roles is still limited. Type strains of *Conexibacter* have been obtained from two soils and were reported to be slow-growing micro-organisms and with G + C content of over 70%, which could favour survival under stressed conditions (Monciardini *et al.* 2003; Seki *et al.* 2012). Spartobacteria comprises one of the primary groups of Verrucomicrobia and is widely distributed in soil and aquatic environments (Janssen *et al.* 2011). Verrucomicrobia were proposed to be responsible for degradation of polysaccharides originated from phytoplankton in an Arctic marine water environment (Cardman *et al.* 2014). A Spartobacteria genome assembled from the Baltic seawater metagenome was also found to be rich in glycoside hydrolases, which probably allowed the use of a range of C sources including

starch, xylan, cellulose, chitin and even sulphated carbohydrates (Herlemann *et al.* 2013). In the tundra soils of this study, Spartobacteria accounted for 4.0% of total prokaryotic community. Although their role in C cycling of the tundra soil ecosystem is not yet clear, the decreased abundance of Spartobacteria in L2 of Ex suggested thaw progression resulted in a less favoured habitat for these micro-organisms.

In L3–L4, among the 38 genera with increased abundances as permafrost thaw progresses, 16 genera belong to Actinomycetales. Actinomycetales is an order of Actinobacteria, which is generally known as *K*-strategists in soil and candidates of degrading relatively complex, recalcitrant C sources (Goodfellow & Williams 1983). Mackelprang *et al.* (2011) observed a quick boost of Actinobacteria abundance upon thaw during a short-term incubation of permafrost soil at 5 °C. Long-term warming of an Arctic tundra soil also led to increased dominance of Actinobacteria, which was proposed to be associated with reduced availability of labile C sources (Deslippe *et al.* 2012). Here, boosted abundances of 16 genera of Actinomycetales in Ex may be associated with reduced substrate quality in the mineral horizon by long-term permafrost warming. In addition, thaw progression increased the abundances of *Chitinophaga*—a genus reported to be strongly chitinolytic (Sangkhobol & Skerman 1981), as well as that of Sphingomonadales, members of which were reported to be capable of degrading aromatic compounds (Balkwill *et al.* 2006). Potential N fixers including *Rhizomicrobium*, *Devosia* and *Rhizobium* were also of higher abundances in Ex compared to Mi and Mo.

Overall, the patterns of changes in the abundances of associated taxa with thaw progression probably suggest different impacts of permafrost thaw on soil microbial communities in the organic (L2) and mineral (L3–L4) horizons. Such observations may indicate different ecosystem processes, for example C dynamics, in the organic and mineral soil horizons and their responses to thaw. In particular, previous analyses of such ecosystems revealed higher plant primary production by thaw progression, with nearly 36% increase in Ex compared to Mi (Schuur *et al.* 2009). Such a boost in plant growth has probably led to increased C input through plant root exudates, especially in the organic horizon and most likely in the form of labile C substrates. On the other hand, ecosystem respiration rate increased by 39% in Ex compared to Mi. Partitioning analyses suggested greater contribution from deeper soil respiration to total ecosystem respiration as thaw progresses. Particularly, the contribution from respiration of old C almost doubled in Ex compared to Mi (16% in Ex, 14% in Mo and 8% in Mi). As old C accumulated during permafrost formation was mostly located in deeper horizons, among which labile C sources were preferentially consumed by micro-organisms, the greater amount of respired old C in Ex suggested further loss of labile C especially in the deeper mineral horizon. Although direct characterization of soil C sources in these sites is not yet available, the above observations suggest a possible scenario that thaw progression could have differentially impacted the C distribution in organic and mineral horizons, which would in turn influence the inhabited microbial communities and ecosystem functioning. In addition, a recent study of the active layer and permafrost microbial communities at a site with comparable levels of C content suggested C limitation in deeper soils based on extracellular enzymatic potentials (Tas *et al.* 2014). In this case, the increased abundance of Actinomycetales in L3–L4 appears to be consistent with such a scenario, that thaw progression led to greater needs to utilize recalcitrant C sources in the mineral horizon. Nonetheless, any conclusion concerning the C dynamics and associated communities should be drawn with caution at the current stage. The lack of knowledge about the physiology and ecology of a significant fraction of archaeal and bacterial taxa hampered our understanding of their roles in the permafrost C cycle. Therefore, more emphasis should be placed on pure-culture studies related to permafrost environments to provide references for their physiology and C decomposition capabilities. Detailed physical and chemical analyses on soil C structure will provide direct evidences for C availability to micro-organisms in these environments. Enzymatic assays and approaches (e.g. single cell genomics) targeting C decomposers will also advance our understanding of C turnover processes and key players in the permafrost C cycle and their dynamics during warming-induced permafrost thaw.

Conclusion

We surveyed the bacterial and archaeal communities in the permafrost-underlain tundra soils at different depths and across a long-term thaw gradient by sequencing the 16S rRNA gene amplicons with the Illumina MiSeq technology. Our results suggested pronounced impacts of soil depth and soil physical–chemical properties on the diversity and composition of microbial communities. Significant effects from thaw progression were revealed in soils of the lower organic and mineral horizons. Patterns of changes in microbial community structure suggested different impacts of thaw in these two soil layers, which was possibly related to the structure of soil C sources. The different responses to permafrost thaw revealed in this study

highlight the importance of considering the net effects of both upper and deeper soils while predicting microbial responses to warming-induced thaw.

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J.D., J.Z., J.M.T., E.E.G.S., L.W. and Z.H. designed research; J.D. performed research, analysed data and wrote the study. J.Z. and M.Y. performed DNA extraction; Y.G. and H.Y. performed PCR and sequencing; K.X. and Y.Q. contributed analytical methods.

Data accessibility

DNA sequences were deposited under NCBI SRA Accession: SRP034636.

OTU table, OTU representative sequences and soil physical–chemical characteristics were uploaded under Dryad doi: doi:10.5061/dryad.p1602.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Variation of soil physical-chemical characteristics with soil depth and soil layers.

Table S2 Dissimilarity test of community structures among soil layers by ADONIS.

Fig. S1 Changes of moisture, pH, C and N content, as well as bulk density along soil depth.

Fig. S2 The maximum (a), minimum (b) daily temperature, and the daily temperature variation (c) of Ex, Mo and Mi sites in May, 2004.

Fig. S3 Association of major classes (>1000 in normalized sequence number) with soil physical-chemical attributes.

Fig. S4 Association of major genera (>500 in normalized sequence number) with soil physical-chemical attributes.

Fig. S5 Proportions of unclassified population at three taxonomic levels (phylum, class and genus) in the four soil layers.

Fig. S6 Abundance of dominant groups involved in methane cycling in the four soil layers.

Fig. S7 Complete list of genera (with relative abundances >0.01%) with different abundance levels among sites within the L2 and L3–L4 soil layers.