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Vertical distribution of bacterial community is associated with the degree of soil organic matter decomposition in the active layer of moist acidic tundra[§]

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The increasing temperature in Arctic tundra deepens the active layer, which is the upper layer of permafrost soil that experiences repeated thawing and freezing. The increasing of soil temperature and the deepening of active layer seem to affect soil microbial communities. Therefore, information on soil microbial communities at various soil depths is essential to understand their potential responses to climate change in the active layer soil. We investigated the community structure of soil bacteria in the active layer from moist acidic tundra in Council, Alaska. We also interpreted their relationship with some relevant soil physicochemical characteristics along soil depth with a fine scale (5 cm depth interval). The bacterial community structure was found to change along soil depth. The relative abundances of *Acidobacteria*, *Gammaproteobacteria*, *Planctomycetes*, and candidate phylum WPS-2 rapidly decreased with soil depth, while those of *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, and candidate AD3 rapidly increased. A structural shift was also found in the soil bacterial communities around 20 cm depth, where two organic (upper Oi and lower Oa) horizons are subdivided. The quality and the decomposition degree of organic matter might have influenced the bacterial community structure. Besides the organic matter quality, the vertical distribution of bacterial communities was also found to be related to soil pH and total phosphorus content. This study showed the vertical change of bacterial community in the active layer with a fine scale resolution and the possible influence of the quality of soil organic matter on shaping bacterial community structure.

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Introduction

Permafrost, distributed across 24% of the terrestrial area in the Northern Hemisphere, is ground that remains frozen for two or more years (Zhang *et al.*, 1999). Permafrost soil contains approximately half of the global terrestrial carbon as soil organic matter (SOM) as low temperatures prevent the rapid decomposition of these carbon stores (Schuur *et al.*, 2009; Tarnocai *et al.*, 2009). According to model projections, temperature will rise faster in the Arctic (ACIA, 2005), which will consequently cause an intensification in permafrost thawing and the release of long-preserved SOM into the atmosphere via microbial decomposition (Schuur *et al.*, 2009; Grosse *et al.*, 2011).

The active layer is the surface of permafrost that undergoes seasonal freezing and thawing. Increasing atmospheric temperature has led to the deepening of the active layer as permafrost thaws (Johnstone *et al.*, 2010). The active layer has gained much scientific attention due to the various ecological, hydrological, and biogeochemical activities that dynamically occur in this layer (Kane *et al.*, 1991). Many studies have reported the higher levels of microbial biomass and diversity, and enzyme activities in the surface soil and a decreasing trend towards the deeper soil in the active layer (Yergeau *et al.*, 2010; Frank-Fahle *et al.*, 2014; Koyama *et al.*, 2014). A recent study showed that the carbon loss of active layer is mediated by microbial communities (Xue *et al.*, 2016).

Although permafrost layers maintain relatively stable environmental conditions compared to the upper active layers, some studies have revealed permafrost thawing caused the change of microbial community members, diversity, and functional gene abundances (Mackelprang *et al.*, 2011; Deng *et al.*, 2015). These studies indicated increasing temperature can facilitate microbial activity and lead to soil carbon vulnerability.

A depth profile of the active layer can serve as a good material in predicting the changes of thawing permafrost. Moreover, soil depth acts as ecological filter to edaphic properties and forms heterogeneous environments for microorganisms along soil depth. Recently, many studies have investigated the depth profile of soil microbial communities in the Arctic (Yergeau *et al.*, 2010; Wilhelm *et al.*, 2011; Frank-Fahle *et al.*, 2014; Gittel *et al.*, 2014; Koyama *et al.*, 2014; Tas *et al.*, 2014; Deng *et al.*, 2015). These studies revealed that abun-

dance and diversity for soil microbial genes or taxa were higher in active layer than in permafrost. The vertical distribution of microbial abundance and diversity correlated with soil properties such as C/N ratio or substrate availability (Frank-Fahle *et al.*, 2014; Gittel *et al.*, 2014; Koyama *et al.*, 2014; Tas *et al.*, 2014; Deng *et al.*, 2015). However, most comparisons were made at broad scales such as between surface and subsurface or between organic and mineral horizons. There still remains a gap to understand the shift of microbial community along soil depth with a fine scale.

This study investigated the vertical distribution of bacterial communities and soil physicochemical properties within the active layer with a fine scale (5 cm depth interval). In addition, bacterial potential functions were inferred from amplicon data using PICRUSt. With this study, we could identify that the soil property strongly affects the shape of bacterial community structure along the active layer from the moist acidic tundra, a region highly responsive and vulnerable to climate change (Anisimov and Fitzharris, 2001).

Materials and Methods

Site description and soil core sampling

The study site was located in Council, Seward Peninsula, Alaska (64° 51′N, 163° 42′W). At the time of sampling (early July 2010), the active layer depth measured by using a steel probe (1 m) was approximately 50 cm. The sampling area in Council was discontinuous permafrost and wet tundra with dwarf shrubs (Yoshikawa and Hinzman, 2003). There were no trees while lichen, moss (*Sphagnum* spp.), bog blueberry (*Vaccinium uliginosum*), and water sedge (*Carex aquatilis*) were dominant (Park and Lee, 2014).

Three sampling points with similar vegetation compositions (dominated by bog blueberry, lichen and moss) were randomly selected within 100 m distance from each other. After removing above ground vegetation and flattening the soil surface, each soil core was taken by hammering a stainless steel pipe (diameter 7.6 cm and length 50.0 cm) into the active layer. The acquired three cores were immediately placed in icebox and transferred to a freezer in the laboratory using Ice Breaker Research Vessel (IBRV) Araon. The soil samples were stored at -20°C for further laboratory analysis.

Soil physical and chemical properties

Frozen soil cores were cut into 5 cm depth increments, and soil horizon was described. A small amount (~5 g) of soil sample was used for microbial analyses, and the rest of the sample was air-dried and sieved through a 2 mm standard mesh for analysis of soil properties. Soil texture was analyzed by wet sieving and a pipette method (Gee and Bauder, 1986), and particle size was classified as sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (< 0.002 mm) according to USDA Soil Taxonomy (Soil Survey Staff, 2014). Soil pH was determined in a soil-water suspension (1:5 ratio, w/v) by a pH meter (Orion 3 star, Thermo Scientific), and then the supernatant was filtered through Whatman No. 42 paper. The filtrate was used to measure electrical conductivity (EC) by an EC meter (PET-2000 Kombi, Stelzner GMBH). The soil was ground to fine powder and used to analyze total carbon (TC) and total nitrogen (TN) contents by an elemental analyzer (Flash EA 1112, Thermo Scientific). For total phosphorus (TP), two different acids were used to digest ground soils depending on the depth and organic residue content (Kuo, 1996): sulfuric acid-peroxide for depths of 0–25 cm soils and perchloric acid-nitric acid for the soil samples in 25–45 cm depth. After digestion, the solution was mixed with an ammonium paramolybdate-vanadate solution, and the absorbance of the mixture solution was determined at 470 nm.

PCR amplification and pyrosequencing

To extract genomic DNA (gDNA), subsampled soils were freeze-dried using a freeze-dry system (LABCONCO). Genomic DNA was extracted from approximately 0.5 g of freeze-dried soil using a FastDNA® SPIN kit for soil and a QuickPrep adapter (MP Biomedicals), according to the manufacturer's protocol. The concentration of gDNA was determined by Hoechst dye 33258 staining followed by spectrophotometry with excitation and emission at 350 nm and 460 nm, respectively (Wallac EnVision 2013 Multilabel Reader, Perkin Elmer). The gDNA was stored at -20°C until further analysis.

Genomic DNA was amplified by PCR using the adapter-multiplex identifier-primer combinations targeting the V1–V3 regions (27F–518R) of the bacterial 16S rRNA gene (Chun *et al.*, 2010). PCR was performed in a total volume of 50 μl containing 1 ng of DNA as a template, 20 pmol of each primer, 1 × PCR buffer (10 mM Tris-HCl; 15 mM $MgCl_2$; 50 mM KCl, pH 8.3), 10 nmol of each dNTP and 1 U of *Taq* polymerase (Roche). The PCR cycle conditions were as follows: an initial denaturation step at 95°C for 7 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. All samples were amplified in triplicate. They were pooled in equal amounts, and purified using the QIAquick PCR Purification Kit (Qiagen). PCR products were quantified using a NanoDrop spectrophotometer. DNA sequencing was performed by ChunLab using a GS-FLX Titanium pyrosequencer (Roche).

Processing of pyrosequencing data

Amplicon pyrosequencing data were processed using the QIIME software package, ver. 1.7 (Caporaso *et al.*, 2010b). Sequencing noise and putative chimeras were removed by AmpliconNoise software, ver. 1.27 (Quince *et al.*, 2011), using the platform option for FLX Titanium sequence data implemented in QIIME. Sequence read length was 378 bp (±52 bp) on average. Sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence similarity level using UCLUST (Edgar, 2010). OTUs were classified against the Greengenes database (release 13.5) (Werner *et al.*, 2012) using the RDP Classifier method (Wang *et al.*, 2007). Sequences were aligned against the Greengenes-aligned reference sequences (DeSantis *et al.*, 2006) using PyNAST software (Caporaso *et al.*, 2010a). A maximum likelihood tree was built using FastTree 2.1 with default settings (Price *et al.*, 2010), and a pairwise beta diversity distance matrix for a randomly selected subset of 1,583 sequences was generated for all samples based on the unweighted UniFrac phyloge-

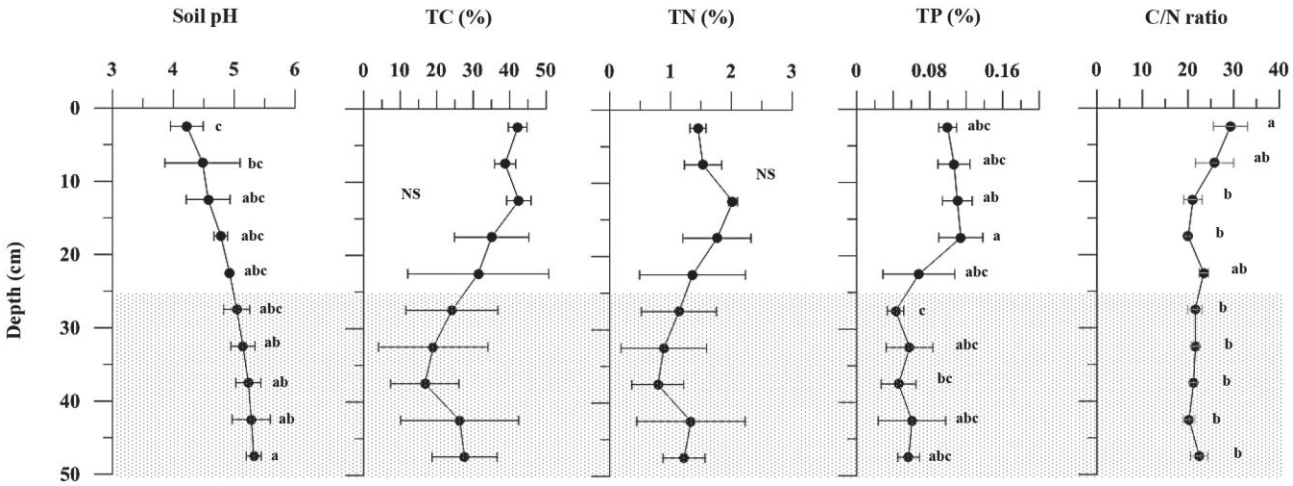

Fig. 1. Soil physical and chemical properties along soil depth. Soil horizon was separated by Oi (fibric) and Oa (sapric, shadow indicated). Different lower case letters among depths indicated significant differences at $P < 0.05$. TC, total carbon content; TN, total nitrogen content; TP, total phosphorus content; C/N, a ratio of carbon to nitrogen content; NS, no significant differences among soil depths.

netic distance metric (Lozupone *et al.*, 2006). Bacterial diversity indices were estimated from a randomly selected subset of 1,583 sequences in each sample to avoid false results due to different sample sizes (Kirchman *et al.*, 2010).

To predict the community's functional capabilities from the 16S rRNA gene data, PICRUSt software (Langille *et al.*, 2013) was implemented in the QIIME package. This software predicts metagenomes of bacterial metabolic pathways based on 16S rRNA gene data and a reference genome database with an extended ancestral-state reconstruction algorithm (Langille *et al.*, 2013).

The FLX Titanium flowgrams have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive database (accession number SRR1312081).

Statistical analysis

All statistical analyses for soil characteristics and bacterial community structure were performed using R (version 3.1.2) (R Core Team, 2014) or PRIMER-E V6 (Clarke and Gorley, 2006). The microbial community and soil properties along depths were compared, and the three cores for the same depth were considered as replicates. One-way ANOVA in conjunction with *post-hoc* Tukey's HSD was performed to determine if there was any significant difference in chemical properties

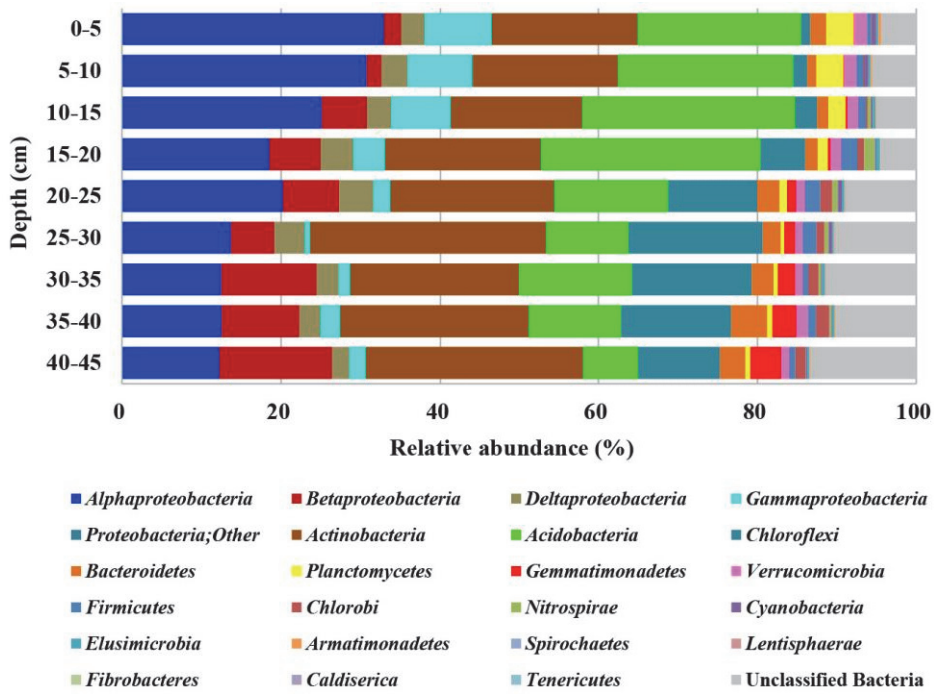

Fig. 2. Vertical distribution of bacterial community composition at phylum level. *Proteobacteria* represented at the class level.

among soil samples with differing depth. A Mantel test was used to determine the soil properties that were significantly correlated with bacterial community composition. Principal Coordinate Analysis (PCoA) was carried out with an unweighted UniFrac distance matrix of bacterial 16S rRNA gene sequences. Analysis of similarity (ANOSIM) with 999 permutations was used to represent significant differences in bacterial community dissimilarity based on unweighted UniFrac distance matrix between soil layers. Cluster analysis was conducted using Primer E with the relative abundance of OTUs and functional gene contents.

Results

Physicochemical characteristics of soil

The soil was classified as organic layer (O layer) on the basis of the TC content (Soil Survey Staff, 2014). Soil profile was composed of two distinct horizons as Oi and Oa according to the decomposition degree of SOM. While soil up to 25 cm depth was the Oi (fibric) horizon composing of less decomposed SOM and plant tissue as fiber, soil between depths of 25–45 cm was the Oa (sapric) horizon consisting of the most highly decomposed SOM. Although the main component of the Oa horizon was organic materials, the horizon was found to contain some mineral soil material which was texturally classified as silt loam (133.1 g/kg for sand, 635.9 g/kg for silt, and 231.1 g/kg for clay on average). Overall, soil properties were distinguished based on the decomposition degree of SOM.

The soil properties in the active layer changed with depth. The soil was acidic (average pH of 4.9 ± 0.4), and the pH increased along soil depth (Fig. 1). The TC and TN contents did not significantly vary by depth. On average, the TC content within the top 25 cm was greater than 30%, while the percentage of TC in the deeper depth ranged from 16.7 to

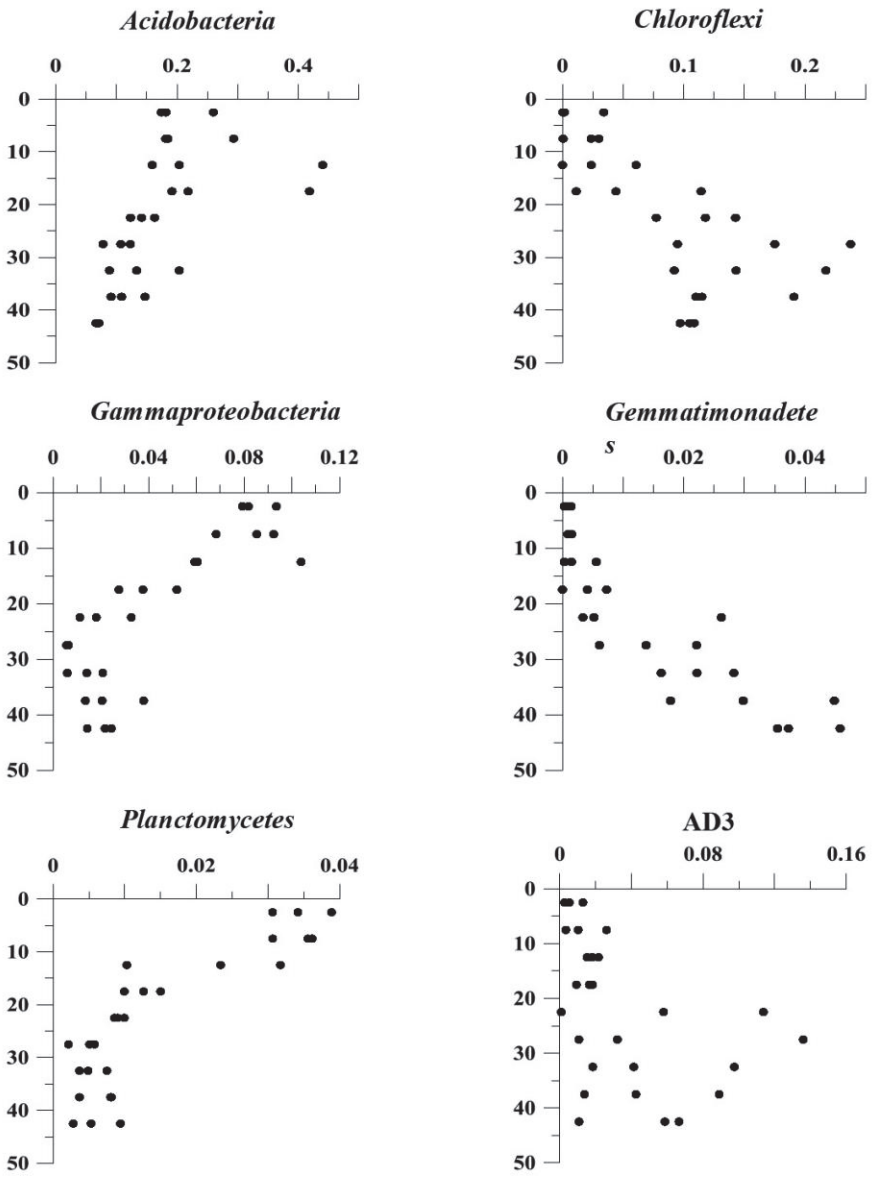

Fig. 3. Changes in relative abundance of representative bacterial groups.

26.3%. Total nitrogen (TN) content ranged from 0.8 to 2.0% throughout the soil core. On the other hand, the TP content and the C/N ratio decreased significantly with soil depth. The ranges of the TP content and C/N ratio were 0.04–0.11% and 20.0–29.3, respectively (Fig. 1). Soil EC did not change with depth and its level was negligible for plant growth (Bernstein, 1975).

Vertical distribution of bacterial diversity

A total of 179,160 bacterial 16S rRNA gene sequences were obtained from 27 samples of the three soil cores. Classifiable sequences were clustered into 6,776 OTUs defined by 97% sequence similarity. The OTUs were assigned into 43 phyla (including four classes of *Proteobacteria*: *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*). Among these phyla, *Acidobacteria*, *Actinobacteria*, and *Alphaproteobacteria* were dominant, accounting for over 45% of the total bacterial abundances from every soil depth (Fig. 2).

The highest number of bacterial OTUs was observed in the upper soil stratum (0–10 cm), while the lowest number of bacterial OTUs was mainly observed at a depth of 25–30 cm (Supplementary data Fig. S1). This result was supported by bacterial richness (Chao-1) and diversity (Shannon's H') from a subset of 1,583 sequences in each soil sample. The bacterial richness and diversity was high in surface soil and linearly decreased until soil depth at 25–30 cm, and did not vary widely below 30 cm (Supplementary data Fig. S1). These results revealed that bacterial richness and diversity in Oi horizon was greater than those in Oa horizon.

Vertical distribution of soil bacterial communities

The bacterial community structure changed along soil depth. At phylum level, the relative abundance of *Betaproteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Gemmatimonadetes*, *Chlorobi*, and candidate phylum AD3 increased along soil depth, whereas *Alphaproteobacteria*, *Gammaproteobacteria*, *Acidobacteria*, *Planctomycetes*, and candidate phylum WPS-2 decreased along soil depth (Fig. 2 and Supplementary data Fig. S2A).

Interestingly, the relative abundance of certain bacterial groups markedly changed around 20–25 cm. For example, the relative abundance of *Acidobacteria*, *Gammaproteobacteria*, and *Planctomycetes*, were rapidly decreased, while *Chloroflexi*, *Gemmatimonadetes*, and candidate phylum AD3 were rapidly increased at around 20–25 cm depth (Fig. 3 and Supplementary data Fig. S2A). This trend was also observed at the lower taxonomic levels of main bacterial groups; members

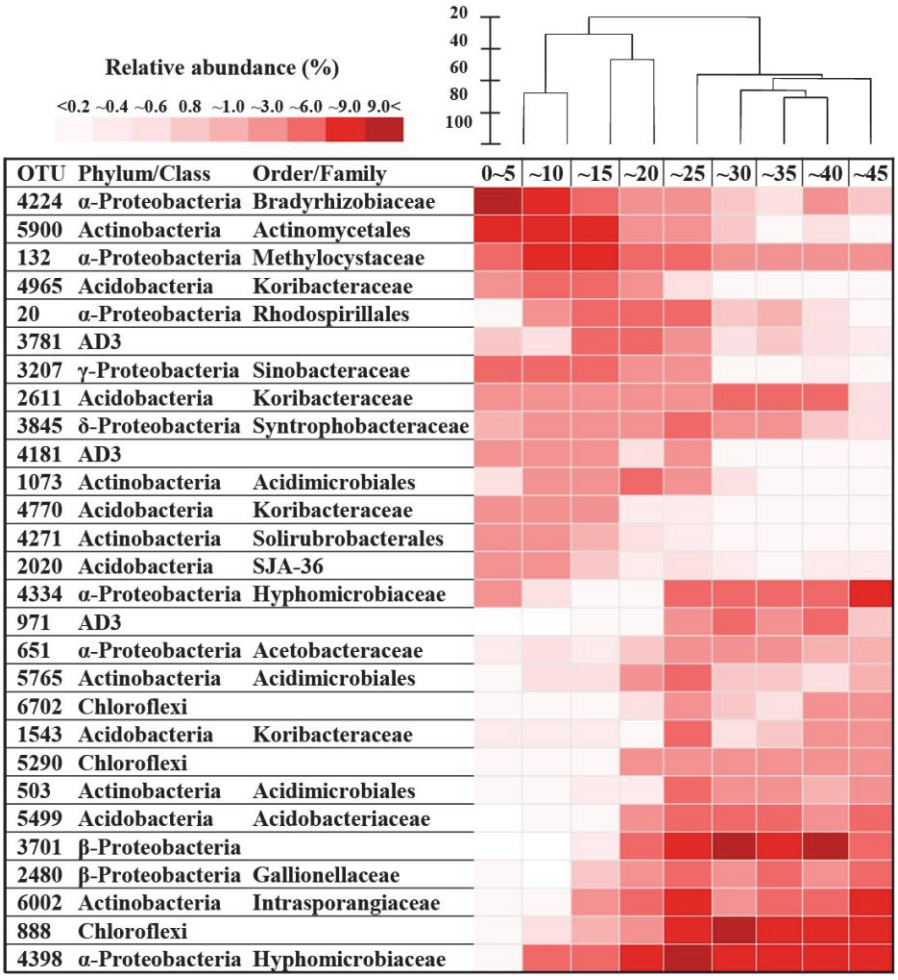

Fig. 4. Relative abundance of dominant bacterial OTUs which represented over 0.5% of total sequences. Upper dendrogram indicates the bacterial community similarity between soil depths.

Table 1. The correlation between soil property and bacterial community within soil horizon. The Pearson correlation (*r*) and significance (*P*) were determined by Mantel tests. Significant correlation between a soil property and bacterial community structure is shown in bold (*$P < 0.05$; ** $P < 0.001$). Benjamini and Hochberg correction was used to adjust *P* values for multiple comparisons by using 'fdrtool' function in R.

Soil[a]	Soil horizon	*Acidobacteria*	*Actinobacteria*	*Alpha proteobacteria*	*Bacteroidetes*	*Beta proteobacteria*	*Chloroflexi*	*Gamma proteobacteria*	AD3
Depth	Whole	**0.426****	0.093	**0.595****	0.061	**0.303****	**0.377***	**0.496***	**0.166***
	Oi	**0.224***	**0.224***	**0.558****	-0.156	0.061	**0.329***	**0.728****	**0.219***
	Oa	0.429	-0.016	-0.111	-0.158	-0.224	0.077	-0.061	-0.158
pH	Whole	**0.412****	-0.034	**0.273***	**0.265***	**0.447****	**0.500***	**0.255***	0.142
	Oi	**0.258***	0.258	-0.023	0.018	**0.211***	**0.453***	0.109	0.099
	Oa	0.089	-0.067	0.035	0.015	**0.354***	-0.162	-0.183	0.111
TC	Whole	**0.210***	-0.054	**0.226****	0.072	-0.001	0.056	**0.181***	0.104
	Oi	**0.300***	**0.301***	0.181	0.052	**0.211***	-0.001	**0.225***	0.153
	Oa	0.034	0.058	-0.105	-0.027	-0.112	-0.157	-0.109	-0.091
TN	Whole	**0.187***	-0.113	0.047	**0.102***	-0.054	-0.057	0.028	0.051
	Oi	0.192	0.192	-0.002	0.010	-0.080	-0.092	0.043	0.089
	Oa	0.025	0.036	-0.100	-0.068	-0.118	-0.178	-0.102	-0.130
TP	Whole	**0.391****	0.093	**0.263****	**0.098***	**0.098***	**0.217***	**0.303***	**0.156***
	Oi	0.192	0.192	-0.002	0.005	0.061	0.110	**0.448***	0.089
	Oa	0.075	-0.093	-0.189	0.047	-0.054	-0.247	-0.255	-0.148
C/N	Whole	-0.132	-0.009	**0.243***	-0.149	0.057	**0.305***	0.141	-0.008
	Oi	-0.233	-0.233	0.132	-0.235	-0.085	0.100	-0.056	-0.127
	Oa	-0.094	-0.162	0.181	-0.274	0.033	0.050	0.328	-0.239
EC	Whole	-0.150	-0.117	0.050	0.110	0.026	-0.095	-0.033	-0.114
	Oi	-0.216	-0.216	0.012	-0.084	-0.041	-0.197	-0.123	-0.205
	Oa	0.043	-0.111	-0.144	**0.722***	0.067	-0.162	-0.214	-0.030

[a] TC, total carbon content; TN, total nitrogen content; TP, total phosphorus content; C/N, a ratio of carbon and nitrogen content; EC, electrical conductivity.

of *Methylocystaceae* (*Alphaproteobacteria*), *Sinobacteraceae* (*Gammaproteobacteria*), *Actinomycetales* (*Actinobacteria*), *Koribacteraceae*, and Ellin6513 (*Acidobacteria*) were rapidly decreased around the depth of 20 cm, while members of *Gallinoellaceae* (*Betaproteobacteria*), *Intrasporangiaceae* (*Actinobacteria*), and SJA-36 (*Acidobacteria*) increased below the depth of 20 cm (Supplementary data Fig. S2). The relative abundance of dominant OTUs also changed at depth of 20–25 cm (Fig. 4).

Bacterial community similarity also considerably changed along soil depth (Supplementary data Fig. S3). Bacterial communities at 0–10 and 10–20 cm soil depths were different from one another, and these communities were clearly distinct from bacterial communities at 20–45 cm soil depths.

Relationships between bacterial communities and soil properties

Vertical distribution of bacterial community structure was related to soil properties, and the primary factor was the decomposition status of SOM. Although TC content was not significantly different with soil depth (Fig. 1), the bacterial community dissimilarity based on unweighted UniFrac distance matrix showed to be significantly correlated with soil horizon ($P < 0.001$). Moreover, the diversity indices were significantly different between Oi and Oa horizon: Chao1 ($P < 0.05$) and Shannon's H' ($P < 0.001$).

The eight most representative bacterial groups were closely related to soil properties. The bacterial communities (except *Actinobacteria*) were significantly correlated with soil pH and/or TP within whole active layer (Table 1). A few bacterial groups correlated with TC, TN, and/or C/N ratio: *Acidobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* with TC ($P < 0.05$), *Acidobacteria* and *Bacteroidetes* with TN ($P < 0.05$), and *Alphaproteobacteria* and *Chloroflexi* with C/N ratio ($P < 0.05$). However, these relationships did always apply in both horizons. For example, *Acidobacteria*, *Actinobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* were correlated with TC ($P < 0.05$), *Acidobacteria*, *Betaproteobacteria*, and *Chloroflexi* were correlated with soil pH ($P < 0.05$), and *Gammaproteobacteria* was correlated with TP ($P < 0.05$) in Oi horizon (Table 1). On the other hand, in the Oa horizon, *Acidobacteria*, *Betaproteobacteria*, and *Bacteroidetes* were correlated with soil depth ($P < 0.05$), soil pH ($P < 0.05$), and EC ($P < 0.05$), respectively (Table 1). These results presented that the influencing soil factors on bacterial community could vary depending on soil horizon.

Inference of potential functions

Various gene categories were detected in this study through the genome data of identified bacteria. PICRUSt functional prediction using KEGG pathway metadata revealed that the bacterial communities possessed various functions along soil depth (Fig. 5). The relative abundance of predicted functional gene categories at Class 1 1evel such as Metabolism (MT), Genetic Information Processing (GIP), Environmental Information Processing (EIP), Cellular Processes (CP), Human Diseases (HD), Organismal Systems (OS), and Unclassified (UN) were quite similar along soil depth. The cluster analysis showed two clusters divided into upper (0–20 cm) and lower layer (below the 20 cm). Although the soil depth at 20–25 cm was classified as Oi horizon, the relative abundance of gene categories was relatively similar with Oa hori-

zon. The bacterial communities harbored the highest relative abundance of functional genes at top soil (0–10 cm) and followed lower depth (20–25 and 30–40 cm). This trend corresponded with bacterial diversity indices (Supplementary data Fig. S1).

The predicted functional gene categories were significantly correlated with soil properties. Among 328 predicted functional gene categories at Class 3 level, approximately 80% of the gene categories was significantly correlated with soil pH, followed by 33% with soil depth, 7.0% with TP, 3.7% with TC, 3.4% with TN, and 3.1% with C/N ratio (Supplementary data Table S1). When we observed the correlations in the carbon related functions such as methane metabolism, carbon fixation, glycolysis, and citrate cycle, most functions showed significant correlations with soil pH, but no relationship with other soil properties (except glycolysis with soil depth, $P < 0.05$) (Supplementary data Table S1).

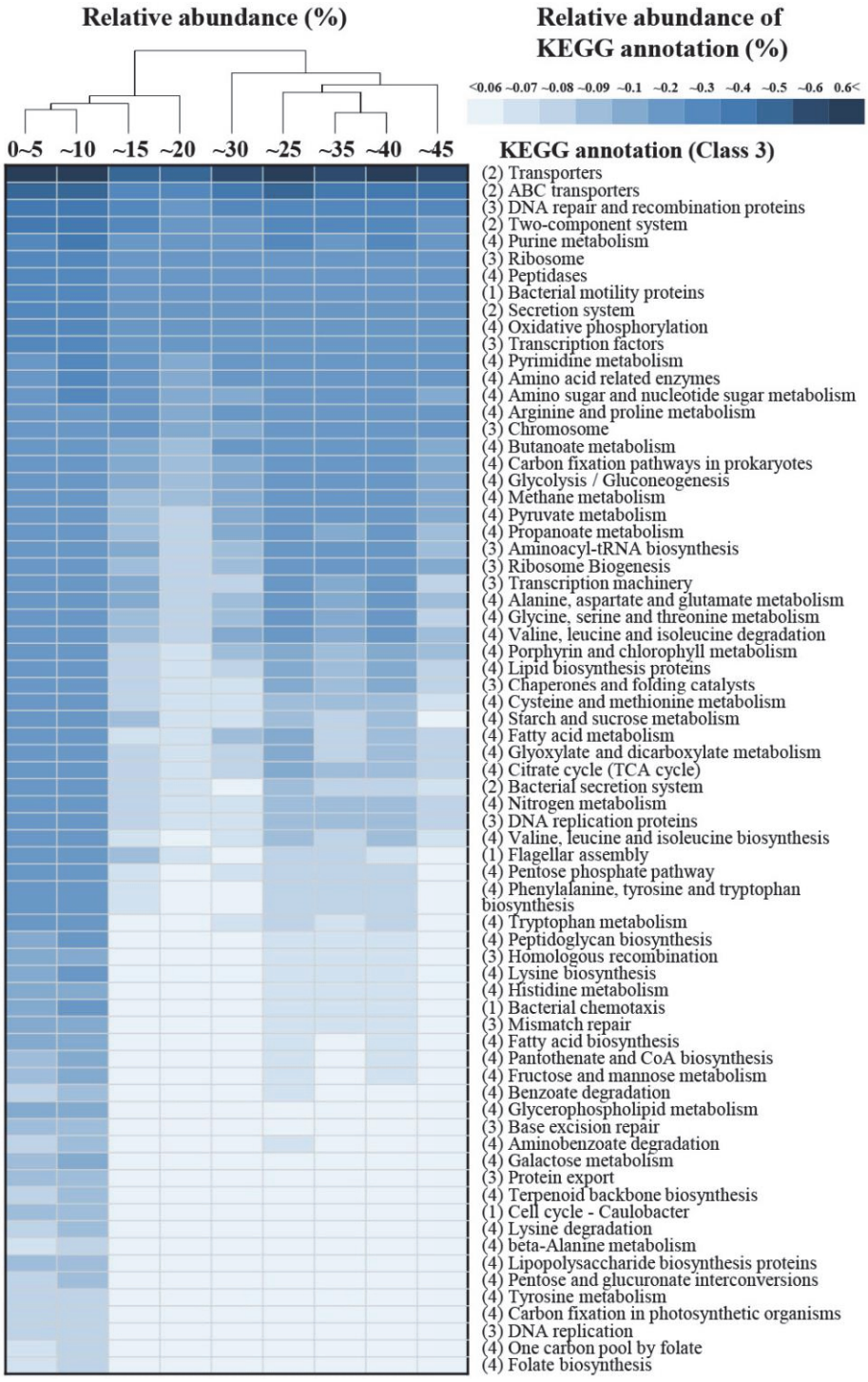

Fig. 5. Relative abundances of gene contents from KEGG annotations. Heatmap for the gene contents at Class 3 representing more than 0.5% from total abundance. Dendrogram is based on hierarchical clustering of relative abundance from total gene contents at Class 3. Functional categories for Organismal systems, Human diseases and Unclassified functions were omitted. Parenthesis represents the KEGG annotation at Level 1: (1), Cellular Processes; (2), Environmental Information Processing; (3), Genetic Information Processing; (4), Metabolism

Discussion

Increasing temperature is causing permafrost thawing and active layer deepening, and accelerating the carbon mineralization by microbial respiration (Luo *et al.*, 2001). Thus, the Arctic soil carbon pool is very vulnerable to climate change (Grosse *et al.*, 2011). The active layer, which is sensitive to atmospheric temperature, provides vertical heterogeneity along the soil depth. Most studies, however, compared bacterial communities in between layers such as between active and permafrost layers or between organic and mineral layers (Gittel *et al.*, 2014; Koyama *et al.*, 2014; Tas *et al.*, 2014; Deng *et al.*, 2015). Moreover, depth distribution of bacterial community at a fine scale had not been fully described yet. This study revealed that the vertical structure of bacterial communities and soil properties were quite different along the soil depth within the active layer at the fine scale.

Bacterial community structure and diversity changed along soil depth in the active layer of moist acidic tundra. The vertical distribution of bacterial community structure in Arctic soils has been reported. (Yergeau *et al.*, 2010; Wilhelm *et al.*, 2011; Frank-Fahle *et al.*, 2014; Koyama *et al.*, 2014; Tas *et al.*, 2014; Deng *et al.*, 2015). The previous studies showed that bacterial richness and diversity were highest in surface layers of soil and decreased towards deeper layers. Our results corresponded with the previous studies (Supplementary data Fig. S1).

The predominant bacterial diversity in surface soil seems to be related with carbon availability. Many terrestrial soils show a significant correlation between microbial biomass and soil carbon contents along depth (Rumpel and Kögel-Knabner, 2011; Eilers *et al.*, 2012). This was also found in some studies on Arctic soils, where they explained that the substrate availability was strongly related to the shift of bacterial community structure between the organic and mineral soil horizons (Koyama *et al.*, 2014; Deng *et al.*, 2015). In this study, soils of all depth were organic horizon, and the carbon contents were not significantly different along the depth. This suggests that the bacterial community structure could be influenced by the carbon quality rather than the carbon quantity. The previous studies have emphasized that the substrate quality is significantly related with the microbial mineralization, which affects the microbial community structure (Merilä *et al.*, 2010), and a high proportion of bacteria in the soil surface was explained by their pre-adaption for rapid metabolism of labile carbon substrate (Fierer *et al.*, 2003). The shift of bacterial community structure at about 20 cm depth coincided with the distinction of Oi and Oa horizons in this study. Therefore, we can explain that the bacterial diversity of this active layer soil in acidic moist tundra is related with carbon quality. The upper Oi layer containing less decomposed organic material could contain more labile carbon and available nutrients compared to the Oa layer. Even though Oa layer consisted of the highly decomposed organic materials, most available carbon seemed to be already consumed and the remaining carbon may be hard for bacteria to use. On the other hand, bacteria can take carbon by decomposing organic materials in Oi layer.

The vertical distribution of bacterial community could be related to the different resource availability of each bacterial group. The higher abundance of *Alphaproteobacteria* and *Gammaproteobacteria* in the upper layer (Oi horizon) could be related to their preference of higher carbon and nutrients. The relative abundance of *Alphaproteobacteria* and *Gammaproteobacteria* increased after fertilization compared to the control plot at Toolik Lake, Alaska (Campbell *et al.*, 2010; Koyama *et al.*, 2014). *Planctomycetes* was also more abundant in Oi than in Oa horizon (Fig. 2). According to the genomic insight, the *Planctomycetes* have large genomes which is a feature of copiotrophs which prefer nutrient-rich environment (Lauro *et al.*, 2009). Oligotrophic-like bacteria such as *Chloroflexi* and AD3 were relatively abundant in Oa horizon (Figs. 2, 3, and Supplementary data Fig. S2). Relative abundance of *Chloroflexi* decreased after the N addition (Fierer *et al.*, 2012a). Although the physiological characteristics of AD3 were not defined due to non-culturability, AD3 has been regarded as an oligotrophic bacterium due to frequent occurrence in considerably mineralized environment such as deep soils (Costello, 2007; Tas *et al.*, 2014). Moreover, *Gemmatimonadetes* and *Chlorobi* have been frequently detected in deeper soil and permafrost (DeBruyn *et al.*, 2011; Wilhelm *et al.*, 2011; Jansson and Tas, 2014; Deng *et al.*, 2015; Schostag *et al.*, 2015). Despite their frequent occurrences in soils, their physiology also has not been characterized. *Betaproteobacteria* interestingly increased along soil depth in this study (Fig. 2), which was in accordance with the study showing higher abundance of *Betaproteobacteria* in mineral than organic layer in Arctic soil (Koyama *et al.*, 2014). Specifically, the relative abundance of *Gallinoellaceae* of *Betaproteobacteria* increased along soil depth in this study (Supplementary data Fig. S2). The *Gallinoellaceae* has been known as microaerophilic and chemolithoautotrophic bacteria having pathways for CO_2-fixation (Emerson *et al.*, 2013). These characteristics may support the bacterial members to be able to survive in deeper soil.

Besides the quality of soil carbon, we found that the vertical distribution of bacterial community was correlated with soil pH and TP (Fig. 1 and Table 1). Soil pH has been known to be an important controlling factor that shapes bacterial community structure across a variety of spatial scales (Fierer and Jackson, 2006; Lauber *et al.*, 2009; Chu *et al.*, 2010; Shen *et al.*, 2013). Previous studies showed that the relative abundance of *Acidobacteria* had a strong positive correlation with soil pH in acidic surface soils and tussock tundra in the Arctic (Neufeld and Mohn, 2005; Wallenstein *et al.*, 2007; Wilhelm *et al.*, 2011; Kim *et al.*, 2014). Moreover, soil pH affected the vertical distribution of bacterial community structure as well as horizontal distribution. *Acidobacteria*, was one of the dominant groups in upper Oi horizon in this study, and decreased toward the lower layer. Dominance of *Acidobacteria* in the upper layer might be related to more acidic condition in this study (Table 1).

The soil phosphorus (P) is an important nutrient and often co-limiting factor together with nitrogen in the subarctic tundra (Chapin *et al.*, 1978). The addition of P resulted in the increase of soil microbial biomass in various soil environments (Griffiths *et al.*, 2012; Liu *et al.*, 2013). The availability of P as well as N for the microorganisms can contribute to the feedback on soil carbon dynamics such as decreasing in soil organic carbon (Mack *et al.*, 2004; Finzi *et al.*,

2011; Griffiths *et al.*, 2012). However, the relationship between soil bacterial community structure and TP has been poorly studied in Arctic soil. Thus, further study is required to investigate the effects of P on microbial community and its interaction with C and N contents along the soil depth.

Other soil properties such as moisture content or O_2 level may also contribute to the differentiation of bacterial community structure. In arid environment, moisture content may be important factor to control the microbial community structure. Our study site, however, was observed moist environment and the surface was seasonally waterlogged due to poor drainage. This feature may develop high water content and low O_2 concentration conditions. Poor drainage environment facilitates anaerobic degradation of organic matter (Preuss *et al.*, 2013). Thus, further observations on moisture content and O_2 level are needed to understand their relationship with the vertical distribution pattern of soil bacteria.

Soil microbial diversity might be a good predictor to explain the ecological functioning (Fierer *et al.*, 2012a, 2012b; Uroz *et al.*, 2013). Focusing on methane metabolism in the functional gene categories, the relative abundance of methane metabolism was highly accounted for all gene categories throughout all soil depth (Fig. 5). One of major OTU, OTU_132 belonged to *Methylocystaceae* (*Alphaproteobacteria*), which is known as methane oxidizing bacteria (MOB), was highly abundant in Oi horizon. MOB has attracted attention because they are the largest biological sink for methane in aerobic soils as oxidization up to 90% of the emitted methane occurs in the deeper soil layers (Bosse and Frenzel, 1997; Wagner and Liebner, 2009).

We also tried to find the relationship between carbon-related functions (e.g. carbon fixation, glycolysis, and citrate cycle) and soil properties. These carbon-related functions were significantly correlated with soil pH than TC (Supplementary data Table S1). Interpretation for the predicted functional gene categories should be cautious, because the functions inferred from amplicon data could be limited and biased. Further metagenomic study is needed to make clear the relationship of bacterial function and soil properties.

In conclusion, this study provided insight on the vertical bacterial community structure in the active layer soil with fine scale depth resolution. The soil cores were divided according to the decomposition status of SOM, into two horizons: Oi and Oa. The bacterial communities of the upper Oi horizon were distinct from those in the lower Oa layer. In addition, a significant correlation was found between bacterial communities and soil pH as well as TP content. Many studies have emphasized the importance of soil pH and carbon for determining bacterial community until now, but the role of other nutrients such as phosphorus has not been studied very well yet. Therefore, a more detailed consideration of a diverse set of soil environmental parameters is required in order to better understand the relationship between microbial community and soil properties in future studies.

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