이학박사학위논문

알래스카 카운실 습지 산성 툰드라 토양 미생물 군집 분석

Study on Prokaryotic Community Structure in Moist Acidic Tundra Soil in Council, Alaska

2016 년 8 월

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Study on Prokaryotic Community Structure in Moist Acidic Tundra Soil in Council, Alaska

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ABSTRACT

The Arctic has gained much scientific attention because global warming is predicted to be greatest and most rapid at high latitudes. A point that should yield scientific attention might be concerned about the loss of soil organic matter (SOM), as it contributes to the positive feedback of global warming. Because a warmer climate will cause carbon stored in the soil to be released into the atmosphere via microbial decomposition. With increasing scientific attention on permafrost environments impacted by global warming, many scientists have focused on the global carbon cycle in Arctic soils observing microbial life. However, knowledge on microbial community and diversity in Arctic soil yet very lacking. Thus, this study investigated prokaryotic community structure, diversity and ecological functions in moist acidic tundra soil of Alaska through the next generation sequencing (NGS) with bioinformatics processing. Moreover, this study investigated the relationship between microbial communities and soil properties.

In chapter1, a general introduction with background information on Arctic environment and on the necessity of research objectives to explain further chapters is given.

In chapter 2, bacterial community structure and its relationship to soil properties in moist acidic tundra soil are described. Although various plants covered the top soil and some vegetation formed a colony, the bacterial communities were not related with vegetation types. Rather, the bacterial community could be markedly differentiated by soil depth and soil pH. The vertical structure of soil profile from active layer to permafrost was observed to be more specific. All soil properties changed along soil depth, and the soil cores were divided by the decomposition status of soil organic matter (SOM). When I observed the shift of bacterial community from active layer to

permafrost, active layer could be divided into Oi, Oe and OA horizons, and permafrost was classified as A horizon. Some bacterial groups abruptly changed near the boundary separating the horizons. Briefly, *Acidobacteria*, *Gammaproteobacteria*, *Planctomycetes*, and WPS-2 were relatively abundant in Oi horizon, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, *Verrucomicrobia* and AD3 were abundant in Oe and OA horizons, and *Actinobacteria*, *Bacteroidetes*, *Caldiserica*, and *Firmicutes* were abundant in A horizon. In archaeal groups, *Crenarchaeota* accounted for approximately 80% from most soil layers. Although the relative abundance of *Euryarchaeota* was insignificant from total archaeal abundance, the relative abundance of *Methanobacteria* and *Methanomicrobia* increased below Oi horizon. Although many studies have emphasized the quantity of soil organic carbon, this study indicated that the soil quality is primary important factor that shapes microbial community structure as well as soil pH.

In chapter 3, bacterial community in Arctic tundra soil was compared with Temperate and Tropical soils. According to a previous study, microbial community significantly interacted with specific soil properties. Thus, I identified the overall bacterial community structure and diversity between biomes, and assessed their relationship with soil properties. From the results of soil properties, Arctic soil was found to be relatively acidic and of nutrient rich environment, and Temperate and Tropical soil showed to be of low nutrient environment. Temperate soil showed highest richness and diversity, while Arctic soil showed the lowest richness and diversity. At phylum level, *Acidobacteria* and *Alphaproteobacteria* were predominant in all biomes. However, specific bacterial groups relatively abundant in each biome; the relative abundance of *Verrucomicrobia* and AD3 were dominant in Arctic soil, and *Bacteroidetes* and *Betaproteobacteria* were dominant in Temperate soil, and *Chloroflexi*, *Cyanobacteria*, and *Ntrospirae* were

dominant in Tropical soil. Most dominant OTUs in all biomes play an important role in biogeochemical cycle in their habitat. Arctic and Tropical soil contained dominant OTUs, which contribute to the reducing of positive feedback of global warming. Although the sample size was limited, this study might help with advancing an understanding the biogeography of bacterial community at regional scales.

The results in this study may contribute to extend our understanding about microbial community in moist acidic tundra soil, as well as help predict the microbial response to warming effect in Arctic soil.

Keywords: active layer, permafrost, microbial community, soil horizon, soil pH, next generation sequencing (NGS)

Student number: 2009-30856

CONTENTS

ABBREVIATIONS

gDNA: Genomic Deoxyribonucleic Acid

MOB: methane oxidizing bacteria

MC: moisture content

NGS: Next Generation Sequencing

NMDS: Non-metric Multidimensional Scaling

OTU: Operational Taxonomic Unit

PCoA: Principal Coordinate Analysis

PCR: Polymerase Chain Reaction

PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

QIIME: Quantitative Insights Into Microbial Ecology

RDP: Ribosomal Database Project

rRNA: Ribosomal Ribonucleic Acid

SOC: soil organic carbon

SOM: soil organic matter

SRA: Short Read Archive

TC: total carbon

TN: total nitrogen

TP: total phosphorus

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|CHAPTER 1|

Introduction: Microbial Ecology in Arctic Tundra Soil

1.1 General characteristics of Arctic soil

The Arctic is part of the Northern Hemisphere. There are three definitions for the Arctic: the Arctic Circle at north of the latitude 66° 32′, the Arctic tree line, and the average daily summer temperature (10°C) line (Figure 1.1). It consists of the Arctic Ocean and terrestrial regions across eight countries: Canada, Denmark (Greenland), Finland, Iceland, Norway, Russia, Sweden, and the United States (Alaska). The terrestrial regions cover a land area of approximately 7.2×10^6 km^2 (Tarnocai *et al.*, 2009).

Located in high latitudes and exposed to low solar radiation energy, the Arctic forms extremely cold environments. Average daily atmospheric temperatures are below 5℃ throughout the year (Zakhia *et al.*, 2008). The Arctic also undergoes seasonal variation experiencing large seasonal temperature fluctuation: winter atmospheric temperature can record below −20 to −40℃ and summer temperature ranges from approximately 3 to 10℃ across large parts of the Arctic region (Tarnocai, 2009). This seasonal temperature fluctuation drives as the cryogenic processes and affects geographical, physical, chemical, and biological properties, altogether making the Arctic a unique environment on Earth. In addition to high seasonal temperature fluctuation, commonly known characteristics in the Arctic area include seasonal high UV radiation exposure, nutrient limitations, limited water availability, low annual precipitation (~160 mm, mostly as snow), simple vegetation structure, and cryogenic disturbance (Tarnocai *et al.*, 2009; Rhodes *et al.*, 2013). Thus, Arctic ecosystems are relatively well preserved from anthropogenic activity, and provide a terra incognita for scientific research.

Frozen Arctic soil is called permafrost, which is soil that remains frozen for two or more years (Tarnocai *et al.*, 2009). Permafrost is generally classified into continuous and discontinuous permafrost depending on the amount and type of ice wedge. Ice wedges actively form mainly in the continuous permafrost zone, and are inactive to weakly active in the discontinuous zone (Péwé, 1975). Sometimes ice wedges form a patterned ground with frequency of freeze-thaw cycles, and have variety of forms such as polygons, circles, steps, and stripes (Drew & Tedrow, 1962). According to the amount and type of ice wedge content, permafrost forms a structurally heterogeneous zone across different climate and geographical locations: Yedoma, Cryopegs, Talik, Thermokarst among others. Briefly, Yedoma is a permafrost type in Northeast Siberia characterized by high level of organic material and up to 90% ice content. Cryopegs is a layer of unfrozen ground prevented by freezing-point depression due to the dissolved-solids content of the pore water. Talik is a layer of unfrozen part of permafrost underlying Thermokarst lake and rivers. Thermokarst is freshwater, formed by the collapse of the underlying permafrost (Kumar, 2011; Jansson & Tas, 2014).

Underlying permafrost is a thin layer called active layer, which undergoes seasonal thaw during the summer (Figure 1.2). Increasing atmospheric temperature has led to the thawing of permafrost and consequently the deepening of the active layer (Johnstone *et al.*, 2010). Permafrost preserves a stable environment, while active layer shows dynamically various ecological, hydrological, and biogeochemical activities (Kane *et al.*, 1991; Hinzman *et al.*, 2003). Thus, the comparison between active layer and permafrost has gained much scientific attention in multidisciplinary approaches.

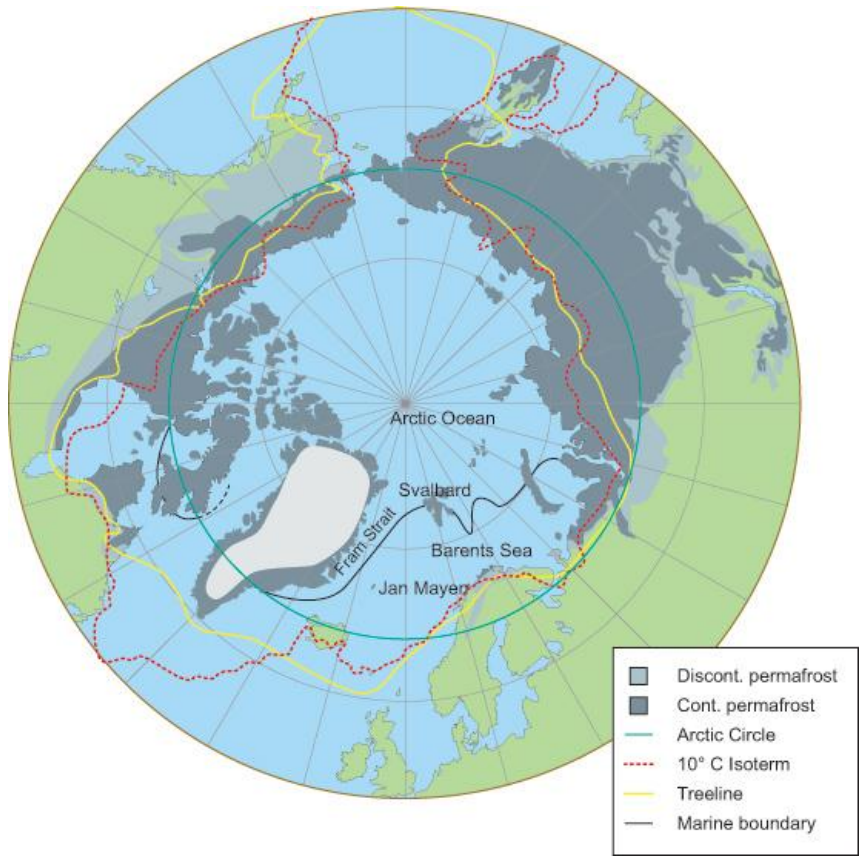


Figure 1.1. Definitions of the Arctic. Arctic soils are consisted of continuous (dark gray) or discontinues permafrost (gray). Green line is defined by region north of the latitude (66°32′); Yellow line is defined by the Arctic tree line; Red dotted line is defined by the average daily temperature (10℃) in summer (Data from http://www.arcticsystem.no).

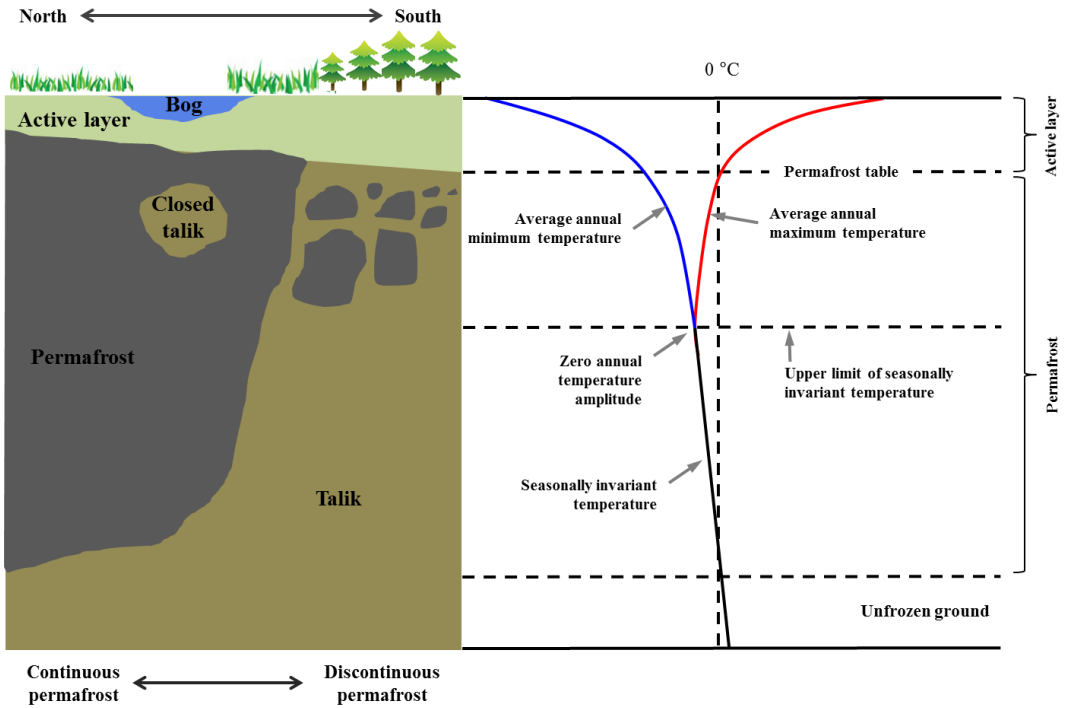


Figure 1.2. Cross-section of permafrost with graph of temperature along soil depth (Image from http://www.scienceinschool.org).

1.2 Current status of global scientific attention for Arctic soil

Arctic soil contains significant amount of carbon, preserving one third of the global soil carbon budget (Gorham, 1991; Zimov *et al.*, 2006). Concretely, 1,035 Pg of organic carbon is estimated to be stored in the Arctic permafrost regions (Hugelius *et al.*, 2013; Hugelius *et al.*, 2014). Low temperatures prevent rapid decomposition and thus allowed huge amount of soil carbon to be stored (Schuur *et al.*, 2009; Tarnocai *et al.*, 2009). Moreover, the anaerobic condition of deep soil makes the fermentation of soil carbon occur more slowly than aerobic condition (Wagner & Liebner, 2009a). Consequentially, these inhospitable conditions have caused soil organic carbon (SOC) in Arctic soil environments to accumulate over thousands of years (Schuur *et al.*, 2009).

In the past decades, Arctic permafrost has received scientific attention because atmospheric temperatures have been on the rise in most permafrost areas. An increasing temperature has led to global warming and climate change. It is widely established that greenhouse gases (GHGs: e.g. CO_2, CH_4 and N_2O) are caused by anthropogenic pollutants (e.g. human activities such as agriculture and waste disposal) and natural events (e.g. fires and erupting volcanoes), and increase the average global temperature. Among the GHGs, many scientists have focused on the flux of CO_2 and CH_4 because of their high contribution to the warming of the atmosphere (Bartlett *et al.*, 1992; Christensen *et al.*, 2000; Wagner & Liebner, 2009b). CO_2 is the primary GHG and most abundant in Earth's atmosphere. While CO_2 causes significant warming as a main GHG, methane is a critical GHG as it traps about 25 times more heat radiated than CO_2 (Schlesinger & Lichter, 2001). Increasing temperature is causing permafrost thawing and degradation (Figure 1.3), and encouraging microbial propagation. Activated microbial respiration

accelerates carbon mineralization (Luo *et al.*, 2001). Through the thawing of permafrost, large quantity of GHGs could be released into the atmosphere. The GHG fluxes from permafrost are influenced by various biotic and abiotic factors (Wagner & Liebner, 2009b). On account of these processes, Arctic permafrost soil is regarded as a considerably major carbon source for global warming (Oechel *et al.*, 1993).

According to model projections, increased GHGs concentrations will lead to the Arctic experiencing a greater warming than that of the global average (ACIA, 2005). With increase of atmospheric temperature, the extent of snow and ice cover will decrease and lead to lower surface reflectivity (albedo). With decrease of snow and ice cover, the darker surfaces, exposed to incoming solar radiation, will absorb a greater amount of heat energy. Actually, the area of Arctic sea ice was recorded to have decreased about 7.4% in 2002 than about 25 years ago (Johannessen *et al.*, 2004). The retreating of the Arctic sea ice is still on-going and is being constantly observed by satellite systems (Figure 1.4). Moreover, increasing temperature have led to degradation of most permafrost (Nelson & Anisimov, 1993; Anisimov *et al.*, 2007). Likewise, thawing permafrost has significant effects on ecosystem diversity, population, productivity, geological structure, and biogeochemical cycles (Schuur *et al.*, 2007; Turetsky *et al.*, 2007). This phenomenon accelerates the positive feedback which amplifies the impacts of global warming and makes the Arctic soil environments to collapse (Figure 1.3 and 1.4). Thus, many scientists are consistently warning about the impacts of climate change, and investigating Arctic ecosystems in relation to climate change to predict the consequences of global warming.

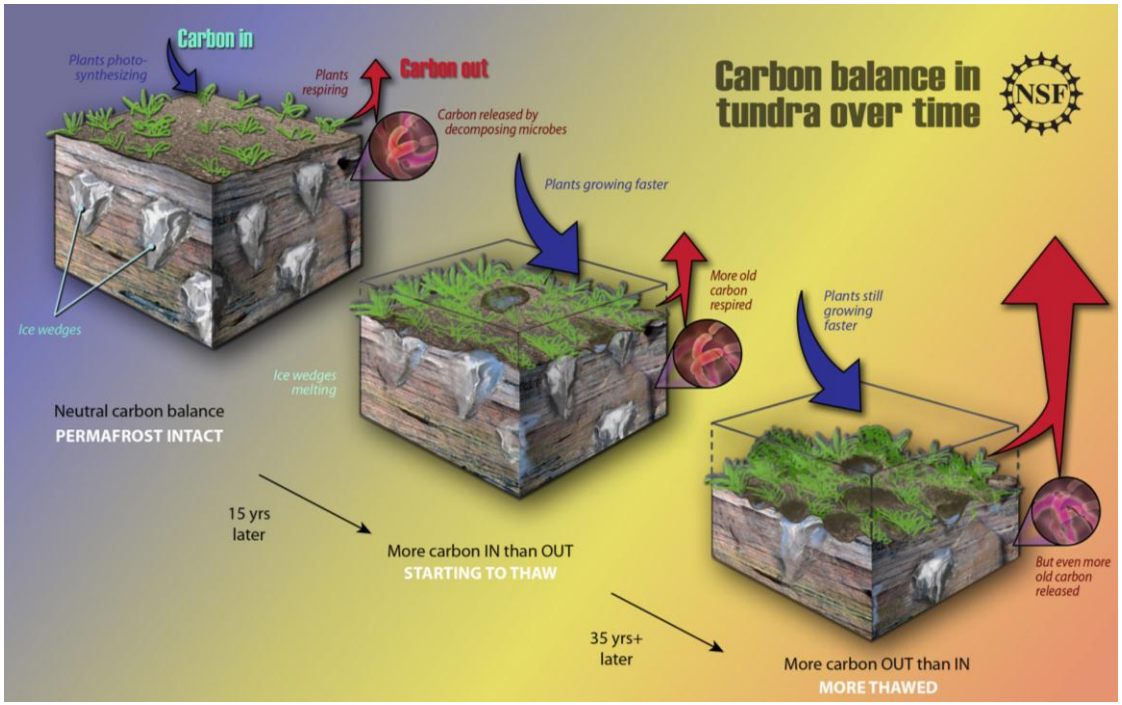


Figure 1.3. Carbon balance in the Arctic soil over time (Image from National Science Foundation).

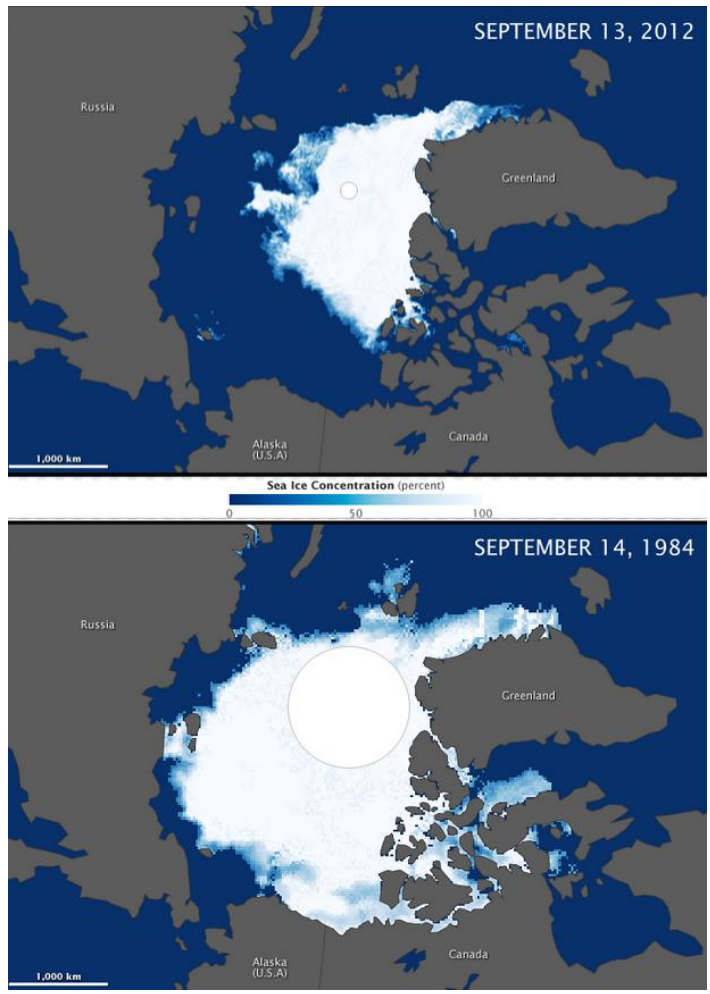


Figure 1.4. Observation of the minimum Arctic sea ice between 2012 (top) to 1984 (bottom). Almost half of the sea ice area in 1984 is lost in 2012 (Image from NASA Earth Observatory images).

1.3 Microbial ecology in the Arctic tundra soil

Arctic soil environment is characterized by extremely cold temperature, high UV radiation exposure, and low water and nutrient availability. Harsh condition of Arctic soil is even considered as analogs for extraterrestrial habitat such as Mars (Gilichinsky, 2002). Despite the harsh environmental conditions, scientists had tried to find viable microorganisms in Arctic permafrost soil. The first report for viable microorganisms was in early 1900s from permafrost soil near soil buried Sanga mammoth (Omelyansky, 1911). Afterwards, many scientists began to investigated microorganisms in Arctic soil. For example, four bacterial genera were isolated from Arctic permafrost near Fairbanks, Alaska (Becker & Volkmann, 1961), and 5 to 130 cells g^{-1} were detected in western Arctic permafrost soil (Boyd & Boyd, 1964). With technological advances, recent developments have greatly improved our ability to recover microorganisms in permafrost (Steven *et al.*, 2009). According to microscopic investigations, in Arctic soil, it was found that the substantial numbers of microorganisms were present with range of 10^3~10^9 cells g^{-1} in Siberian permafrost and Spitsbergen Island, respectively (Rivkina *et al.*, 1998; Hansen *et al.*, 2007). The advent of sequencing technologies has revealed that soil bacterial diversity is not fundamentally different in the Arctic from that found in other biomes (Chu *et al.*, 2010). This suggests that cosmopolitan groups of Bacteria adapted to subzero environments (Steven *et al.*, 2009). However, the study has ambiguity at taxonomic level resolution which means that the understanding of bacterial diversity and community structure at phylum level could lead overestimate or underestimate the actual taxonomic groups. Taxonomic level resolution with the advent of sequencing technologies will lead to a greater understanding of microbial diversity and community structure. Recent studies

have shown that a number of microorganisms survive in Arctic soil. The research investigating microbial populations is being carried out on an on-going basis using new and advanced sequencing technologies (representative sequencers listed in Table 1.1).

Arctic soil provides various heterogeneous habitats for microbial life. The microbial community structure changes according to environmental heterogeneity. For example, the amount of ice content in permafrost affects microbial biomass. Ice in the permafrost soil has a high salt content and can drop the freezing point. Thus, ice forms brine veins which can function as a habitat for microbial life in permafrost (Jansson & Tas, 2014). Relatively high abundance of microbial cells usually can be detected in ice-contained soils than other soils with no ice. Besides the amount of ice content, there are various soil factors which can affect the microbial community structure such as soil texture, temperature, moisture, oxygen content, organic matter content, etc. and these soil environmental soil factors differently distributed along soil horizontal and vertical structure (Steven *et al.*, 2006; Jansson & Tas, 2014).

In horizontal structure, soil factors change along latitudinal gradients. Many scientists have tried to describe patterns of microbial communities with various scales (local to global). However, the latitudinal differences in life are well established in higher organisms not microorganisms. For example, some studies revealed that not only was bacterial diversity in continental scales unrelated to site temperature and latitude, but that community composition was largely independent of geographic distance. Indeed, the studies showed that the diversity of bacterial communities was strongly correlated to soil pH (Fierer & Jackson, 2006; Chu *et al.*, 2010). The previous studies suggest that microbial biogeography is controlled primarily by edaphic variables than by geographical distance. As was shown in the previous abovementioned studies,

a similar pattern was observed for Arctic soil in this study: microbial community structure was significantly correlated to soil pH than sampling distance (Chapter 2.1). However, bacterial community showed distinctive structure along latitudinal gradients (Chapter 3). These contradictory results have caused some controversy. Thus, more studies on microbial patterns across various horizontal distance scales are needed for a better understanding of microbial communities.

In vertical structure, soil factors changes along the soil depth. Soil depth acts as ecological filter to edaphic properties (soil texture, soil pH, nutrient contents, moisture and oxygen contents, temperature, etc.) and provides heterogeneous environments for microorganisms along depth. As mentioned above, although the soil surface environment in Arctic is too severe for the propagation of microbial cells, many studies have reported that biomass, microbial diversity, and enzyme activity were much higher in surface soil and decreased at deeper soil (Kobabe *et al.*, 2004; Yergeau *et al.*, 2010; Frank-Fahle *et al.*, 2014; Koyama *et al.*, 2014). Results similar with the previous studies was observed in this study (Chapter 2.1 and 2.2), which described the change of microbial community structure and diversity indices with soil properties along soil depth.

Microbial cells are metabolically active in cold environments. Cold adapted microbial cells generally need specific survival adaptations for their cellular components to remain fluid and functional at subzero temperature. Specifically, adaptations include regulation of membrane fluidity, cold-adapted proteins, cold-shock proteins, cold-acclimation proteins, antifreeze and ice-binding proteins, and osmolytes (Jansson & Tas, 2014). Using the specific adaptations, microbial cells can enter a dormant state or can metabolize and propagate at low temperature. Some studies revealed that CH_4

emissions were detected in the non-growing season (Song *et al.*, 2015). This result indicated that microbial cells are metabolically active in Arctic soils. CH_4 is a metabolic byproduct produced by Archaea (specifically, members of *Euryarchaeota*) in anoxic environmental conditions, such as in deeper soil. CH_4 produced from deeper anoxic soils is oxidized approximately 90% in upper aerobic conditions, and finally up to 10% of CH_4 is emitted to the atmosphere (Bosse & Frenzel, 1997; Wagner & Liebner, 2009a). That is, a large proportion of CH_4 emitted is consumed by methane oxidizing bacteria (MOB) in upper soil. This paper observed the microorganisms that produce methane and those that oxidize along the soil depth (Chapter 2.1 and 2.2). Certainly, MOB was relatively abundant in upper soils and methanogens showed increasing abundance in deeper soils, including permafrost. Although we did not check the methane flux in this study, we could alternatively check the potentiality through a prediction tool (PICRUSt, a tool that predicts the gene content of a microbial community from a marker gene survey, using an existing database of microbial genomes (Langille *et al.*, 2013)) (Chapter 2.2).

To sum up, the warming effect is making soil organic carbon more vulnerable (Grosse *et al.*, 2011). A warmer climate will cause carbon stored in the soil to be released into the atmosphere via microbial decomposition. Finally, thawing permafrost will lead to a rapid cycling of C and N. That is, with increasing temperature facilitating the microbial activity, more instability will follow for permafrost.

Table 1.1. A list of representative next generation sequencing (NGS)

Platform	Read length (bp)	Throughput per run	Library amplification	Carrier of library molecules or beads during sequencing	Sequencing principle
Roche					
454 FLX+	700	900 Mb	emPCR on microbeads	Picotiterplate	Pyrosequencing
454 FLX Titanium	400	500 Mb			
454 GS	400	50 MB			
Illumina					
Hiseq 2500	2x250	150 Gb	Bridge-OCR on microbeads	Flow cell	Reversible terminator sequencing by synthesis
Miseq	2x150	1000 Mb			
Life technologies					
SOLiD 4	50x35	71,000 Mb	emPCR on microbeads	Flow Chip	Sequencing by ligation
SOLid 5500	60x60	155,000 Mb			
Ion torrent					
PGM	200	1000 Mb	emPCR on microbeads	Ion Chip	Semiconductor-based sequencing by synthesis
Proton					
Pacific biosciences					
PacBio RS	1500	45/SC	Not applied	SMRT cell	Single-molecule, real-time DNA sequencing by synthesis

1.4 Objectives of this study

Considering approximately 25% of Earth's terrestrial surface is underlain by permafrost, our knowledge of the microbial life is surprisingly limited. As mentioned above, Arctic soil contains a huge amount of organic carbon of the global soil carbon, and a significant part of carbon deposits could be vulnerable to increasing temperature. Microorganisms will contribute to the organic carbon vulnerability because a warmer climate will cause carbon stored in the soil to be released into the atmosphere via microbial decomposition. However, many things need further clarification. Questions that require further investigation are: How is the microbial community in Arctic soils different from other biomes? What are the indigenous microorganisms of Arctic soils? Which soil properties affect microorganisms? How will microbial communities respond to the changing environment?

To answer these questions, microbial community structure and soil properties were investigated in the moist, acidic, and Alaska tundra, where it is highly responsive and vulnerable to climate change (Christensen *et al.*, 1998). To identify the soil microbial community structure and diversity, microbial 16S rRNA gene was sequenced using high-throughput sequencing (GS-FLX 454 pyrosequencing). Various soil properties were analyzed (soil texture, pH, electrical conductivity, and total carbon, nitrogen, and phosphorus contents).

The objectives of this study were to:

1) describe the microbial community structure of moist acidic tundra in Council, Alaska,

2) compare the microbial community structure between active layer and permafrost, and Arctic soil and other geographical located soils,

3) identify the relationships between microbial communities and soil edaphic properties, and

4) predict the microbial functional potential through a prediction tool using an existing database of microbial genomes.

This study can provide information on the spatial distribution (horizontal and vertical distribution) of microbial diversity and community structure, and similarity and/or difference on the bacterial diversity between Arctic soil and non-Arctic soils. Moreover, this study can contribute to understanding biogeochemical cycles in Arctic soil through observing the microbial physiological characteristics.

|CHAPTER 2|

Spatial Distribution of Prokaryotic Community Structure in Moist Acidic Tundra Soil

2.1 Horizontal and vertical distribution of bacterial community and relationships with soil properties in active layer

2.1.1 Introduction

Permafrost, distributed across 24% of the terrestrial area in the Northern Hemisphere, is soil that remains frozen for two or more years (Tarnocai *et al.*, 2009). Permafrost soil contains approximately half of the global terrestrial carbon as soil organic matter (SOM) because low temperatures prevent the rapid decomposition of these carbon stores (Ping *et al.*, 2008; Schuur *et al.*, 2009; Tarnocai *et al.*, 2009). 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). While permafrost soil is relatively a stable environment, active layer is a dynamic environment where ecological, hydrological, and biogeochemical activities dynamically occur (Kane *et al.*, 1991; Hinzman *et al.*, 2003).

Soil affected by Arctic permafrost has gained much scientific attention because global warming is predicted to be greatest and most rapid at high latitudes (IPCC, 2007). Evidence collected from the past few decades indicates that warming is already underway in the Arctic (ACIA, 2005; Chapin *et al.*, 2005). As containing a significant amount of soil carbon in permafrost soils including active layer, the warming effect is making soil organic carbon more vulnerable (Grosse *et al.*, 2011). A warmer climate will cause carbon stored in the soil to be released into the atmosphere via microbial

decomposition (Bardgett *et al.*, 2008; Schuur *et al.*, 2009). A recent study showed that the carbon loss of active layer is mediated by microbial communities (Xue *et al.*, 2016).

Understanding soil microbial community structure is essential to elucidate microbial processes. Many scientists have characterized microbial communities across Arctic soil environments using molecular works such as DGGE, T-RFLP, clone libraries, and next-generation sequencing (Männistö *et al.*, 2007; Steven *et al.*, 2007; Wallenstein *et al.*, 2007; Lauber *et al.*, 2009; Margesin *et al.*, 2009; Campbell *et al.*, 2010; Chu *et al.*, 2010; Larose *et al.*, 2010; Schuette *et al.*, 2010; Yergeau *et al.*, 2010; Coolen *et al.*, 2011). From these previous studies, interestingly, bacterial communities in Arctic soil are similar in structure and diversity to bacterial communities of other biomes at the phylum level (Chu *et al.*, 2010). However, few studies have examined the Arctic bacterial community structure at a lower taxonomic level (Männistö *et al.*, 2007; Steven *et al.*, 2007; Campbell *et al.*, 2010; Larose *et al.*, 2010), and such studies may reveal important differences in the actual functional groups of bacteria present in the Arctic.

Then, what is the vertical distribution of microbial communities in Arctic soil? Many studies have reported that microbial biomass and diversity, and enzyme activities were much higher in the surface soil and decreased towards the deeper soil in the active layer (Yergeau *et al.*, 2010; Frank-Fahle *et al.*, 2014; Koyama *et al.*, 2014). These vertical distribution of microbial abundance and diversity related with soil properties related changes along soil depth such as C/N ratio (Frank-Fahle *et al.*, 2014) or substrate availability (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 in a fine scale.

In this study, the horizontal and vertical distribution of bacterial community structures were observed in moist acidic tundra soils located in Council, Alaska, and explored the relationships between bacterial communities and soil properties. In addition, the vertical distribution of bacterial potential functions was inferred from amplicon data using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). For the investigations, a large volume of bacterial sequence data was obtained from soil samples through pyrosequencing to examine the bacterial community at a deep phylogenetic level. The relationships between bacterial communities and soil properties were investigated in horizontal (area of approximately 300 m × 50 m) and vertical (within active layer at 5 cm depth interval) structures.

2.1.2 Materials and Methods

Site description and sampling design

The study sites are located in Council, on the Seward Peninsular in Northwest Alaska (64°51′ N, 163°39′ W; Figure 2.1 and 2.2), an Arctic region. The site is approximately 30 m above sea level, and the annual mean air temperature and precipitation are –3.1 ± 1.4℃ and 258 mm, respectively (climate data were obtained from the International Arctic Research Center of the University of Alaska, Fairbanks). The active layer depth measured by using a steel probe (1 m). At the time of sampling (early July 2010 for vertical sampling and mid-August 2011 for horizontal sampling), the depth of the active layer was approximately 50–70 cm. Vegetation was observed by using quadrate (40 x 40 cm) and the sampling site was composed of moist acidic tussock tundra, and the dominant vegetation was cotton grass (*Eriophorum vaginatum*) or tussock, blueberry (*Vaccinium uliginosum*), and lichen and moss (*Sphagnum* spp.) beds (Figure 2.3).

To observe the horizontal distribution of the bacterial community structure, thirty-six points were selected over an area of ca. 300 m × 50 m. The points were spaced at 25 m intervals, resulting in a latticework of 12 points × 3 points (Figure 2.1). Before acquiring soil samples, we removed the aboveground vegetation and litter layer, and cleaned the shovel with 70% ethanol to prevent contamination between samples. At each site, soil samples were collected from a depth of 0–10 cm (upper-layer soil) and a depth of 10–20 cm (lower-layer soil). The collected soil samples were wrapped in autoclaved foil, placed in zip-lock bags, and transported to the laboratory in a frozen state. The soils in sites 4 and 35 were saturated with water, and only

the upper-layer soil was collected. Total 70 soil samples were collected for this study.

To observe the vertical distribution of the bacterial community structure, three sampling points with similar vegetation compositions (dominated by blueberry, lichen and moss) were randomly selected within 100 m-distance from each other (Figure 2.2). After removing aboveground 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 soil samples were immediately placed in icebox and transferred to a freezer in the laboratory using icebreaking research vessel (IBRV) Araon. The soil samples were stored at –20℃ for further laboratory analysis.

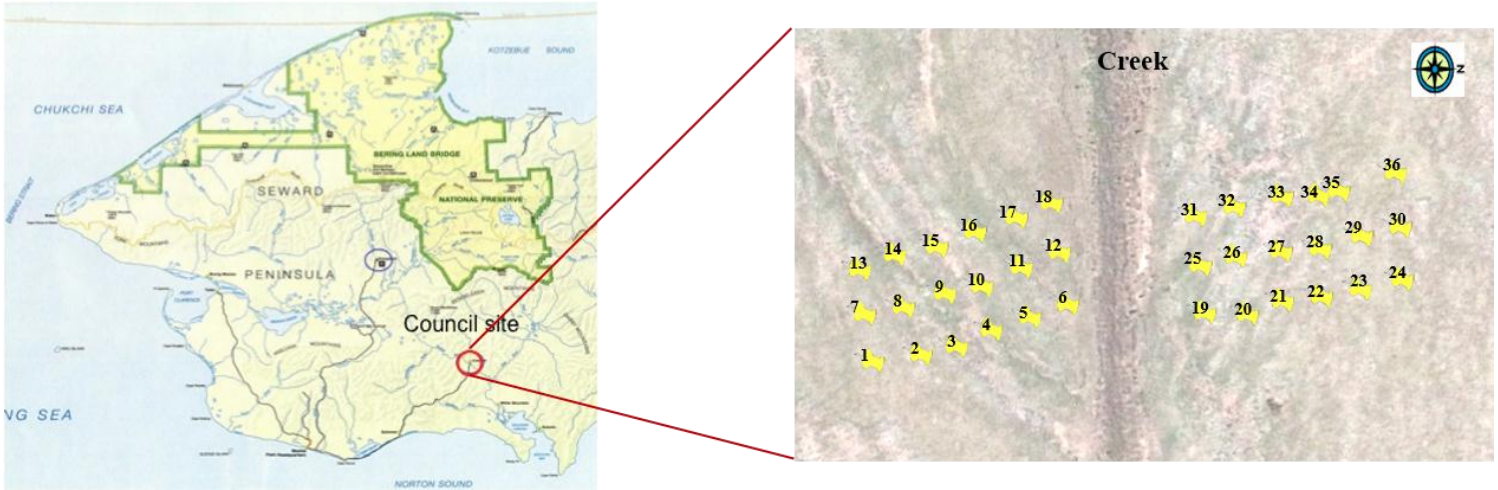
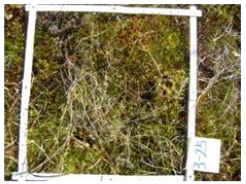


Figure 2.1. Sampling area located in Council, Alaska (left) and horizontal sampling points (right). Total 70 samples with two-soil depth (0–10 and 10–20 cm) at 36 sampling points were horizontally chosen in Council, Alaska. There is no data for lower-layer for sampling points 4 and 35 because these points were waterlogged.

Figure 2.2. Vertical soil core sampling points in this study. Each sampling point contains 9 horizons with 5 cm increments.


Site 13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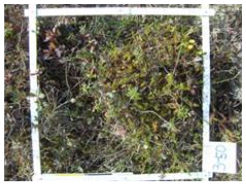
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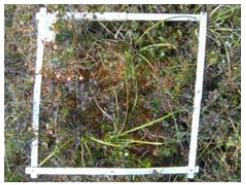
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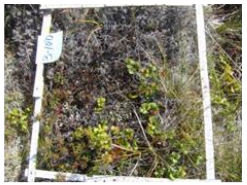
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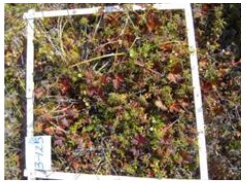
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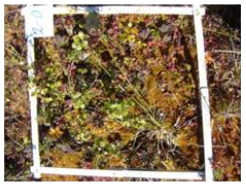
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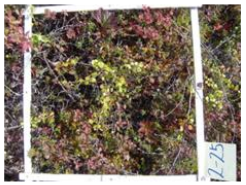
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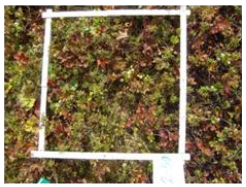
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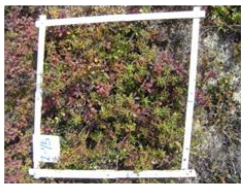
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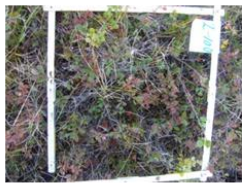
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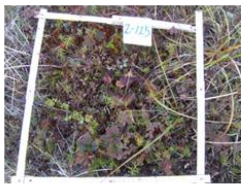
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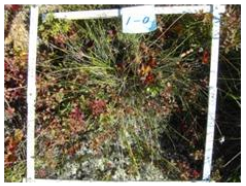
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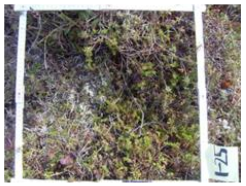
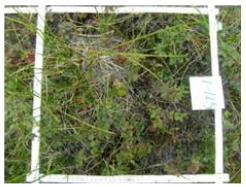

Site 2


Site 3


Site 4


Site 5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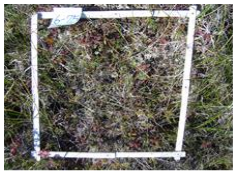
Site 31

Site 32

Site 33



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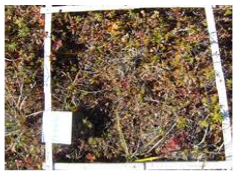

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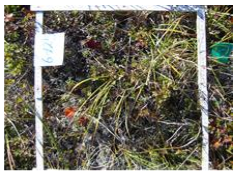

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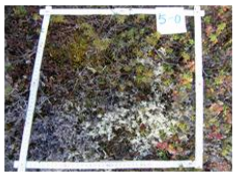

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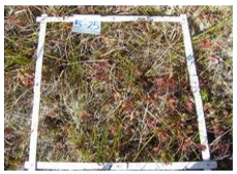
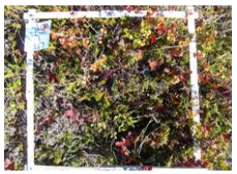


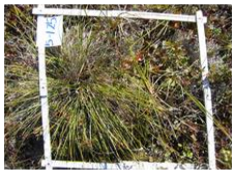
Site 26

Site 27



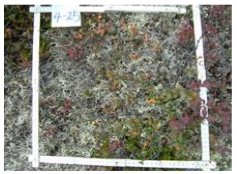
Site 28

Site 29



Site 30

Site 19



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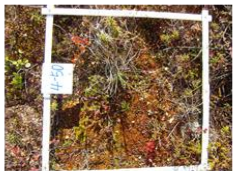
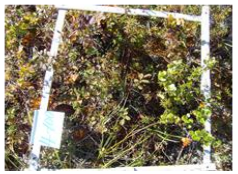


Site 21

Site 22



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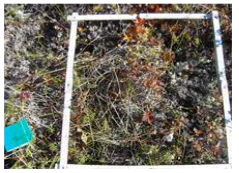


Site 24

Figure 2.3. Vegetation survey using quadrate (40 x 40 cm). Most sampling sites were composed of lichen and moss (*Sphagnum* spp.) bed. The dominant vegetation was cotton grass (tussock; *Eriophorum vaginatum*) and blueberry (*Vaccinium uliginosum*).

Physical and chemical properties of soil

For horizontal sampling: gravimetric moisture content (MC) was determined by measuring the difference in weight between the field-moist soil samples and the same soil samples dried at 105℃ for 48 hours. For inorganic nitrogen (N) analyses, approximately 7 g of fresh soil was immediately set aside after sampling and kept frozen until extraction. Inorganic N (NH_4^+ and NO_3^-) was extracted using a 2 M KCl solution and filtered through Whatman #42 paper. The filtrate was analyzed with an Auto-analyzer (Quaatro; Seal Analytical, Inc.). The remaining soil was air-dried and sieved through 2 mm mesh for further analyses. Soil pH was determined in a 1:10 soil:water (w/v) solution (Thomas *et al.*, 1996). Soil was ground and passed through a 53 μm sieve to determine total carbon (TC) and total nitrogen (TN) content. TC and TN contents were measured by combustion (950℃) (FlashEA 1112; Thermo Fisher Scientific). Total inorganic carbon was negligible in all soil samples.

For vertical sampling: 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 & Bauder, 1986). Soil pH was determined in a soil-water suspension (1:5 ratio, w/v) by a pH meter (Orion 3 star, Thermo Scientific, USA), 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, Germany). 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, Cambridge, UK). For total phosphorus

(TP), two different acids were used to digest ground soils depending on the depth and organic residue content: 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 from both soil samples, the soils were sub-sampled from the soil samples, freeze-dried (LABCONCO, USA), and homogenized. Genomic DNA was extracted from 0.5 g of the homogenized soil samples using a FastDNA® SPIN kit for soil (MP Biomedicals) and a QuickPrep adapter (MP Biomedicals), according to the manufacturer's recommended protocol. The total DNA was quantified by Hoechst dye 33258 staining using a spectrophotometer with excitation and emission at 350 nm and 460 nm, respectively (Wallac EnVision 2013 Multilabel Reader, Perkin Elmer). The extracted DNA was stored at -20℃ until further analysis.

Extracted genomic DNA was amplified by PCR using the adapter-multiplex identifier-primer combinations targeting the V1–V3 regions (27F–518R) of bacterial 16S rRNA gene (Chun *et al.*, 2010).

For horizontal sampling, the PCR reaction mixture (50 μL) contained 25 μL of master mix (DreamTaq™ Green PCR Master Mix [2×]), 1.4 μL of the forward and reverse primers (20 pmol of each primer), 1 μL of template DNA (1 ng/μL), and 22.6 μL of deionized distilled water (DDW). PCR program was as follows: an initial denaturation step at 95℃ for 3 min

followed by 30 cycles of denaturation at 95℃ for 30 s, annealing at 56℃ for 30 s, and extension at 72℃ for 90 s, with a final extension at 72℃ for 7 min.

For vertical sampling, the 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 program was as follows: an initial denaturation step at 95℃ for 7 min followed by 30 cycles of denaturation at 95℃ for 1 min, annealing at 57℃ for 1 min, and extension at 72℃ for 1 min, with a final extension at 72℃ for 7 min.

All samples were amplified in triplicate, pooled in equal amounts, and purified using the QIAquick PCR Purification Kit (Qiagen). The PCR products were quantified with a NanoDrop. DNA sequencing was performed using a GS-FLX 454 pyrosequencer (Roche).

Processing of pyrosequencing data

PCR amplicon pyrosequencing data were processed using the QIIME software package, ver. 1.7 (Caporaso *et al.*, 2010a). Briefly, raw flowgrams (sff files) were filtered and noise and chimeras were removed using AmpliconNoise software, ver. 1.27 (Quince *et al.*, 2011), using the platform option for FLX Titanium sequence data implemented in QIIME. Sequences were clustered based on operational taxonomic units (OTUs) at 97% similarity using UCLUST (Edgar, 2010). OTUs were assigned to taxa using the RDP Classifier method (Wang *et al.*, 2007) with a training set based on the Greengenes database (Werner *et al.*, 2012). Sequence alignments for phylogenetic reconstruction were generated using PyNAST software (Caporaso *et al.*, 2010b) and the Greengenes database (DeSantis *et al.*, 2006). Using additional downstream tools in QIIME, a phylogenetic tree was built

from the aligned sequences using FastTree 2.1 (Price *et al.*, 2010), and a pairwise beta diversity distance matrix for a randomly selected subset of 700 sequences for horizontal sampling and 1,583 sequences for vertical sampling was generated for all samples based on the unweighted UniFrac phylogenetic distance metric (Lozupone *et al.*, 2006).

To facilitate diversity comparisons among bacterial communities, we estimated diversity indices, including the Chao1, Shannon, and Simpson indices, for a randomly selected subset of 700 sequences for horizontal sampling and 1,583 sequences for vertical sampling from each sample to avoid effects of different sample sizes (Kirchman *et al.*, 2010).

To predict the community's functional capabilities from the 16S rRNA gene data for vertical sampling, 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 454 FLX Titanium flowgrams have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive database (accession number, SRP026166 for horizontal sampling and SRR1312081 for vertical sampling).

Statistical analysis

Statistical analyses were performed using R (version 3.0.0; The R Foundation for Statistical Computing) and PRIMER-E V6 (Clarke & Gorley, 2006). For analysis of horizontal sampling, analysis of similarity (ANOSIM)

with 999 permutations and non-metric multidimensional scaling (NMDS) were conducted to compare bacterial community structure. Classification and regression tree (CART) analyses were conducted using RPART in the R software package (CP value set at 0.001) to determine which environmental variables explained the deviance of the dominant bacterial groups. A Mantel test was used to determine which physical and chemical properties of soil were significantly correlated with the bacterial community. To assess how the bacterial community changed with sampling distance (ca. 22 to 427 m), a distance-decay relationship analysis, which assumes that community similarity will decrease with increasing geographical distance, was performed with some modification (Martiny *et al.*, 2011). Briefly, the rate of distance-decay of the bacterial communities was calculated as the slope of a linear least squares regression on the relationship between geographic distance (m) versus the jackknifed unweighted UniFrac distance of bacterial similarity, which is a qualitative metric of beta-diversity and is unaffected by the presence of duplicate sequences.

For analysis of vertical sampling, 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 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 Coordinates 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 alpha diversity indices and 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 (operational taxonomic units) and functional gene contents.

2.1.3 Results

Physicochemical characteristics of soil

The results from the horizontal sampling, the soil at the study site was acidic and moist. The soil pH ranged from 3.90 to 5.02 (Table 2.1 and 2.2). The upper-layer soil pH was slightly more acidic than the lower-layer soil pH (Table 2.1). Gravimetric MC was greater than 100%, except at site 17. The upper-layer soil contained higher MC than the lower-layer soil at most sites. In the upper-layer soil, the average total carbon (TC) and total nitrogen (TN) contents were 40% and 1.5%, respectively. The lower-layer soil had a lower TC content (36%), but the same TN content as the upper-layer soil. Therefore, the C/N ratio was higher in the upper-layer soil. The ammonium ion (NH_4^+) concentration was higher than the nitrate ion (NO_3^-) concentration at both depths and at all sites. Ammonium concentrations ranged from 8.6 $\mu g \cdot g^{-1}$ to 93.1 $\mu g \cdot g^{-1}$ soil, but the nitrate concentrations were negligible. The soil properties of site 17 were completely different from those of the other sites. This site contained lower MC, TC, and TN contents, and higher soil pH because it was primarily composed of mineral layers rather than organic layers, which comprised the other sampling sites.

The results from the horizontal sampling, most depths of soil were classified as organic layer (O layer) on the basis of the TC content (Soil Survey Staff, 2014), and soil profile was composed of two distinct horizons

as Oi and Oa according to the decomposition degree of SOM (Figure 2.4). While soil up to 25 cm depth was the Oi (fibric) horizon that was composed of less decomposed SOM and plant tissue as fiber, soil between the depths of 25–45 cm was the Oa (sapric) horizon which consisted of the most highly decomposed SOM. Although the main component of the Oa horizon was organic materials, the horizon was found to be containing some mineral soil material which was texturally classified as silt loam (133.1 g kg^{-1} for sand, 635.9 g kg^{-1} for silt, and 231.1 g kg^{-1} for clay on average). Overall soil properties were distributed based on the decomposition degree of SOM (Figure 2.5).

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 (Figure 2.4). The TC and TN contents did not significantly differ 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 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 (Figure 2.4). Soil EC did not change with depth and its level was negligible for plant growth (Bernstein, 1975).

Table 2.1. The summary of physical and chemical properties of the soil samples

	pH		TC (%)		TN (%)		C/N		MC (%)		NO_3^- ($\mu g\ N\ g^{-1}$ soil)		NH_4^+ ($\mu g\ N\ g^{-1}$ soil)	
	U	L	U	L	U	L	U	L	U	L	U	L	U	L
Mean	4.35	4.53*	39.94	35.94*	1.50	1.54	28.52	24.15*	628.2	438.4*	0.80	0.75	32.53	29.06
SD	0.29	0.28	6.81	12.35	0.46	0.58	9.05	6.56	222.5	252.6	0.48	0.53	22.25	17.91
CV (%)	6.61	6.25	17.04	34.36	30.38	37.52	31.71	27.14	35.4	57.6	59.18	70.56	68.40	61.61
MAX	5.02	5.01	43.87	48.55	2.23	2.38	66.30	56.93	1070.3	1201.6	3.19	3.29	93.08	91.55
MIN	3.90	3.96	2.10	1.85	0.09	0.08	19.31	18.26	53.4	32.1	0.27	0.21	8.62	9.79

TC, total carbon; TN, total nitrogen; C/N, a ratio of carbon to nitrogen; MC, moisture content; SD, standard deviation; CV, coefficient of variation, U, upper; L, lower.

* denotes significant differences (p <0.05) of soil properties between the upper- and lower-layers.

Table 2.2. The physical and chemical properties of the soil samples

Sampling sites	pH		TC (%)		TN (%)		C/N		MC (%)		NO_3^- ($\mu g\ N\ g^{-1}$ soil)		NH_4^+ ($\mu g\ N\ g^{-1}$ soil)	
	U	L	U	L	U	L	U	L	U	L	U	L	U	L
1	4.04	4.08	39.45	42.91	1.00	1.52	39.56	28.20	667.6	752.5	0.58	0.49	23.90	16.74
2	4.00	4.33	41.92	24.84	1.60	1.13	26.18	22.06	585.4	187.8	0.53	0.21	17.71	20.03
3	4.55	4.50	42.76	45.38	2.22	1.72	19.31	26.41	506.8	405.9	1.09	0.69	93.08	30.88
4	4.63	ND	40.54	ND	1.89	ND	21.46	ND	746.5	ND	0.55	ND	80.26	ND
5	4.51	4.66	36.56	42.32	1.16	1.96	31.54	21.58	960.2	789.5	0.59	0.42	21.01	35.62
6	4.65	4.64	40.05	48.55	1.54	1.99	25.98	24.45	618.3	484.2	0.27	0.28	45.09	26.18
7	4.29	4.41	43.64	47.21	1.73	2.13	25.29	22.22	609.4	547.2	0.48	0.55	28.46	91.55
8	4.23	4.59	40.52	41.85	1.04	1.75	38.89	23.95	871.2	264.9	0.42	0.34	12.18	30.88
9	4.14	3.96	38.08	48.33	1.32	1.85	28.89	26.18	536.9	509.4	1.15	1.10	63.81	37.69
10	4.10	4.34	40.61	44.37	1.67	1.91	24.27	23.27	368.5	382.8	0.39	1.28	53.67	36.44
11	4.66	4.72	37.18	43.18	1.67	2.14	22.32	20.22	464.5	441.8	0.41	0.50	17.08	12.96
12	4.38	4.69	41.78	9.38	1.30	0.36	32.14	26.05	984.7	143.1	0.55	0.39	33.80	11.10

Table 2.2. Continued.

13	4.10	4.43	41.49	45.52	1.28	2.17	32.31	21.02	760.9	556.8	0.52	0.74	20.55	21.49
14	4.14	4.67	42.40	40.21	1.71	1.62	24.87	24.84	302.6	292.0	0.71	0.55	55.50	45.18
15	4.70	4.93	41.66	17.71	1.80	0.79	23.20	22.47	518.5	311.7	1.16	0.58	43.16	28.81
16	4.53	4.85	43.12	15.44	1.98	0.75	21.74	20.53	439.8	123.6	1.15	0.39	15.58	19.47
17	5.02	4.83	2.10	1.85	0.09	0.08	22.57	22.51	53.4	32.1	0.38	0.36	32.99	22.32
18	4.04	4.13	43.84	45.85	1.79	2.14	24.53	21.44	680.3	261.0	1.01	0.49	17.11	26.65
19	4.07	4.34	42.79	48.07	1.86	2.32	22.97	20.73	451.0	366.0	1.00	0.52	12.73	27.39
20	4.50	4.82	37.36	39.91	1.66	1.94	22.51	20.56	533.1	477.7	3.19	3.29	12.65	30.01
21	4.48	4.80	41.08	26.44	1.78	1.25	23.12	21.12	644.6	395.2	0.90	0.77	42.24	46.15
22	4.47	4.44	41.33	44.35	1.27	1.84	32.59	24.12	1058.9	767.0	0.60	0.80	36.13	22.27
23	4.03	4.19	41.73	37.37	1.23	1.34	33.90	27.84	906.0	605.1	0.58	0.85	25.22	19.44
24	3.90	4.19	40.94	41.82	1.01	1.40	40.61	29.89	906.1	684.6	0.82	1.04	10.62	16.20
25	4.15	4.51	37.82	46.01	1.13	2.38	33.50	19.31	484.0	365.0	0.82	0.83	8.62	12.27
26	4.65	4.82	41.87	32.48	1.96	1.45	21.33	22.34	677.2	199.3	0.93	0.69	13.07	10.27

Table 2.2. Continued.

27	4.57	4.64	43.02	41.92	2.10	2.14	20.54	19.57	777.0	632.9	0.83	1.05	72.65	69.96
28	4.28	4.29	41.46	45.32	1.42	1.97	29.14	23.03	620.6	407.6	0.79	0.77	15.40	11.92
29	4.14	4.33	40.78	38.99	0.97	1.22	42.22	31.91	791.2	891.1	1.00	1.27	13.99	18.65
30	4.03	4.33	43.25	23.87	1.58	1.02	27.46	23.33	423.4	289.4	0.74	0.87	14.66	9.79
31	4.62	4.96	42.60	24.69	1.89	1.22	22.59	20.31	558.2	139.4	0.84	0.47	21.27	16.60
32	4.81	5.01	43.87	33.48	2.23	1.46	19.64	22.88	590.2	339.1	0.96	0.64	55.57	25.28
33	4.58	4.85	41.45	15.80	1.90	0.87	21.79	18.26	351.2	111.2	0.72	0.56	48.95	29.78
34	4.61	4.74	35.88	37.13	1.34	1.71	26.88	21.68	472.3	546.4	0.57	0.55	62.47	61.47
35	4.07	ND	41.35	ND	1.19	ND	34.75	ND	626.3	ND	0.91	ND	15.83	ND
36	3.95	4.12	41.44	39.57	0.63	0.70	66.30	56.93	1070.3	1201.6	0.79	1.14	13.99	46.69

TC, total carbon; TN, total nitrogen; C/N, a ratio of carbon to nitrogen; MC, moisture content; SD, standard deviation; CV, coefficient of variation. ND, no data. U, upper; L, lower.

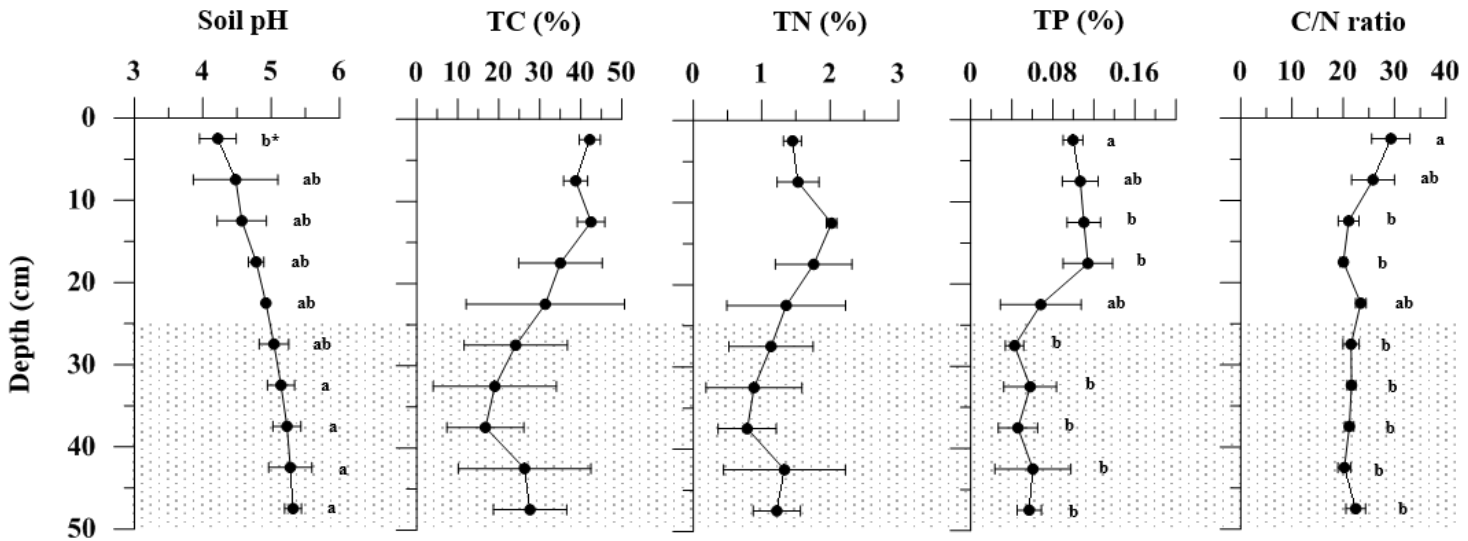


Figure 2.4. Soil physical and chemical properties along soil depth. Soil horizon was separated by Oi (fibric) and Oa (sapric, shadow indicated). TP, total phosphorus content; C/N, a ratio of carbon and nitrogen content; TC, total carbon content; TN, total nitrogen content.

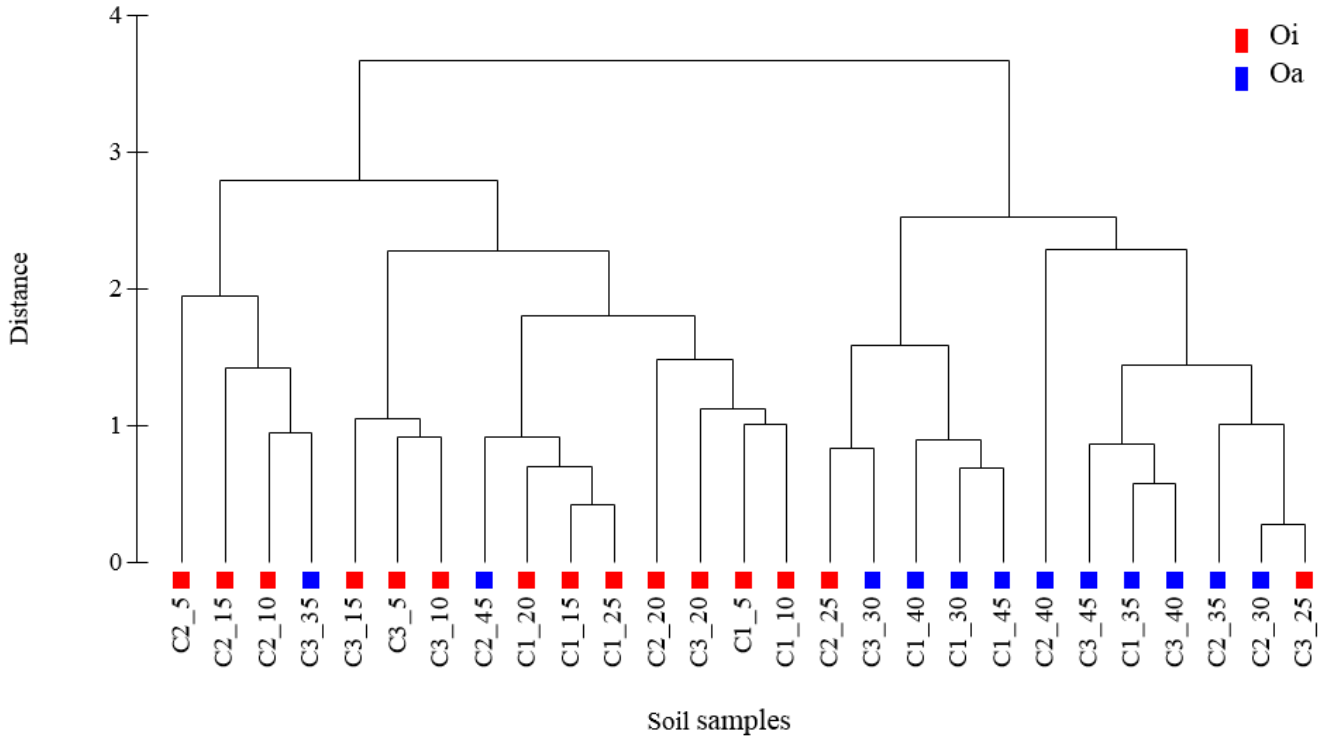


Figure 2.5. Cluster analysis based on Euclidean distances between soil physical and chemical properties.

General description of sequencing results

A total of 91,742 good quality 16S rRNA gene sequences (V1–V3 region) was obtained from all the soil samples for horizontal sampling. On average, 1,311 sequences (range, 718–1,944 sequences) were obtained per sample. When I compared the soil bacterial communities using the same number of reads (700 sequences per sample), bacterial abundance and bacterial diversity were significantly higher in the upper-layer soils, as indicated by the Chao1 ($p < 0.001$), Shannon ($p < 0.001$), and Simpson ($p < 0.05$) indices (Table 2.3). OTUs accounted for 25.1–43.7% of the diversity, according to the Chao1 index.

A total of 179,160 bacterial 16S rRNA gene sequences were obtained from 27 samples of the three soil cores for vertical sampling. 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*, *Beta-proteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*). Among these phyla, *Acidobacteria*, *Actinobacteria*, and *Alphaproteobacteria* were dominant, accounting for over 45% of the total bacterial abundance from every soil depth (Figure 2.6). 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 (Figure 2.7). 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 (Figure 2.7). The bacterial richness and diversity was high in surface soil and linearly decreased until soil depth at 25–30 cm, and did not varied widely below 30 cm (Figure 2.7). Our results revealed that the bacterial abundance, richness, and diversity in Oi horizon was greater than Oa horizon.

Table 2.3. Summary statistics of pyrosequencing 16S rRNA gene sequences of soil samples

Sampling sites	No. of bacterial reads		No. of observed OTUs[a]		Alpha diversity measures[*]									
					OTUs		Chao1		Shannon (*H*')		Simpson		Estimated overage (%)	
	U	L	U	L	U	L	U	L	U	L	U	L	U	L
1	1870	1590	474	397	260	211	611	517	6.87	6.89	0.97	0.98	86.1	86.7
2	1944	1573	368	275	201	134	502	372	6.32	5.98	0.97	0.96	89.7	91.5
3	1062	923	231	213	118	113	352	352	6.11	6.27	0.96	0.97	88.9	87.8
4	1353	-	495	-	296	-	795	-	7.58	-	0.99	-	78.1	-
5	1298	908	429	285	247	175	681	599	7.25	6.97	0.98	0.98	81.0	80.7
6	988	984	362	307	222	183	661	562	7.16	6.94	0.98	0.98	77.5	81.4
7	1372	1360	375	204	202	111	529	289	6.86	5.60	0.98	0.96	85.3	91.8
8	1225	1706	409	304	260	161	679	439	7.13	6.17	0.98	0.97	78.8	90.6
9	1448	718	463	178	273	107	741	426	7.18	6.23	0.98	0.97	81.1	85.1
10	1580	1153	433	339	249	208	620	588	6.87	6.62	0.97	0.97	84.2	82.0
11	1693	876	497	260	296	149	678	467	7.24	6.70	0.99	0.98	82.5	83.0
12	1373	1397	388	274	224	142	596	395	6.83	6.16	0.98	0.97	83.7	89.8
13	1765	1262	379	229	213	127	503	349	6.52	5.57	0.98	0.95	87.9	89.9
14	1687	1584	356	333	186	195	447	463	6.63	6.20	0.98	0.96	89.0	87.7
15	1694	1574	334	269	173	139	442	383	6.39	6.06	0.97	0.97	89.8	91.2
16	1266	1346	470	388	283	210	742	541	7.36	6.87	0.98	0.98	77.6	84.4
17	1416	1455	348	319	181	163	459	429	6.80	6.52	0.98	0.97	87.2	88.8

Table 2.3. Continued.

18	1635	1090	450	154	256	87	658	258	7.10	4.80	0.99	0.91	84.3	92.0
19	1510	927	381	246	205	138	520	416	6.75	6.38	0.97	0.97	86.4	85.1
20	1432	1389	452	286	250	164	671	420	7.16	6.22	0.98	0.97	82.5	88.2
21	1351	1222	339	252	196	142	507	383	6.71	6.07	0.98	0.96	85.5	88.4
22	1341	1602	410	201	237	88	623	240	7.02	5.56	0.98	0.96	82.3	94.5
23	1297	1493	329	275	180	128	485	365	6.81	6.26	0.98	0.97	86.1	91.4
24	1303	1838	371	175	220	73	603	175	7.00	5.16	0.98	0.93	83.1	96.0
25	1706	1445	515	393	311	227	783	562	7.24	6.53	0.99	0.96	81.8	84.3
26	1255	1080	422	261	253	141	674	417	7.29	6.32	0.99	0.97	79.8	86.9
27	1182	999	427	325	251	182	647	536	7.65	7.18	0.99	0.99	78.8	81.8
28	842	1593	310	312	199	168	649	470	6.95	6.48	0.98	0.98	76.4	89.5
29	1314	1388	306	275	154	146	416	363	6.49	6.17	0.97	0.97	88.3	89.5
30	973	1388	214	227	107	106	327	286	6.22	6.06	0.97	0.97	89.0	92.4
31	1261	1036	345	257	192	133	499	392	6.80	6.46	0.98	0.98	84.8	87.2
32	1098	1279	341	318	198	177	582	467	6.97	6.65	0.98	0.98	82.0	86.2
33	1223	1278	373	398	212	220	592	578	7.00	7.22	0.98	0.98	82.7	82.8
34	777	745	258	234	160	129	534	442	6.66	6.72	0.98	0.98	79.4	82.7
35	1139	-	294	-	156	-	417	-	6.74	-	0.98	-	86.3	-
36	857	1011	265	351	155	213	483	649	6.76	7.24	0.98	0.98	81.9	78.9

[a] The number of OTUs was generated at the 97% sequence similarity cutoff.

[*] Diversity indices represent the randomly selected subsets (n=700) for each sample. -, no data. U, upper; L, lower.

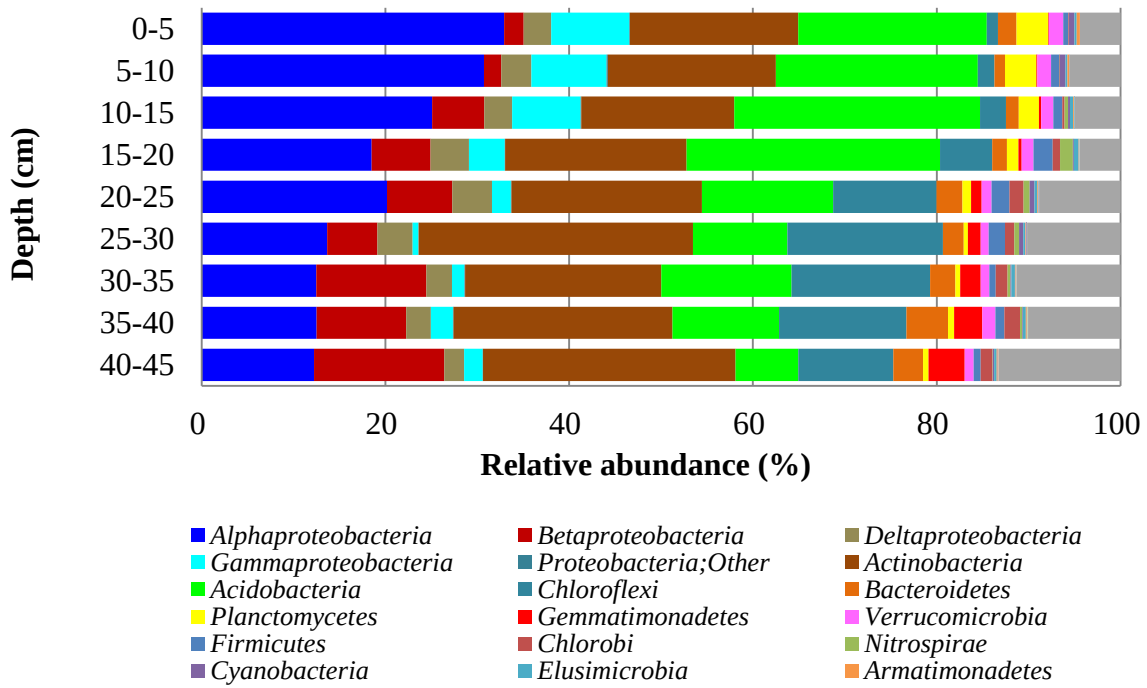


Figure 2.6. Vertical distribution of bacterial community composition at phylum level (*Proteobacteria* represented at the class level).

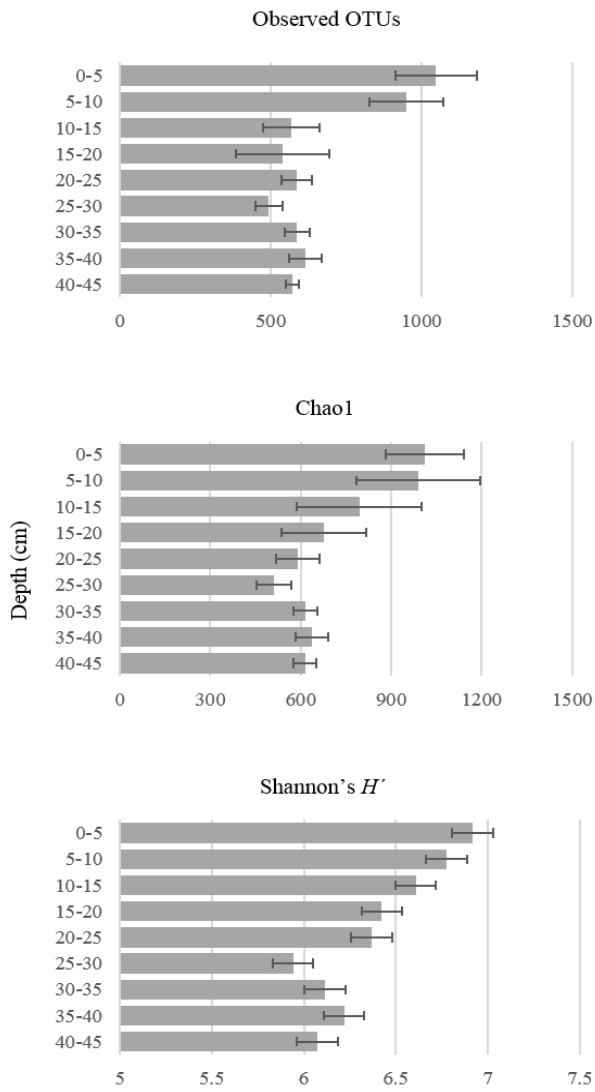


Figure 2.7. Statistical summary of pyrosequencing of 16S rRNA gene from vertical soil sampling. The number of OTUs was generated at the similarity cutoff of 97%. Diversity indices (Chao1 and Shannon's *H´*) were calculated from the randomly selected subsets (n=1,583) per sample.

Bacterial community structure within and between sites

The classifiable sequences comprised members of 50 phyla, including candidate phyla. The dominant phyla were *Acidobacteria*, *Alphaproteobacteria*, and *Actinobacteria*, which accounted for more than 40% of the bacterial sequences in all soil samples (Figure 2.8). In addition, sequences of *Betaproteobacteria*, *Gammaproteobacteria*, *Chloroflexi*, *Deltaproteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Planctomycetes*, *Chlorobi*, *Firmicutes*, *Elusimicrobia*, *Nitrospira*, *Armatimonadetes* (former candidate division OP10), *Gemmatimonadetes*, *Cyanobacteria*, *Spirochaetes*, *Fibrobacteres*, *Caldiserica* (former candidate division OP5), *Lentisphaerae*, and *Epsilonproteobacteria* were also identified at relatively low abundances, as well as members of 27 candidate phyla and several unclassified bacteria (Figure 2.8).

In general, the bacterial community structures in the upper- and lower-layer soils were different. *Alphaproteobacteria*, *Gammaproteobacteria*, and *Planctomycetes* were more abundant in the upper-layer soils, whereas *Actinobacteria*, *Betaproteobacteria*, *Chloroflexi*, and AD3 were more abundant in the lower-layer soils (Figure 2.9). At the family level, *Methylocystaceae*, *Acetobacteraceae*, *Sinobacteraceae*, and Ellin6513 were more abundant in the upper-layer soil, whereas *Gallionellaceae*, *Solibacteraceae*, *Intrasporangiaceae*, and Ellin6529 were more abundant in the lower-layer soil (Figure 2.9). The 16S rRNA gene sequences of the dominant OTUs, which accounted for more than 1% of the total sequences, were identified (Table 2.9). Only one OTU (OTU_1) showed greater than 97% sequence similarity with cultured bacteria, and most of the dominant OTUs have yet to be cultured (Table 2.4). The dominant bacterial OTUs accounted for 9.7% and 15.0% of the total sequences in the upper and lower-layer soil

samples, respectively. Among the 11 dominant OTUs, three accounted for over 1% of total sequence in both soil layers. The nonmetric multidimensional scaling (NMDS) plots indicated that the bacterial communities showed greater similarity across horizontal layers than through vertical depth (Figure 2.10a). This pattern was confirmed by a significant ANOSIM value ($r = 0.338$, $p < 0.001$) between the two depths. Bacterial communities were similar between sampling sites (Figure 2.11). There was significant correlation between bacterial community similarity and sampling distance in the lower-layer soils ($p < 0.05$; Figure 2.11), however, the relationship was not observed in upper layer soils ($p > 0.05$).

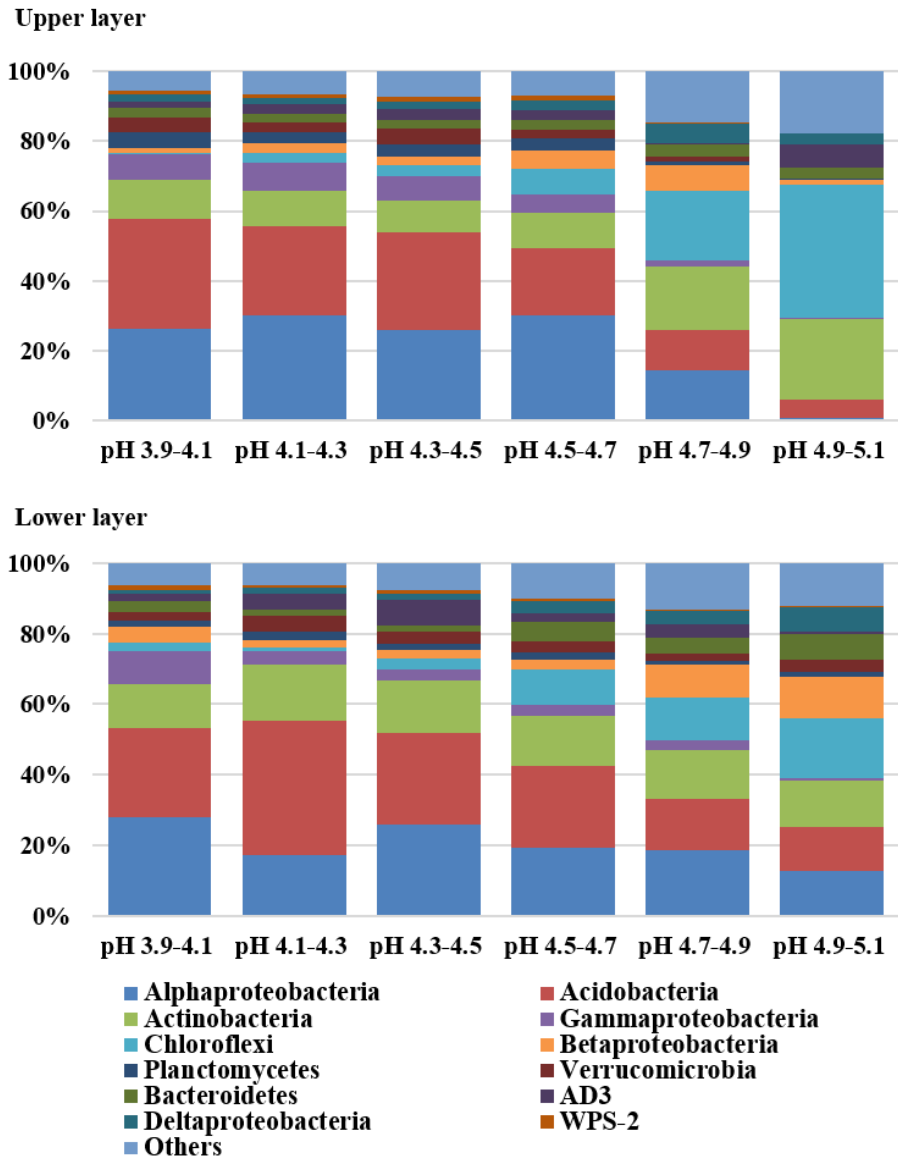


Figure 2.8. Relative abundance of phyla in the soil bacterial communities in the upper- and the lower-layer soils, separated according to pH. Others contain minor groups.

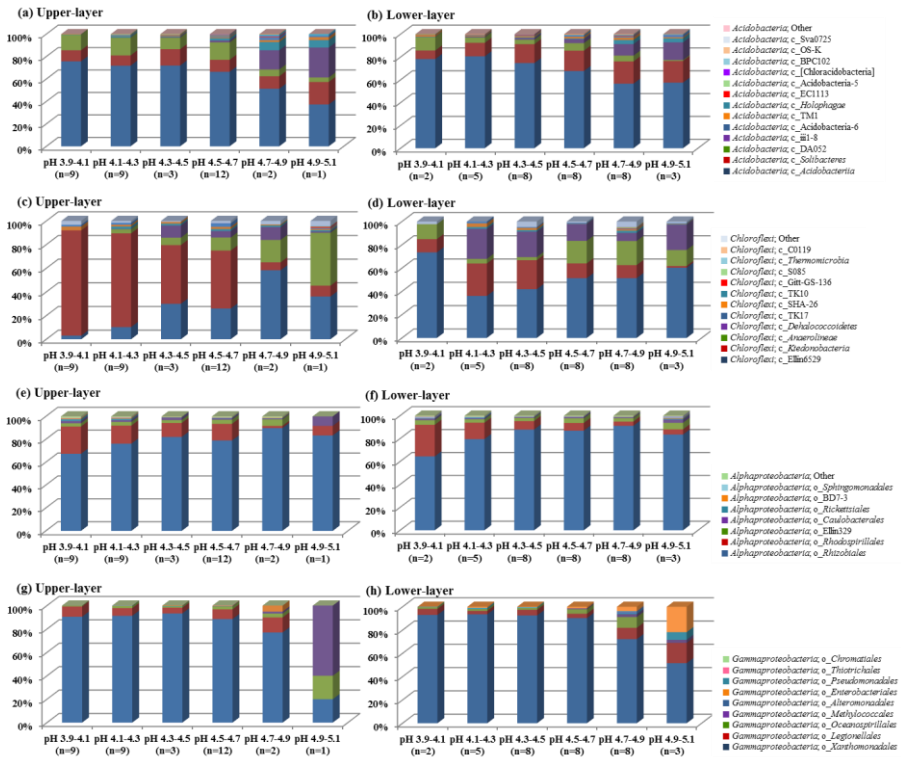


Figure 2.9. Bacterial community structures at class level of *Acidobacteria* (a and b) and *Chloroflexi* (c and d), and at order level of *Alphaproteobacteira* (e and f) and *Gammaproteobacteria* (g and h) with soil pH category.

Table 2.4. A list of dominant OTUs which were accounted for over 1% among total reads through EzTaxon-e[†] database

OTU no.	The closest species (accession no.)	Detection source[*]	Pairwise similarity (%)	Lineage	Relative abundance (%)	
					Upper	Lower
1	*Afipia broomeae* (KB375282)	Human	99.5	*Alphaproteobacteria*	3.61	2.38
2	*Pseudolabrys sp.* (EU937836)	Biofilm	98.5	*Alphaproteobacteria*	1.44	2.91
3	*Telmatobacter sp.* (AJ292586)	Polychlorinated biphenyl-polluted soil	98.8	*Acidobacteria*	1.38	1.87
4	EU150278_s in *Steroidobacter*_f (EU150278)	Soil	100	*Gammaproteobacteria*	2.04	0.62
5	*Koribacter sp.* (AY913298)	Forest	98.8	*Acidobacteria*	0.01	1.88
6	*Koribacter sp.* (GQ339162)	Iron(II)-rich seep	99.3	*Acidobacteria*	0.54	1.41
7	*Koribacter sp.* (EU150193)	Soil from spruce fir forest	99.8	*Acidobacteria*	1.24	0.40
8	*Oryzihumus sp.* (4P001838)[**]	ND	98.3	*Actinobacteria*	0.51	1.19
9	*Granulicella sp.* (FJ466102)	Volcanic deposit	99.8	*Acidobacteria*	0.97	1.13
10	EU861899_s in *Solirubrobacterales* (EU861899)	Meadow surface soil	100	*Actinobacteria*	0.69	1.10
11	*Gallionella sp.* (4P002107)[**]	ND	99.3	*Betaproteobacteria*	0.43	1.08

[*] Data for detection sources were from NCBI or publications.

[**] Accession number was from EzTaxon-e.

[†] EzTaxon-e database (Kim *et al.*, 2012).

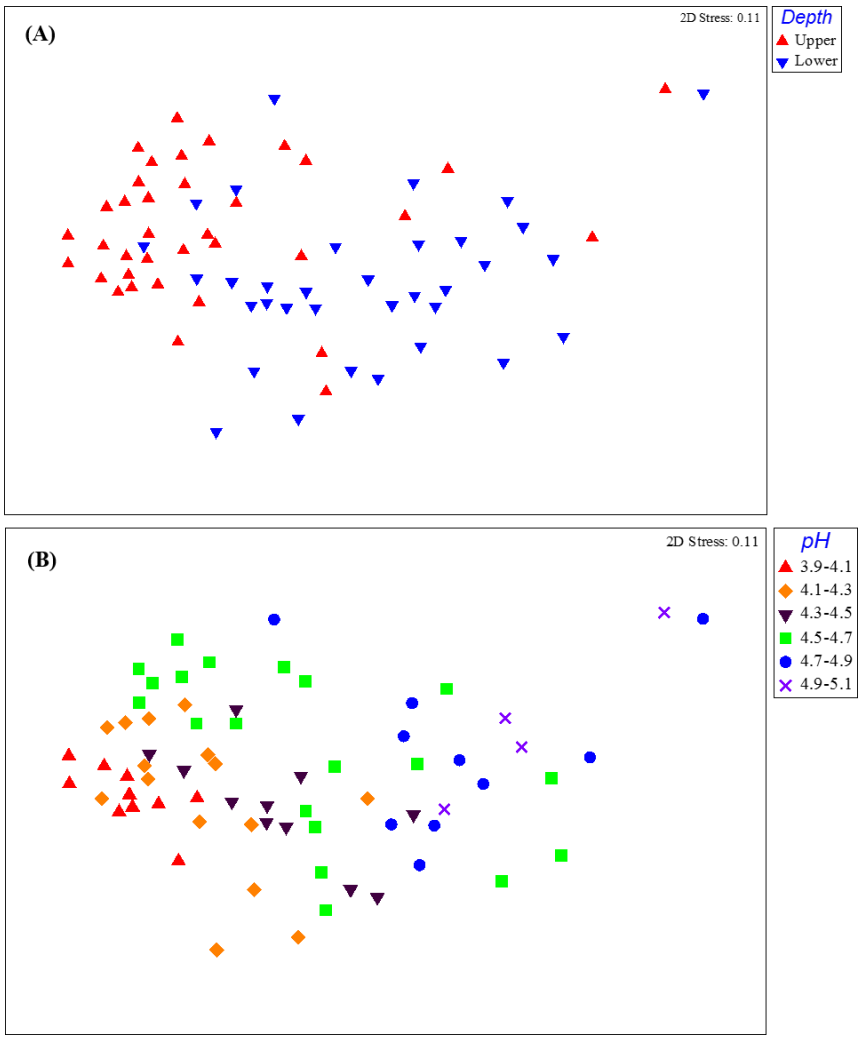


Figure 2.10. Nonmetric multidimensional scaling (NMDS) plots derived from phylogenetic similarity based on jackknifed unweighted UniFrac distances between soil samples, with symbols coded by depth (A) and pH (B).

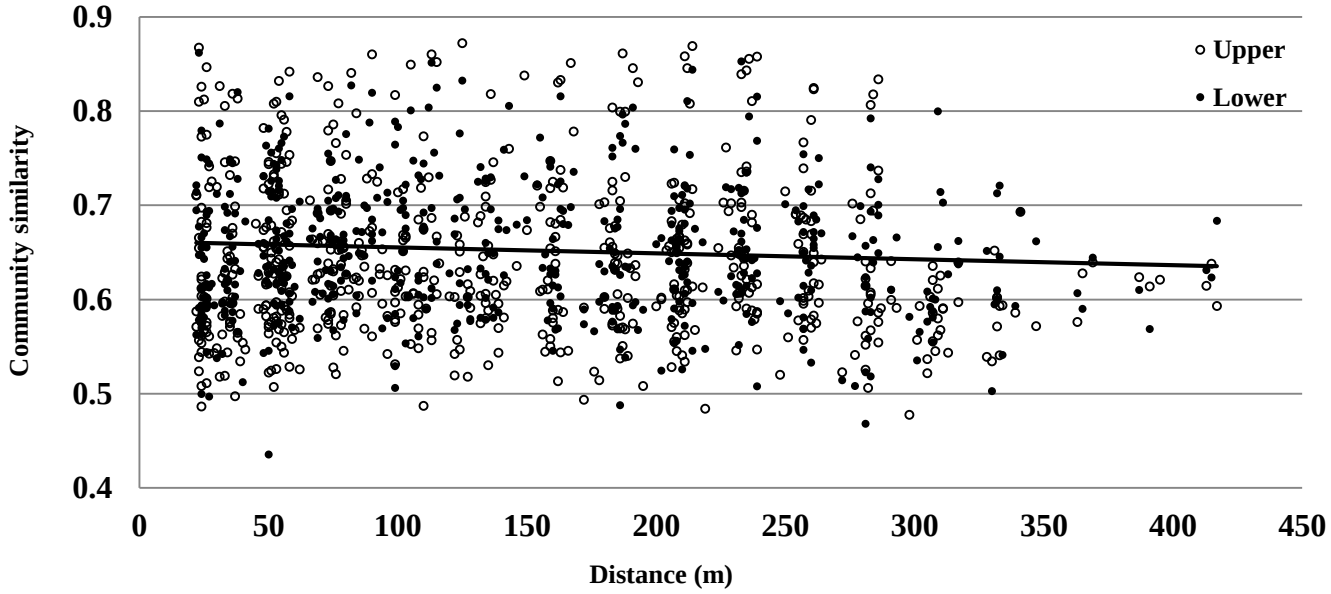


Figure 2.11. Distance-decay analysis of the relationship between geographic distance and bacterial community distance based on jackknifed unweighted UniFrac distance in both layers. The slope was significant in the lower-layer soils ($p < 0.05$; y = -6E-05x + 0.6615, solid line).

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 (Figure 2.6 and 2.12).

Interestingly, the relative abundance of certain bacterial groups markedly changed around 20–25 cm. For example, the relative abundance of *Acidobacteria*, *Gammaproteobacteria*, and *Plancto-mycetes* rapidly decreased, while *Chloroflexi*, *Gemma-timonadetes*, and candidate phylum AD3 rapidly increased at around 20–25 cm depth (Figure 2.13). This trend was also observed at the lower taxonomic levels of main bacterial groups; members of *Methylocystaceae* (*Alphaproteobacteria*), *Sinobacteraceae* (*Gamma-proteobacteria*), *Actinomycetales* (*Actinobacteria*), *Kori-bacteraceae* and Ellin6513 (*Acidobacteria*) rapidly decreased around the depth of 20 cm, while members of *Gallionella* (*Betaproteobacteria*), *Intrasporangiaceae* (*Actinobacteria*), and SJA-36 (*Acidobacteria*) increased below the depth of 20 cm (Figure 2.12b and c). The relative abundance of dominant OTUs also changed at the depth of 20–25 cm (Figure 2.14). This trend corresponded with the result of a principal coordinates analysis (PCoA) plot based on unweighted UniFrac distance (Figure 2.15).

Bacterial community similarity also considerably changed along soil depth. Briefly, 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 (Figure 2.15).

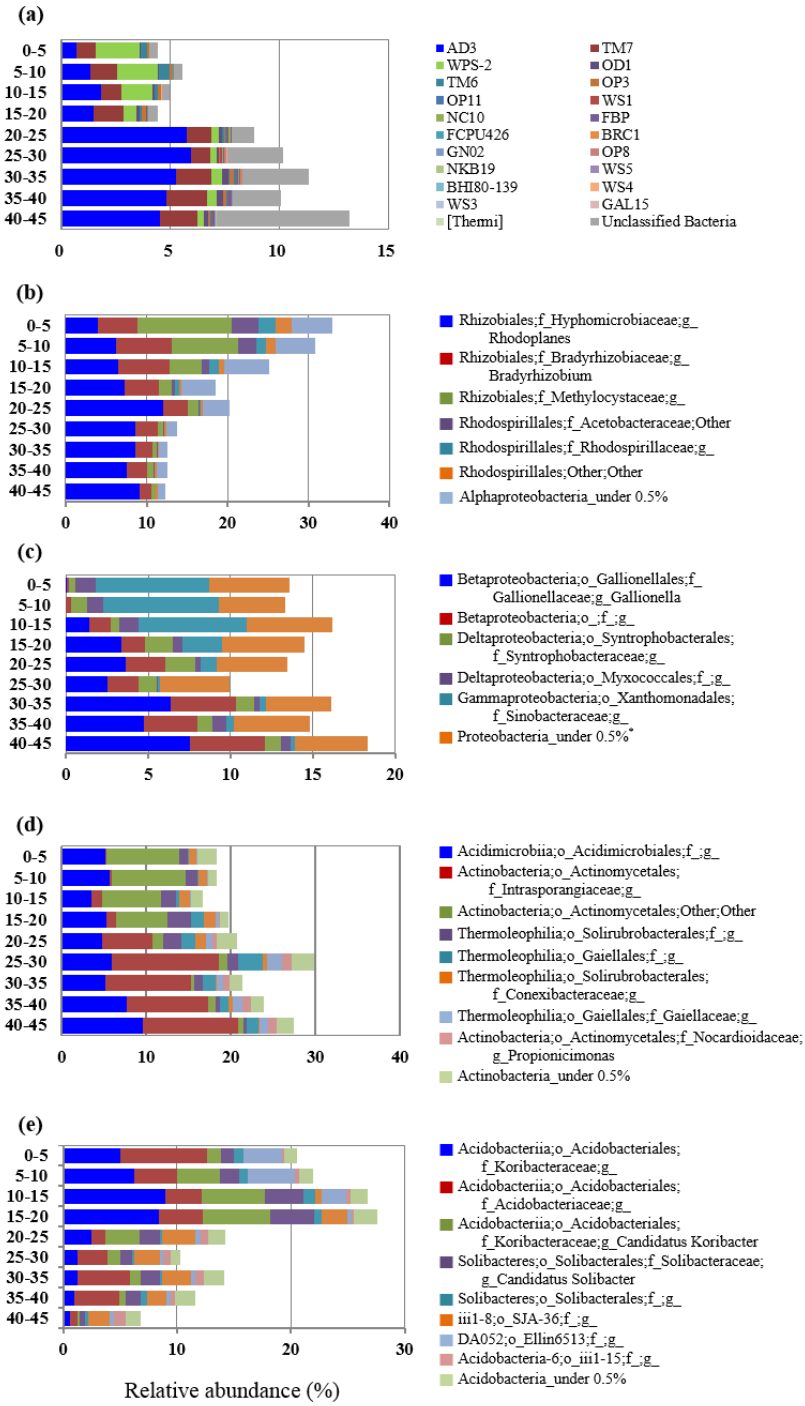

(a)
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
0
5
10
15
AD3
TM7
WPS-2
OD1
TM6
OP3
OP11
WS1
NC10
FBP
FCPU426
BRC1
GN02
OP8
NKB19
WS5
BHI80-139
WS4
WS3
GAL15
[Thermi]
Unclassified Bacteria
(b)
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
0
10
20
30
40
Rhizobiales;f_Hyphomicrobiaceae;g_ Rhodoplanes
Rhizobiales;f_Bradyrhizobiaceae;g_ Bradyrhizobium
Rhizobiales;f_Methylocystaceae;g_
Rhodospirillales;f_Acetobacteraceae;Other
Rhodospirillales;f_Rhodospirillaceae;g_
Rhodospirillales;Other;Other
Alphaproteobacteria_under 0.5%
(c)
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
0
5
10
15
20
Betaproteobacteria;o_Gallionellales;f_ Gallionellaceae;g_Gallionella
Betaproteobacteria;o_;f_;g_
Deltaproteobacteria;o_Syntrophobacterales; f_Syntrophobacteraceae;g_
Deltaproteobacteria;o_Myxococcales;f_;g_
Gammaproteobacteria;o_Xanthomonadales; f_Sinobacteraceae;g_
Proteobacteria_under 0.5%*
(d)
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
0
10
20
30
40
Acidimicrobiia;o_Acidimicrobiales;f_;g_
Actinobacteria;o_Actinomycetales; f_Intrasporangiaceae;g_
Actinobacteria;o_Actinomycetales;Other;Other
Thermoleophilia;o_Solirubrobacterales;f_;g_
Thermoleophilia;o_Gaiellales;f_;g_
Thermoleophilia;o_Solirubrobacterales; f_Conexibacteraceae;g_
Thermoleophilia;o_Gaiellales;f_Gaiellaceae;g_
Actinobacteria;o_Actinomycetales;f_Nocardioidaceae; g_Propionicimonas
Actinobacteria_under 0.5%
(e)
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
0
10
20
30
Acidobacteriia;o_Acidobacteriales; f_Koribacteraceae;g_
Acidobacteriia;o_Acidobacteriales; f_Acidobacteriaceae;g_
Acidobacteriia;o_Acidobacteriales; f_Koribacteraceae;g_Candidatus Koribacter
Solibacteres;o_Solibacterales;f_Solibacteraceae; g_Candidatus Solibacter
Solibacteres;o_Solibacterales;f_;g_
iii1-8;o_SJA-36;f_;g_
DA052;o_Ellin6513;f_;g_
Acidobacteria-6;o_iii1-15;f_;g_
Acidobacteria_under 0.5%
Relative abundance (%)

Figure 2.12. Vertical distribution of candidate phyla and unclassified bacteria (a), and the genus with the relative abundance over 0.5% from total abundance from the dominant bacterial groups of *Alpha-proteobacteria* (b), *Betaproteobacteria*, *Deltaproteobacteria*, and *Gammaproteobacteria* (c), *Actinobacteria* (d), and *Acidobacteria* (e). * without *Alphaproteobacteria*.

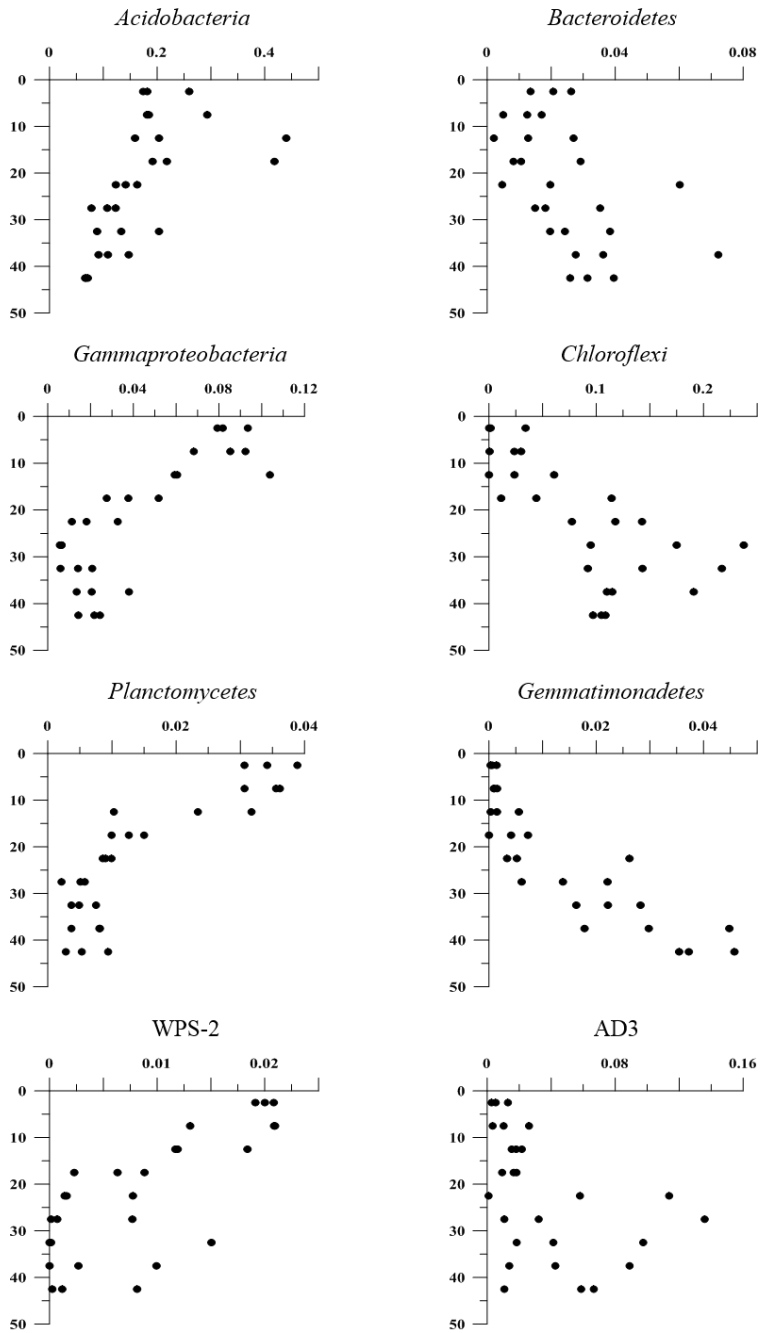


Figure 2.13. Plots show depth profile of representative bacterial groups.

OTU ID	Phylum	Class	Order	Family	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45
OTU_4224	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae									
OTU_5900	Actinobacteria	Actinobacteria	Actinomycetales										
OTU_132	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae									
OTU_4965	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae									
OTU_20	Proteobacteria	Alphaproteobacteria	Rhodospirillales										
OTU_3781	AD3	JG37-AG-4											
OTU_3207	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae									
OTU_2611	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae									
OTU_3845	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae									
OTU_4181	AD3	ABS-6											
OTU_1073	Actinobacteria	Acidimicrobiia	Acidimicrobiales										
OTU_4770	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae									
OTU_4271	Actinobacteria	Thermoleophilia	Solirubrobacterales										
OTU_2020	Acidobacteria	iii1-8	SJA-36										
OTU_4334	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae									
OTU_971	AD3	JG37-AG-4											
OTU_651	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae									
OTU_5765	Actinobacteria	Acidimicrobiia	Acidimicrobiales										
OTU_6702	Chloroflexi	Ellin6529											
OTU_1543	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae									
OTU_5290	Chloroflexi	Ellin6529											
OTU_503	Actinobacteria	Acidimicrobiia	Acidimicrobiales										
OTU_5499	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae									
OTU_3701	Proteobacteria	Betaproteobacteria											
OTU_2480	Proteobacteria	Betaproteobacteria	Gallionellales	Gallionellaceae									
OTU_6002	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae									
OTU_888	Chloroflexi	Ellin6529											
OTU_4398	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae									

Figure 2.14. Relative abundance of dominant bacterial OTUs which represented over 0.5% from total sequences. Upper dendrogram indicates the bacterial community similarity between soil depths.

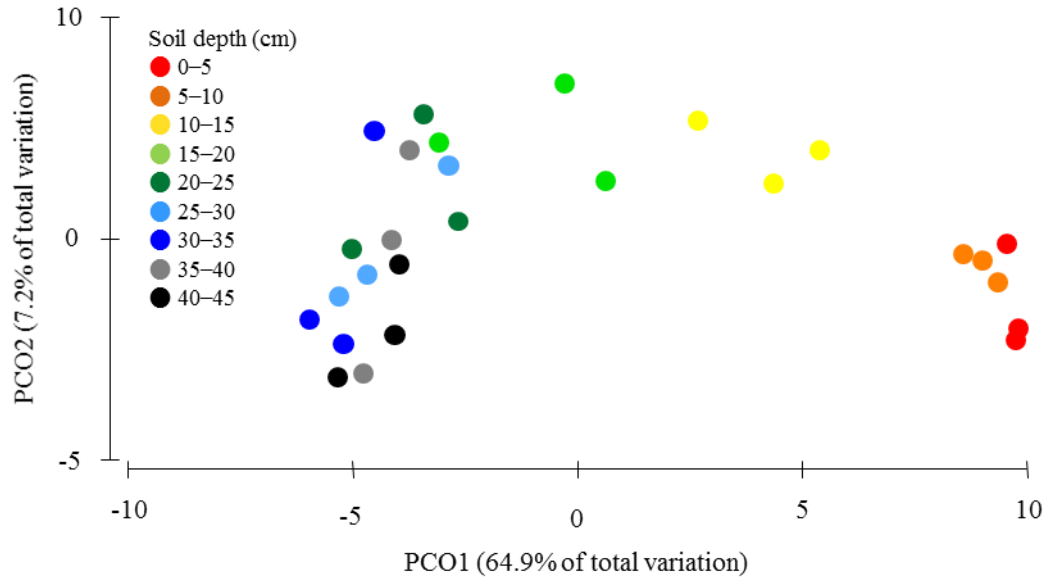


Figure 2.15. Principal coordinate analysis (PCoA) ordination. PCoA based on an unweighted UniFrac distance matrix of bacterial 16S rRNA gene sequences.

Relationships between bacterial communities and soil properties

Significant associations were observed between soil bacterial community and physical and chemical properties of soil in horizontal sampling. In general, pH showed the highest correlation ($r = 0.392$, $p < 0.001$) with bacterial community composition in all soil samples (Table 2.5 and Figure 2.10b). *Acidobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* decreased with increasing soil pH, whereas *Chloroflexi*, *Betaproteobacteria*, *Bacteroidetes* and *Delta-proteobacteria* increased with increasing soil pH (Figure 2.8). Similar results were observed in the upper- and lower-layer soils. At the class or order level, the bacterial community structure changed along the pH gradient, and some taxa showed opposite responses to pH compared to that at the phylum level (Figure 2.9). For example, uncultured iii1-8 and *Acidobacteria*-6 of the *Acidobacteria*, *Rhizobiales* of the *Alphaproteobacteria*, and *Legionellales* of the *Gammaproteobacteria* increased with increasing soil pH, whereas *Ktedonobacteria* of the *Chloroflexi* decreased with increasing soil pH.

Besides soil pH, C/N ratio ($r = 0.112$, $p < 0.05$), MC ($r = 0.212$, $p < 0.001$), TC ($r = 0.196$, $p < 0.005$), and TN ($r = 0.171$, $p < 0.005$) showed significant correlation with the overall soil bacterial community composition (Table 2.5). However, different soil properties were associated with the bacterial community structure in the two soil layers; in the upper-layer soils, TN ($r = 0.323$, $p < 0.005$) and NH_4^+ ($r = 0.167$, $p < 0.05$) were significantly associated with the community composition, whereas C/N ratio ($r = 0.213$, $p < 0.05$) and MC ($r = 0.257$, $p < 0.005$) were significantly associated with the community composition in the lower-layer soils (Table 2.5).

To identify the most influential soil properties, correlation between physical and chemical properties of soil and the dominant groups were determined using CART analysis (Figure 2.16). The soil characteristics affecting each dominant group differed between the soil depths. Soil pH was the best predictor for the presence of *Acidobacteria* in both soil layers. The presence of *Alphaproteobacteria* was related to pH in the upper-layer soils and was related to TN in the lower-layer soils. *Actinobacteria* was related to pH in the upper-layer soils and was related to C/N ratio in the lower-layer soils (Figure 2.16).

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 (Figure 2.4), the bacterial community dissimilarity based on unweighted UniFrac distance matrix showed significant correlation with soil horizon (ANOSIM, $R = 0.55$, $p < 0.001$). Moreover, the diversity indices were significantly different between Oi and Oa horizon: Chao1 (ANOSIM, $R = 0.253$, $p < 0.05$) and Shannon's H' (ANOSIM, $R = 0.31$, $p < 0.001$).

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 2.6). 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 not always apply in both horizons. For example, *Acidobacteria*, *Actinobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* were correlated with TC ($p < 0.05$), *Acidobacteria*,

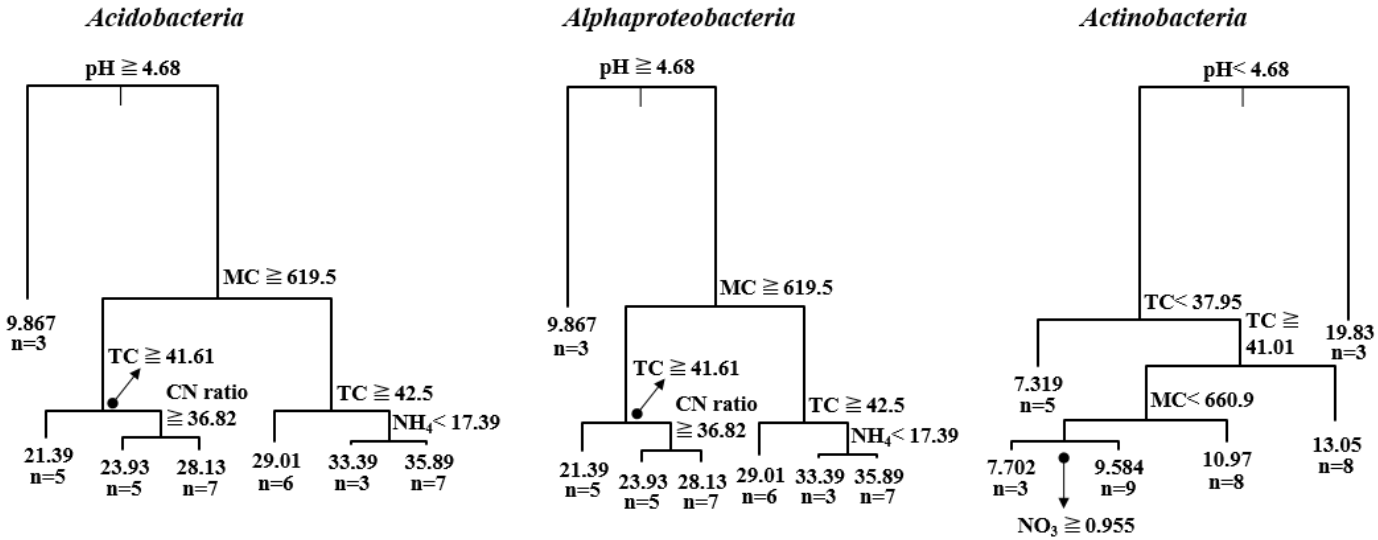
Betaproteobacteria, and *Chloroflexi* were correlated with soil pH ($p < 0.05$), and *Gamma-proteobacteria* was correlated with TP ($p < 0.05$) in Oi horizon (Table 2.6). On the other hand, in the Oa horizon, *Acidobacteria*, *Betaproteobacteria*, and *Bacteroidetes* were correlated with soil depth ($p < 0.05$), soil pH ($p < 0.05$), and EC ($p < 0.05$), respectively (Table 2.6). These results presented that the influencing soil factors on bacterial community could vary depending on soil horizon.

Table 2.5. The significant correlations between physicochemical properties of soil and bacterial communities

Soil physical and chemical properties	All soil samples (n=70)		Upper-layer (n=36)		Lower-layer (n=34)	
	r	*p*	*r*	*p*	*r*	*p*
pH	0.392	**0.001**	0.393	**0.001**	0.395	**0.001**
C/N	0.112	**0.021**	0.148	0.054	0.213	**0.025**
MC	0.212	**0.001**	0.122	0.094	0.257	**0.005**
TC	0.196	**0.003**	0.168	0.062	0.116	0.137
TN	0.171	**0.005**	0.323	**0.002**	0.137	0.077
NO_3^-	0.001	0.375	0.020	0.392	-0.039	0.656
NH_4^+	0.035	0.191	0.167	**0.016**	-0.044	0.691

The Spearman's rank correlations (*r*) and significance (*p*) were determined by Mantel tests.
Bold *p*-value defines the significant difference ($p < 0.05$).
C/N, a ratio of carbon and nitrogen; MC, moisture content; TC, total carbon; TN, total nitrogen.

(A) Upper-layer



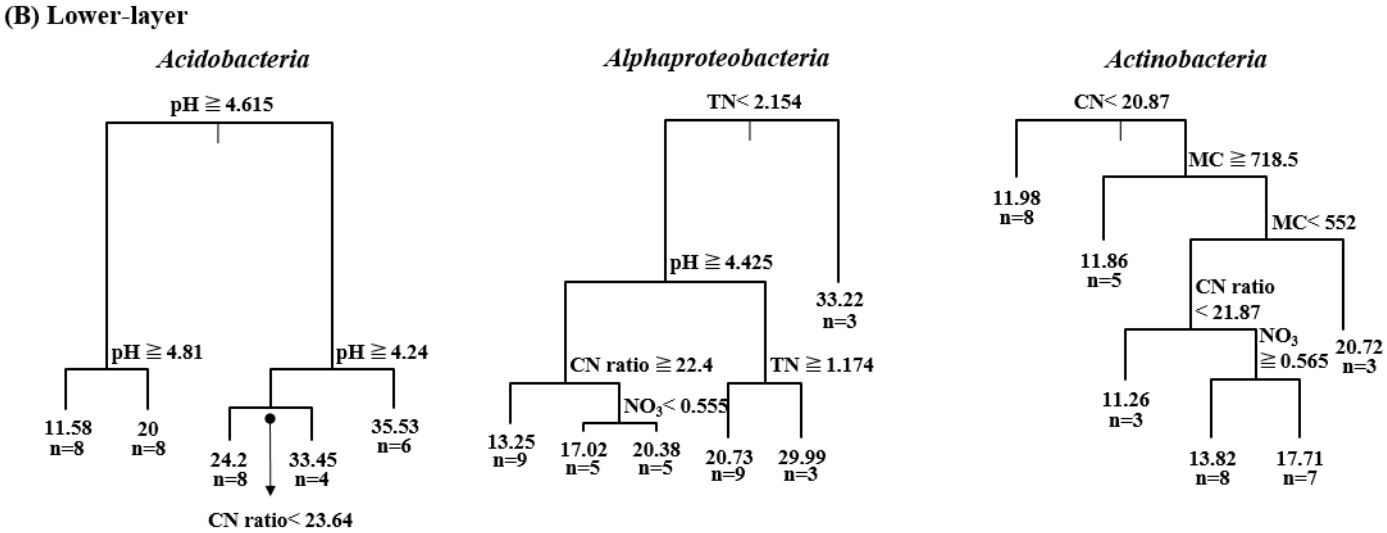


Figure 2.16. Classification and regression tree (CART) analysis. Description of the main properties for the dominant phyla *Acidobacteria*, *Alphaproteobacteria*, and *Actinobacteria* in the upper-layer (A) the lower-layer (B) soil samples.

Table 2.6. The correlation between soil property and bacterial community within soil horizon. The Pearson correlation (*r*) and significance (*p*) determined by Mantel tests

Soil properties	Soil horizon	*Acidobacteria*	*Actinobacteria*	*Alpha*	*Bacteroidetes*	*Beta*	*Chloroflexi*	*Gamma*	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	0.01	-0.08	-0.092	0.043	0.089
	Oa	0.025	0.036	-0.100	-0.068	-0.118	-0.178	-0.102	-0.13
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	0.1	-0.056	-0.127
	Oa	-0.094	-0.162	0.181	-0.274	0.033	0.05	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

TC, total carbon content; TN, total nitrogen content; TP, total phosphorus content; C/N, a ratio of carbon and nitrogen content; EC, electron conductivity; *Alpha*, *Alphaproteobacteria*; *Beta*, *Betaproteobacteria*; *Gamma*, *Gammaproteobacteria*.

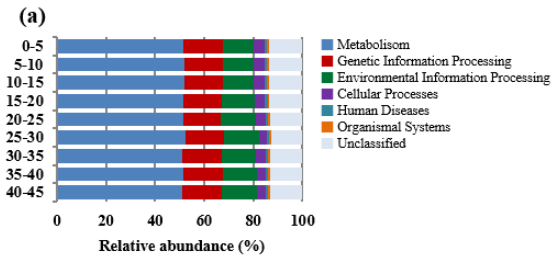
Significant correlation between a soil property and bacterial community structure is shown in bold. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. *p*-values corrected using 'fdrtool' function in R.

Inference of potential functions

Various gene categories were detected in this study through genome data of identified bacteria. PICRUSt functional prediction using KEGG pathway metadata revealed that the bacterial communities possessed various functions along soil depth (Figure 2.17). The relative abundances 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 (Figure 2.17a). However, the relative abundance of functional gene categories at Class 3 level showed different distribution along soil depth (Figure 2.17b). The cluster analysis showed two cluster 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 horizon. 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; Figure 2.17b). This trend corresponded with bacterial diversity indices (Figure 2.7).

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 (Table A1). When we observed the correlation functions related with carbon 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, $p < 0.05$) (Table A1).


(a)
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
0
20
40
60
80
100
Relative abundance (%)
Metabolism
Genetic Information Processing
Environmental Information Processing
Cellular Processes
Human Diseases
Organismal Systems
Unclassified

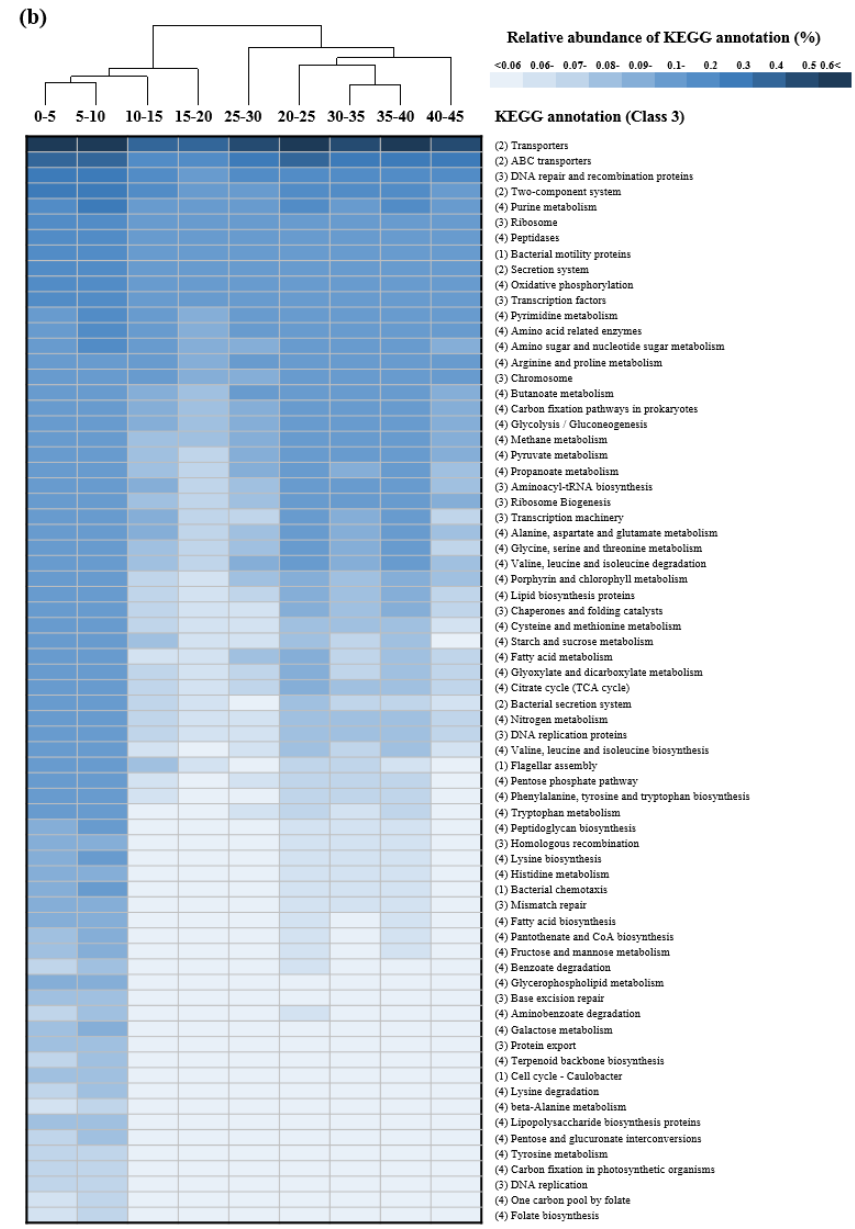

(b)
0-5
5-10
10-15
15-20
25-30
20-25
30-35
35-40
40-45
Relative abundance of KEGG annotation (%)
<0.06
0.06-
0.07-
0.08-
0.09-
0.1-
0.2
0.3
0.4
0.5
0.6<
KEGG annotation (Class 3)
(2) Transporters
(2) ABC transporters
(3) DNA repair and recombination proteins
(2) Two-component system
(4) Purine metabolism
(3) Ribosome
(4) Peptidases
(1) Bacterial motility proteins
(2) Secretion system
(4) Oxidative phosphorylation
(3) Transcription factors
(4) Pyrimidine metabolism
(4) Amino acid related enzymes
(4) Amino sugar and nucleotide sugar metabolism
(4) Arginine and proline metabolism
(3) Chromosome
(4) Butanoate metabolism
(4) Carbon fixation pathways in prokaryotes
(4) Glycolysis / Gluconeogenesis
(4) Methane metabolism
(4) Pyruvate metabolism
(4) Propanoate metabolism
(3) Aminoacyl-tRNA biosynthesis
(3) Ribosome Biogenesis
(3) Transcription machinery
(4) Alanine, aspartate and glutamate metabolism
(4) Glycine, serine and threonine metabolism
(4) Valine, leucine and isoleucine degradation
(4) Porphyrin and chlorophyll metabolism
(4) Lipid biosynthesis proteins
(3) Chaperones and folding catalysts
(4) Cysteine and methionine metabolism
(4) Starch and sucrose metabolism
(4) Fatty acid metabolism
(4) Glyoxylate and dicarboxylate metabolism
(4) Citrate cycle (TCA cycle)
(2) Bacterial secretion system
(4) Nitrogen metabolism
(3) DNA replication proteins
(4) Valine, leucine and isoleucine biosynthesis
(1) Flagellar assembly
(4) Pentose phosphate pathway
(4) Phenylalanine, tyrosine and tryptophan biosynthesis
(4) Tryptophan metabolism
(4) Peptidoglycan biosynthesis
(3) Homologous recombination
(4) Lysine biosynthesis
(4) Histidine metabolism
(1) Bacterial chemotaxis
(3) Mismatch repair
(4) Fatty acid biosynthesis
(4) Pantothenate and CoA biosynthesis
(4) Fructose and mannose metabolism
(4) Benzoate degradation
(4) Glycerophospholipid metabolism
(3) Base excision repair
(4) Aminobenzoate degradation
(4) Galactose metabolism
(3) Protein export
(4) Terpenoid backbone biosynthesis
(1) Cell cycle - Caulobacter
(4) Lysine degradation
(4) beta-Alanine metabolism
(4) Lipopolysaccharide biosynthesis proteins
(4) Pentose and glucuronate interconversions
(4) Tyrosine metabolism
(4) Carbon fixation in photosynthetic organisms
(3) DNA replication
(4) One carbon pool by folate
(4) Folate biosynthesis

Figure 2.17. Relative abundances of gene contents from KEGG annotations. (a) Distribution of gene contents at Class 1. (b) 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.

2.1.4 Discussion

Increasing temperature is causing the permafrost thawing and active layer deepening, accelerating the carbon mineralization via increases in 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 horizontal and vertical heterogeneity along the soil depth. Most studies, however, compared bacterial communities between layers such as active and permafrost layers, and 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 in active layer at a fine scale was not fully described yet. This study can provide information on the spatial distribution of bacterial community structure in moist acidic tundra soil, and study on depth profile can provide some clues to predict the shift of bacterial community structure according to the thawing.

The similarity of the bacterial community was more different through the vertical depth (10 cm) than across the horizontal layers (>25 m). Soil depth is one of the major parameters influencing microbial community. Some study showed that microbial biomass and activity significantly decreased at the surface following wildfire, however, the effects of wildfire those changes were not obvious at 20 cm depth (Waldrop & Harden, 2008). Furthermore, while long-term warming significantly decreased the evenness of bacterial communities at the surface organic layer soils, the effect of warming was relatively minor in the mineral layer (Deslippe *et al.*, 2012). Previous studies indicated that this vertical variation was due to numerous soil properties that change with soil depth, such as pH, nutrient and water availability, plants, soil structure, oxygen, and temperature (Fierer *et al.*, 2003;

Ström *et al.*, 2003; Kobabe *et al.*, 2004; Hansel *et al.*, 2008). In this study, we also found that there were significant differences in soil pH, MC, TC concentration, and C/N ratio between the two soil depths ($p < 0.05$, Table 2.1), whereas no obvious trends in soil properties were observed among soils obtained at the same depth.

Among the three major groups, the relative abundances of *Alphaproteobacteria* decreased with depth, although those of *Acidobacteria* and *Actinobacteria* were similar in both soil layers (Figure 2.18). This observation corresponded with other observations of bacterial community composition changes with soil depth (Eilers *et al.*, 2012; Frank-Fahle *et al.*, 2014). *Alphaproteobacteria* prefer nutrient-rich environments (Nemergut *et al.*, 2010; Thomson *et al.*, 2010; Goldfarb *et al.*, 2011). Moreover, Fierer *et al.*, (2012) showed the increase in the relative abundance for *Alphaproteobacteria* with additional N input. The decomposition degree of plant and moss differed at different soil depths. The lower C/N ratio in the lower-layer soil reflected more decomposition in the lower-layer than in the upper-layer (Table 2.1). Therefore, labile materials that provide nutrients for bacteria might be more abundant in the upper-layer soils. In addition, the concentrations of TC, TN, and NH_4^+ were higher in the upper-layer soils than in the lower-layer soils (Table 2.2). The environment in the upper-layer would favor *Alphaproteobacteria* propagation.

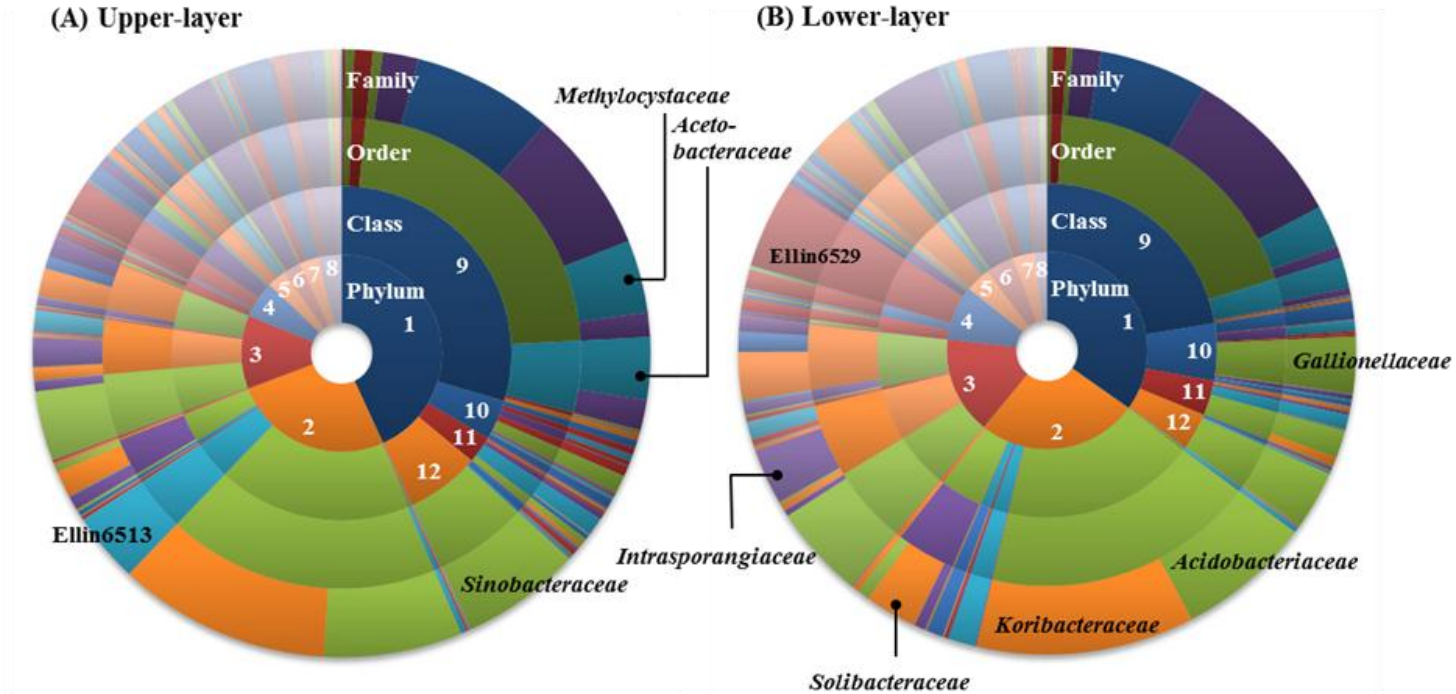

(A) Upper-layer
Family
Order
Class
Phylum
1
2
3
4
5
6
7
8
9
10
11
12
Methylocystaceae
Aceto-
bacteraceae
Ellin6513
Sinobacteraceae
(B) Lower-layer
Family
Order
Class
Phylum
Ellin6529
Gallionellaceae
Intrasporangiaceae
Acidobacteriaceae
Koribacteraceae
Solibacteraceae

Figure 2.18. Hierarchical classifications of 16S rRNA gene sequences in the eight main bacterial communities. The rings show the soil bacterial community composition at different taxonomic levels, the innermost ring indicates the composition at the phylum level, and the other rings show the composition at the class, order, and family. 1, *Proteobacteria*; 2, *Acidobacteria*; 3, *Actinobacteria*; 4, *Chloroflexi*; 5, *Bacteroidetes*; 6, AD3; 7, *Verrucomicrobia*; 8, *Planctomycetes*; 9, *Alphaproteobacteria*; 10, *Betaproteobacteria*; 11, *Deltaproteobacteria*; 12, *Gamma-proteobacteria.*

Soil pH was significantly correlated with bacterial community structure in both layers (Table 2.5 and Figure 2.10). Specifically, *Acidobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* decreased with increasing soil pH, whereas *Betaproteobacteria* and *Chloroflexi* increased with increasing pH (Figure 2.8). Even when we excluded the dominant groups (*Alphaproteobacteria*, *Acidobacteria*, and *Actinobacteria*) from the statistical analyses, the minor groups also showed significant correlations with soil pH ($p < 0.001$) in both upper- and lower-layer soils (Table 2.7). These results corresponded with other studies of Arctic soils (Männistö *et al.*, 2007; Chu *et al.*, 2010). Männistö *et al*. (2007) showed that the soil pH of parent materials had greater influence on bacterial community structure than changes in soil temperature in the Arctic region. Chu *et al*. (2010) compared bacterial community structure on a global scale, and concluded that bacterial community composition in Arctic soil was strongly influenced by local environmental factors associated with soil acidity than by other factors. Moreover, soil pH is known as a strong driver shaping bacterial community structure in various soil ecosystems; including a wide range of soils in North and South America and agricultural soil in Scotland (Fierer & Jackson, 2006; Lauber *et al.*, 2009; Bartram *et al.*, 2014).

Table 2.7. The significant correlations between physicochemical properties of soil and bacterial minor groups*

Soil physical and chemical properties	All soil samples (n=70)		Upper layer (n=36)		Lower layer (n=34)	
	r	*p*	*r*	*p*	*r*	*p*
pH	0.422	0.001	0.427	0.001	0.350	0.001
C/N	0.032	0.251	-0.012	0.496	0.178	0.185
MC	0.081	0.04	-0.011	0.515	0.140	0.043
TC	0.212	0.002	0.147	0.091	0.157	0.040
TN	0.140	0.003	0.300	0.003	0.126	0.047
NO_3^-	0.002	0.466	0.049	0.285	0.014	0.392
NH_4^+	0.062	0.111	0.312	0.004	-0.045	0.693

*Bacterial minor groups except major groups (*Alphaproteobacteria*, *Acidobacteria*, and *Actinobacteria*).
The Spearman's rank correlations (*r*) and significance (*p*) were determined by Mantel tests.
C/N, ratio of carbon and nitrogen; MC, moisture content; TC, total carbon; TN, total nitrogen.

At lower taxonomic levels, some taxa showed responses to pH opposite to that observed at the phylum level. For example, *Acidobacteria*-6 and iii1-8 (*Acidobacteria*), and *Legionellales* (*Gammaproteobacteria*) increased with increasing soil pH, whereas *Ktedonobacteria* (*Chloroflexi*) decreased with increasing soil pH (Figure 2.9). Our results agreed with other studies to show the relationship between soil pH and the lower taxonomic levels of bacteria (Jones *et al.*, 2009; Bartram *et al.*, 2014). Although these taxa were not the dominant groups, it is noteworthy to examine the different responses of bacterial groups at the lower taxonomic levels because not all members of the same phylum behaved in the same way.

The dominant bacterial phyla in the moist acidic tussock tundra soil in this study were *Acidobacteria*, *Alphaproteobacteria*, and *Actinobacteria*. It is consistent with other studies of various subarctic and Arctic soils, such as tundra soil from Nunavut, the Toolik Lake area, and spanning the Arctic region (Neufeld & Mohn, 2005; Wallenstein *et al.*, 2007; Campbell *et al.*, 2010; Chu *et al.*, 2010; Nemergut *et al.*, 2010; Schuette *et al.*, 2010). Bacterial composition, including minor groups, was also similar to that of other Arctic soils (Zhou *et al.*, 1997; Neufeld & Mohn, 2005; Wallenstein *et al.*, 2007; Campbell *et al.*, 2010; Chu *et al.*, 2010; Nemergut *et al.*, 2010; Schuette *et al.*, 2010). Moreover, the three dominant phyla are also dominant in soils from non-polar areas (Fierer & Jackson, 2006; Will *et al.*, 2010; Li *et al.*, 2012; Shen *et al.*, 2013). According to a review, these phyla are ubiquitous, and they are the most abundant phyla in soils from various ecosystems (Janssen, 2006). However, soil bacterial community in Hess Creek, Alaska showed different community structure since *Actinobacteria*, *Proteobacteria*, and *Chloroflexi* were dominant (Mackelprang *et al.*, 2011). Because *Chloroflexi* increased with increasing soil pH, the abundant *Chloroflexi* in Hess Creek can be

attributed to higher soil pH range of 6.43-6.52 in this area (Mackelprang *et al.*, 2011).

The higher resolution of pyrosequencing data allowed us to look into the information on the potential ecological roles of bacteria in the arctic tundra soil. At the species level, OTU_1 (99.5% sequence similarity with *Afipia broomeae*) and OTU_2 (98.5% sequence similarity with *Pseudolabrys sp.*) were predominant in this study (Table 2.4). They belonged to *Rhizobiales* (*Alphaproteobacteria*) which are known to fix nitrogen as plant root symbionts. The genus *Steroidobacter* of *Gammaproteobacteria* can reduce nitrate to dinitrogen monoxide and further to dinitrogen (Fahrbach *et al.*, 2008). *Gallionella* of *Betaproteobacteria* is characterized by its oxidation of Fe (II) (Hedrich *et al.*, 2011). Methane-consuming bacteria which belongs to the family *Methylocystaceae* (*Alphaproteobacteria*) were detected in this study as well (Figure 2.18). These results provide some information on the ecological roles of bacteria in tundra soil.

The distance-decay relationships showed that bacterial community similarity decreased with increasing sampling distance in lower-layer soils, whereas no significant relationship was detected in the upper-layer soils (Figure 2.11). Decreasing bacterial community similarity with distance can be explained by increasing differences in environmental properties (Nekola & White, 1999). Although it may not be direct evidence of dissimilarity in environmental properties across distance, we observed greater variation in soil properties (TC and TN concentrations and MC) in the lower-layer soils than in the upper-layer soils (Table 2.1). There is currently no consensus on the biogeographical patterns of bacterial communities. Several studies showed that bacterial community similarity decreased with increasing sampling distance. Some study reported that bacterial community

composition changed with geographic distance (1–200 km range) (Monroy *et al.*, 2012); however, they could not explain this relationship using measured soil properties. Other study also observed an increasing pattern of bacterial community dissimilarity with distance in topographically complex high-altitude slopes in the Himalaya (1–1,200 m range) (Stres *et al.*, 2013). However, other studies reported no biogeographical pattern in microbial community similarity, including bacteria, on either a local or a global scale (Ritz *et al.*, 2004; Chu *et al.*, 2010; Queloz *et al.*, 2011). We also found contrasting results for the relationship between bacterial community similarities and sampling distance between the two depths; no differences were observed in the upper layer, whereas increasing dissimilarity with distance was observed in the lower layer. Therefore, additional research is needed to determine the relationship between geographical distance and bacterial community structure.

Bacterial community structure and diversity was found to change along soil depth (Figure 2.6 and 2.7). The vertical distribution of bacterial community structure has been reported in Arctic soils (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 soils and decreased towards deeper layers. Our results corresponded with the previous studies. In this study, the bacterial community structure shifted at a certain soil depth. This phenomenon commonly found in many terrestrial soils shows significant correlation between microbial biomass and soil carbon contents along depth (Rumpel & Kögel-Knabner, 2011; Eilers *et al.*, 2012). This was also found in some studies in Arctic soils, and they explained that the substrate availability strongly related to the shift of bacterial community structure between the

organic and mineral soil horizons (Koyama *et al.*, 2014; Deng *et al.*, 2015). Although our study agrees with the explanation that substrate availability affected the shaping of the bacterial community structure, we could not find any explanation for why bacterial community shifted within organic layer. The shift of bacterial community structure at about 20 cm depth coincided with the distinction of Oi and Oa horizons in this study. This suggested that the bacterial community structure could be influenced by the SOM quality rather than the quantity of carbon.

Previous studies have emphasized that the substrate quality is significantly related with the microbial mineralization, affecting the microbial community structure (Merilä *et al.*, 2010). The vertical distribution of bacterial communities was related with carbon availability, 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). 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.

The vertical distribution of bacterial community could be related to the different resource availability of each bacterial groups. The higher abundance of *Alphaproteobacteria* and *Gammaproteobacteria* in the upper

layer (Oi) could be related to their preference of higher C 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). In case of *Planctomycetes,* the phylum was more abundant in Oi than Oa horizon (Figure 2.6). According to the genomic insight, the *Planctomycetes* have large genomes which is a feature of copiotrophs (Lauro *et al.*, 2009). 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 to use for bacteria. On the other hand, bacteria can take C by decomposing organic materials in Oi layer.

Oligotrophic-like bacteria such as *Chloroflexi* and AD3 were relatively abundant in Oa horizon. 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 bacteria due to frequent occurrence in considerably mineralized environment such as deep soils (Costello, 2007; Tas *et al.*, 2014).

Moreover, *Gemmatimonadetes* and *Chloribi* have been frequently detected in deeper soil and permafrost (DeBruyn *et al.*, 2011; Wilhelm *et al.*, 2011; Jansson & 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 (Figure 2.6), 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 genus *Gallionella* of *Betaproteobacteria* increased along soil depth in this study (Figure 2.12c). The genus *Gallionella* 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 could 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 (Figure 2.4 and Table 2.6). Soil pH has known as the important controlling factor to shape bacterial community structure across a variety of spatial scales (Fierer & Jackson, 2006; Lauber *et al.*, 2009; Campbell *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 & Mohn, 2005; Wallenstein *et al.*, 2007; Wilhelm *et al.*, 2011; Kim *et al.*, 2014a). Moreover, soil pH affected the vertical distribution of bacterial community structure as well as horizontal distribution. For example, *Acidobacteria* which is known as oligotrophic bacteria (Fierer *et al.*, 2007) was one of the dominant groups in upper Oi horizon in this study and decreased toward the lower layer. Thus, dominance of *Acidobacteria* in the upper layer might be related to more acidic condition in this study (Table 2.6).

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.

Soil microbial diversity might be a good predictor to explain the ecological functioning (Fierer *et al.*, 2012a; Fierer *et al.*, 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 (Figure 2.17). One of major OTU, OTU_132 belonged to *Methylocystaceae* (*Alphaproteobacteria*), which is known as methane oxidizing bacteria (MOB), was highly abundant in Oi horizon (Figure 2.14). MOB has been attracted attention because they are the largest biological sink for methane in aerobic soils as oxidization up to 90% of the emitted methane from the deeper soil layers (Bosse & Frenzel, 1997; Wagner & Liebner, 2009a).

We also tried to find the relationship between carbon-related functions (e.g. carbon fixation, glycolysis, and citrate cycle) and soil properties. Unexpectedly, these carbon-related functions as well as other functions were significantly correlated with soil pH than TC (Table A1). This may be determined by which functional gene potential was drawn from bacterial community information showing positive correlation between bacterial community structure and soil pH. Therefore, interpretation for the predicted functional gene categories should be cautious because the functions inferred from amplicon data could be limited and biased.

In conclusion, this study provided insight into the spatial distribution of bacterial community structure and relations with soil properties in the active layer:

- Although various plants covered the top soil, the bacterial communities were relatively similar across the horizontal layers compared to the communities through the vertical depth.

- The soil cores were divided into two horizons according to the decomposition status of SOM, and the bacterial communities of the upper Oi horizon were also distinct from those of the lower Oa layer. Some bacterial groups abruptly changed between Oi and Oa horizon (*Acidobacteria*, *Gammaproteobacteria*, *Planctomycetes*, and WPS-2 decreased, and *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, and AD3 increased).

- *Alphaproteobacteria* and *Gammaproteobacteria* closely related with TC.

- The vertical distribution of bacterial communities was significantly correlated with soil pH and TP content.

- Certain phylogenetic groups at lower taxonomic levels showed a different response to pH from that at the phylum level. This indicated the necessity of analyzing bacterial communities at lower taxonomic levels such as species, which actually perform various functions in the environment.

Although the bacterial community structure was primarily distinguished by SOM quality, spatial distribution of bacterial communities was closely related with soil pH and TP. It is important to monitor the change

of soil properties to predict microbial community structure shift according to the warming effect. Many studies emphasized the importance of soil pH and carbon for determining bacterial community up to now, but the role of other nutrients such as phosphorus has not studied very well. Therefore, more detailed and diverse soil environmental parameters should be considered in order to better understand the relationship between microbial community and soil properties in the future studies.

2.2 Comparing prokaryotic community structure between active layer and permafrost

2.2.1 Introduction

Soil is probably the most complex environment for microbes on Earth. The soil microbial diversity and community structure are influenced by a wide range of biotic (any living component that affects another organism) and abiotic factors (soil carbon, nitrogen, moisture content, climate, pH, texture, temperature, etc.). The environmental gradient for these soil factors was observed with soil depth. The highest richness and diversity have been observed on the surface soil, decreasing with soil depth (Fierer *et al.*, 2003). Soil abiotic factors also varied along soil depth, briefly showing decreasing soil carbon and nitrogen or increasing soil pH (Eilers *et al.*, 2012; Frank-Fahle *et al.*, 2014; Deng *et al.*, 2015). The heterogeneity of soil factors related with soil depth can affect to the microbial community structure and diversity along soil depth.

Microorganisms play an essential role in biogeochemical cycling and ecosystem functioning (Falkowski *et al.*, 2008). Increasing the scientific attention of global warming on permafrost environments, many scientists have focused on the global carbon cycle in Arctic soils. If there is no cryoturbation, namely the mixing of materials from various horizons of the soil from the bedrock due to freezing and thawing, a considerable amount of organic carbon would be found in surface and decreasing toward deeper soil depth. As a result of increase of global temperature, a significant part of the buried organic carbon could be lost to the atmosphere via c-related gas

emission by microbial decomposition in the next 100 years, which can contribute to positive feedback of global warming (Knoblauch *et al.*, 2013).

Soil depth can be one of the environmental factors to affect shaping microbial community structure. Depth profile of the active layer can be a good material to predict the changes of thawing permafrost. Soil depth acts as ecological filter to edaphic properties and forms heterogeneous environments for microorganisms along soil depth. According to the studies on the vertical distribution of soil microbial communities (Chapter 2.1), the microbial abundance and diversity were significantly correlated with the quality of soil organic matter (especially soil carbon), which is the degree of decomposition rate of organic matter (Fierer *et al.*, 2003; Deng *et al.*, 2015). We can easily observe the change of quality in soil organic matter by investigating the soil depth profile. Then, microbial communities may differ along soil depth and may have different metabolic functions in lower soil depth compared to the upper communities. Actually, the soil microbes inhabiting the soil surface and subsurface soil showed to have physiologically or phylogenetically adapted for appropriate metabolism of substrates (Fierer *et al.*, 2003). However, most studies have mainly focused on microbial communities found on the surface of soil, where density of microorganisms is highest, and the distribution of microbes through the soil profile from surface to permafrost remain poorly understood.

The microbial community composition and structure are expected to differ along soil depth, especially soil horizon based on soil texture, and those are significantly different between active layer and permafrost. Then, are microbial physiological characteristics different between active layer and permafrost? To answer the question, we observed the microbial community structure along soil horizon based on the quality of soil organic matter, and

compared the communities with soil properties between active layer and permafrost. To obtain the answer, we sequenced the 16S rRNA gene for Bacteria and Archaea of soil cores from surface to permafrost from moist acidic tundra, Alaska using 454 GS FLX Titanium pyrosequencing. Abiotic soil factors, such as soil pH, carbon and nitrogen contents, and moisture content were analyzed and statistically analyzed with soil microbial communities along soil depth. This study may provide clues about the physiological characteristics in active layer and permafrost, and may help with the prediction of microbial community with permafrost thawing.

2.2.2 Materials and Methods

Site description and soil core sampling

The study site is located in Council (64º 51′N, 163º 42′W), Seward Peninsula, Alaska (Figure 2.18). At the time of sampling (July 2014), a thawing depth of active layer depth was approximately 30 cm. Lichen, moss (*Sphagnum* spp.), blueberry (*Vaccinium uliginosum*), and water sedge (*Carex aquatilis*) were dominant in the sampling area (Park & Lee, 2014).

Three sampling points were selected based on Ground Penetrating Radar (GPR)* survey (Figure 2.18). Based on the GPR survey, Core 2 contained relatively higher moisture than other Cores. Core 3 showed relatively strong resistivity than Core 1. However, there were no relationships between core sampling points and GPR (data not shown). After removing aboveground vegetation and flattening the soil surface, each soil core was taken using a corer into the permafrost (up to 1.5 m). Each core site comprised three replicates. The acquired nine cores were immediately placed in icebox

and transferred to a freezer in the laboratory. The soil samples were stored at –20°C for further laboratory analysis.

*Ground-penetrating radar (GPR) is a geophysical method that uses radar pulses to image the subsurface. This nondestructive method uses electromagnetic radiation in the microwave band (UHF/VHF frequencies) of the radio spectrum, and detects the reflected signals from subsurface structures (https://en.wikipedia.org).

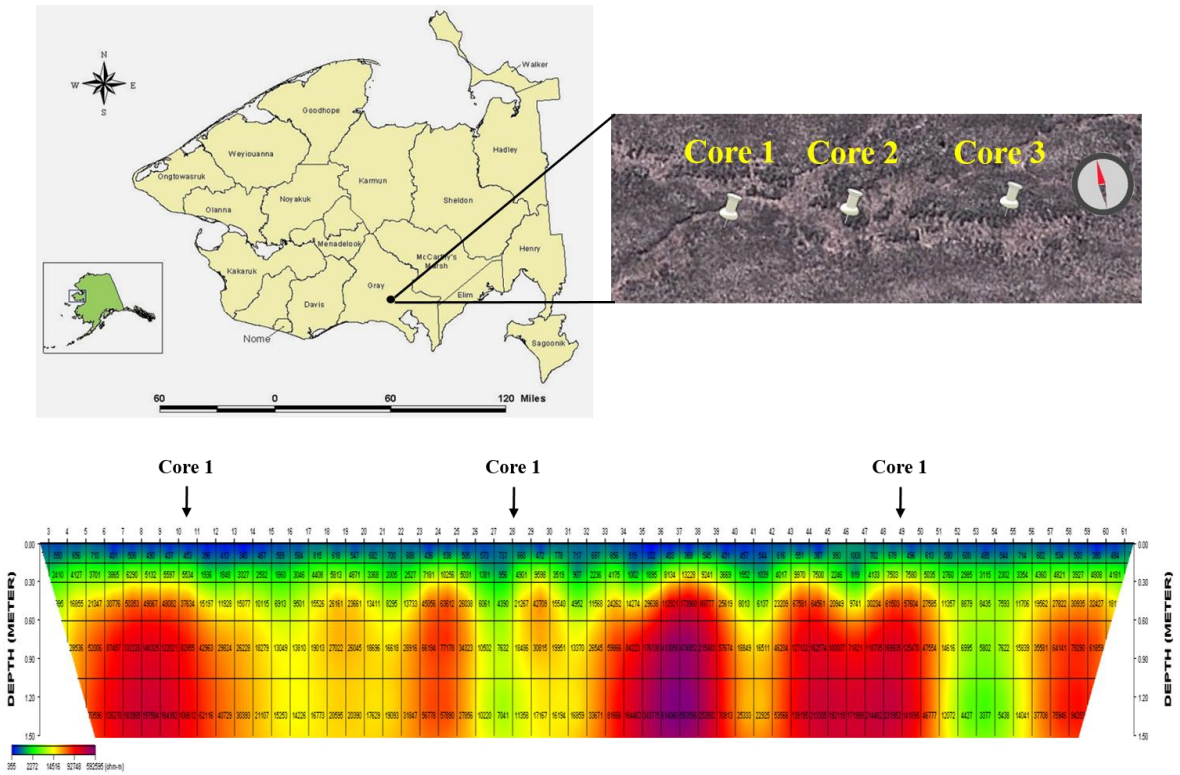


Figure 2.19. Sampling points based on Ground Penetrating Radar (GPR) survey.

Soil physical and chemical properties

Up to 15 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. Soil horizon was separated by O (organic materials) and A (sand and silt) based on the soil texture followed by USDA scheme (sand 2–0.05 mm, silt 0.05–0.002 mm, and clay < 0.002 mm) from USDA Soil Taxonomy (Soil Survey Staff, 2014). Soil texture was analyzed by wet sieving and a pipette method (Gee & Bauder, 1986). Soil pH was determined in a soil-water suspension (1:5 ratio, w/v) by a pH meter (Orion 3 star, Thermo Scientific, USA), and then the supernatant was filtered through Whatman No. 42 paper. 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, Cambridge, UK). Gravimetric moisture content (MC) was determined by measuring the difference in weight between the field-moist soil samples and the same soil samples dried at 105℃ for 48 hours.

PCR amplification and pyrosequencing

To extract gDNA, ~15 g of soils were freeze-dried using a freeze-dry system (LABCONCO, USA). gDNA from freeze-dried soil was extracted using a FastDNA® SPIN kit for soil and a QuickPrep adapter (MP Biomedicals, USA), according to the manufacturer's protocol. The concentration of gDNA was determined by fluorescent nucleic acid stain using PicoGreen® dsDNA reagent kit (Invitrogen) by spectrofluorometer with excitation and emission at 480 nm and 520 nm, respectively (Wallac EnVision 2013 Multilabel Reader, Perkin Elmer). The extracted gDNA was

purified using PowerClean® DNA clean-up kit (MOBIO, USA) and purified gDNA was stored at –20℃ 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), and V3–V6 regions (A519F–A1017R) of the archaeal 16S rRNA gene (Klindworth *et al.*, 2012). The PCR was performed using ready-to-use solution of DreamTaq Green PCR Master Mix (2X), which containing DreamTaq DNA Polymerase, optimized DreamTaq Green buffer, $MgCl_2$, and dNTPs (Thermo Scientific™). The PCR program was as follows: an initial denaturation step at 95℃ for 7 min followed by 30 cycles (35 cycles for Archaea) of denaturation at 95℃ for 1 min, annealing at 57℃ for 1 min, and extension at 72℃ for 1 min, with a final extension at 72℃ for 7 min. All samples were amplified in triplicate, pooled in equal amounts, and purified using the QIAquick PCR Purification Kit (Qiagen). PCR products were quantified with a NanoDrop spectrophotometer. DNA sequencing was performed using a GS-FLX Titanium pyrosequencer (Roche).

Processing of pyrosequencing data

Amplicon pyrosequencing data were processed using the QIIME software package, ver. 1.9.1 (Caporaso *et al.*, 2010a). 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. Sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence similarity level using UCLUST (Edgar, 2010). Singleton OTUs are excluded because many more of the

sequences can be clustered using closed-reference OTU picking in this workflow, it can run in far less time than classic open-reference OTU picking (Rideout *et al.*, 2014). 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.*, 2010b). 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 1,051 sequences for Bacteria and 134 sequences for Archaea was generated for all samples based on the unweighted UniFrac phylogenetic distance metric (Lozupone *et al.*, 2006). Microbial diversity indices were estimated from a randomly selected subset of 1,583 and 134 sequences of Bacteria and Archaea, respective in each sample to avoid false results due to different sample sizes (Kirchman *et al.*, 2010).

Statistical analysis

To identify the relationships between microbial community and soil properties, statistical analyses were performed using R (version 3.0.0) (R, 2006) and PRIMER-E V6 (Clarke & Gorley, 2006). Boxplots were created for soil properties and diversity indices that were significantly different among the soil layers. Analysis of similarity (ANOSIM) with 999 permutations was used to represent significant differences in soil properties and alpha diversity indices between soil layers. Non-metric multidimensional scaling (NMDS) was generated using Bray-Curtis dissimilarity based on microbial community frequency to compare microbial community structure among soil layers. Venny (http://bioinfogp.cnb.csic.es/tools/venny) was used

to draw Venn diagrams from OTUs presence-absence data. Mantel test was calculated to test an association between bacterial community and soil properties along soil layers. Linear discriminant analysis coupled with effect size (LEfSe) was performed to identify the microbial taxa differentially represented between soil layers at OTU level (https://huttenhower.sph.harvard.edu/galaxy).

2.2.3 Results

General characteristics of soil properties

Soil profile was mostly classified as O and A horizon. The distinction among horizons was based on the relative ratio between organic and mineral materials (O, a layer dominated by organic matter; A, a mineral horizon) along soil depth (Table 2.8). Active layer depth was observed at ~70 cm in this sampling area and the O horizon was consisted with Oi, Oe, and OA horizons in detail. Below the 70 cm was determined permafrost. To summarize, active layer and permafrost were composed with O and A horizon, respectively (Table 2.8).

The soil properties changed with depth. The soil pH was ranged 3.3 to 5.6, and the pH increased from surface to permafrost table and decreased to permafrost (Table 2.8). The soil in sampling area was slightly acidic. The TC and TN contents, and C/N ratio were highest in surface and decreased to deeper soil depth with range from 47.9 to 1.3%, 2.4 to 0.06%, and 64.5 to 14.9 respectively. However, moisture content was highest in surface and decreased toward deeper depth, and re-increased in permafrost layer (Table 2.8). Overall soil properties were distributed based on the decomposition degree of SOM (Table 2.8). The vertical distribution of soil properties was

summarized using boxplots (Figure 2.20). The measured values of most soil properties were highly diverse in active layer and lowest in permafrost. There were significant differences in the soil properties between soil layers (Figure 2.20).

Table 2.8. Soil physical and chemical properties along soil depth

Core No.	Soil horizon	Soil layer	pH	TC (%)	TN (%)	CN (%)	MC (%)
Core 1	Oi	Active layer	3.9 ± 0.3	41.6 ± 4.0	0.9 ± 0.3	48.9 ± 13.6	329.4 ± 76.0
	Oe		5.0 ± 0.2	21.9 ± 17.8	0.8 ± 0.7	25.9 ± 2.5	224.2 ± 118.9
	OA		4.6 ± 0.6	5.7 ± 3.4	0.2 ± 0.1	28.2 ± 6.4	64.5 ± 29.4
	A	Permafrost	4.8 ± 0.5	1.8 ± 0.5	0.1 ± 0.0	19.3 ± 3.2	88.4 ± 30.3
Core 2	Oi	Active layer	5.0 ± 0.8	41.4 ± 4.0	1.0 ± 0.1	41.8 ± 1.7	487.4 ± 144.6
	Oe		5.1 ± 0.3	23.7 ± 17.6	1.1 ± 0.7	22.0 ± 2.7	208.5 ± 117.0
	OA		5.2 ± 0.1	7.4 ± 6.6	0.3 ± 0.2	24.1 ± 3.7	90.8 ± 34.9
	A	Permafrost	5.4 ± 0.3	2.8 ± 1.8	0.1 ± 0.0	20.1 ± 6.2	144.4 ± 33.4
Core 3	Oi	Active layer	5.2 ± 0.2	41.3 ± 1.3	1.1 ± 0.3	39.7 ± 10.0	516.5 ± 248.8
	Oe		5.0 ± 0.4	29.1 ± 18.1	1.3 ± 0.9	24.0 ± 3.5	222.7 ± 131.8
	OA		5.1 ± 0.4	10.8 ± 4.9	0.4 ± 0.2	26.7 ± 3.9	129.1 ± 106.2
	A	Permafrost	4.5 ± 0.8	3.9 ± 1.9	0.2 ± 0.1	19.1 ± 2.5	153.4 ± 36.6

TC, total carbon content; TN, total nitrogen content, C/N, a ratio of carbon and nitrogen content; MC, moisture content.

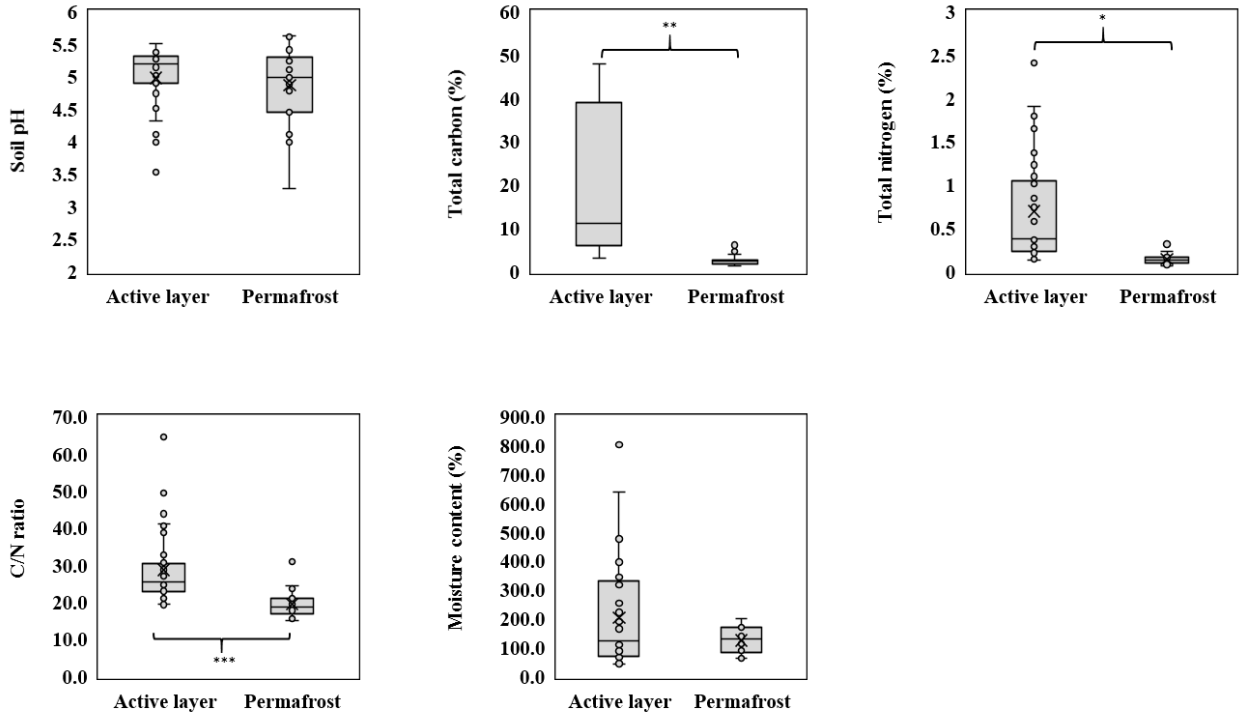


Figure 2.20. Boxplots of soil properties. Whiskers indicate the highest and lowest values, and asterisks indicate significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

General characteristics of microbial communities between soil layers

Quality checked 245,315 bacterial reads and 56,435 archaeal reads were finally processed to next OTUs and other statistical analyses. The number of OTUs was generated at the 97% sequence similarity cutoff. On average 3,407 reads (1,051 to 13,861) for Bacteria and 1,307 reads (134 to 3,380) for Archaea per sample were obtained. To facilitate diversity comparisons among microbial communities, we estimated diversity indices, including the Chao1, Shannon, phylogenetic diversity and observed OTUs, for a randomly selected subset of 1,051 sequences and 134 sequences from each bacterial and archaeal sample, respectively, to avoid effects of different sample sizes.

In bacterial alpha diversity indices, active layer was significantly different with permafrost (Figure 2.21). The highest number of bacterial OTUs was observed in active layer. This result was supported by bacterial richness (Chao 1) and diversity (Shannon's *H′*). Phylogenetic diversity in active layer was significantly higher than permafrost. In archaeal alpha diversity indices, active layer was significantly different with permafrost in all diversity indices (Figure 2.22). The highest number of bacterial OTUs was observed in permafrost. This result was supported by archaeal richness (Chao 1). However, archaeal diversity (Shannon's *H′*) varied in permafrost. Phylogenetic diversity was also highest in permafrost. These results indicated that bacterial and archaeal diversity indices had different characteristics along soil layers.

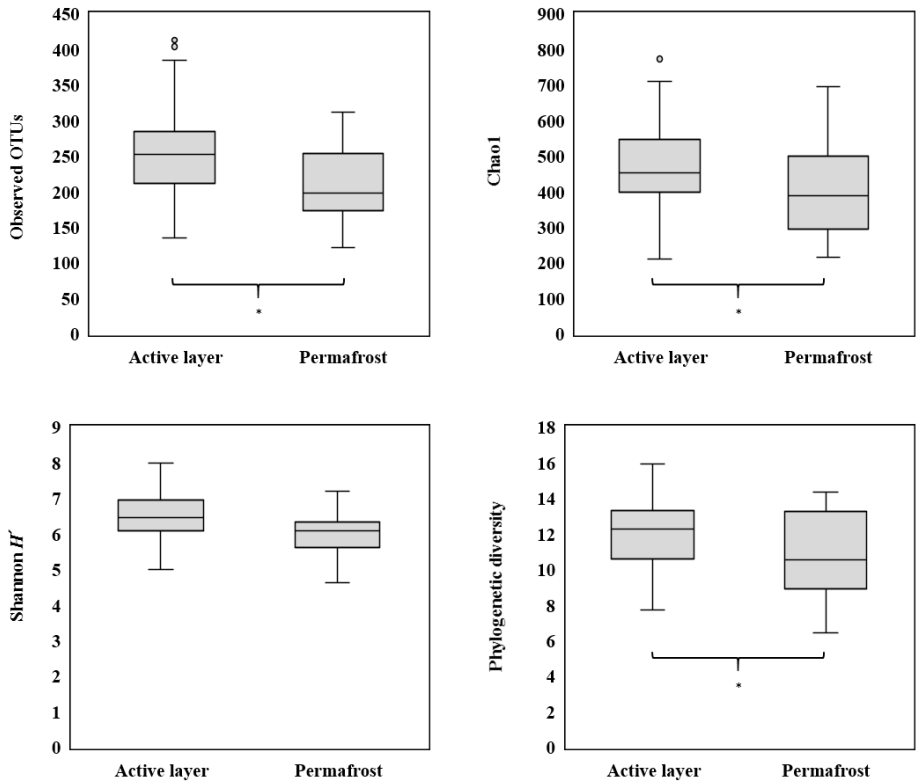


Figure 2.21. Boxplots of bacterial alpha diversity indices. Diversity indices represent the randomly selected subsets (n=1,051) for each sample. Asterisks indicate significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

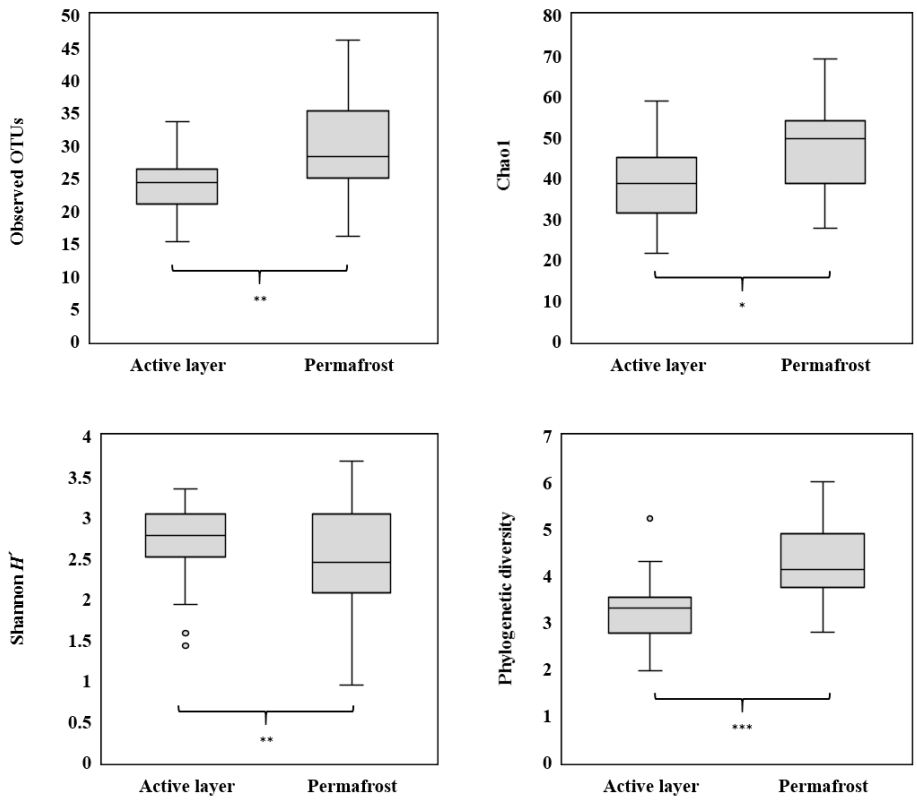


Figure 2.22. Boxplots of archaeal alpha diversity indices. Diversity indices represent the randomly selected subsets (n=134) for each sample. Asterisks indicate significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Microbial community structure between active layer and permafrost

The vertical distribution of bacterial community structure changed along soil depth (Figure 2.23A). In bacterial phyla, the relative abundances of *Alphaproteobacteria*, *Acidobacteria*, *Gamma-proteobacteria*, *Planctomycetes*, and *Verrucomicrobia* were abundant in soil surface and decreased toward deeper soil depth, while the relative abundances of *Actinobacteria*, *Bacteroidetes*, *Caldiserica*, and *Firmicutes* were increased toward deeper soil depth. Interestingly, the relative abundance of bacterial communities markedly differentiated within soil layer. For example, three soil horizons consisted active layer, and bacterial community structure of each soil horizon was distinct from each other: the relative abundances of *Acidobacteria*, *Gammaproteobacteria*, *Planctomycetes*, and *Verrucomicriboa* were higher in Oi horizon, AD3 was higher in Oe horizon, *Betaproteobacteria*, *Chloroflexi*, and *Gemmatimonadetes* were higher in OA horizon. In O horizon of permafrost, *Bacteroidetes*, *Caldiserica*, *Deltaproteobacteria*, *Firmicutes*, and OD1 were higher than other soil horizons. Overall bacterial communities were clearly distinguished between active layer and permafrost (Figure 2.24A). This pattern was confirmed by a significant ANOSIM value (Global R = 0.563, $p < 0.001$) between layers.

In vertical distribution of archaeal taxa at class level, the community composition was relatively simpler than Bacteria (Figure 2.23B). Three archaeal phyla detected: *Crenarchaeata*, *Euryarchaeota*, and candidatus *Parvarchaeota*. Generally, *Crenarchaeota* accounted for approximately 80% from most soil layers (Figure 2.23B). The relative abundance of Marine Benthic Group (MBGA) belonging *Crenarchaeota* was highest in surface soil (Oi horizon) in active layer and consistently decreased to the deeper soils. On the other hand, the relative abundance of Miscellaneous Crenarchaeotal

Group (MCG) was lowest in Oi horizon and increased toward deeper soils (Figure 2.23B). Although the relative abundance of *Euryarchaeota* was insignificant from total archaeal abundance, the relative abundance of *Methanobacteria* and *Methanomicrobia* increased below Oi horizon and showed the highest abundance in permafrost soil in Core 3 (Figure 2.23B). Generally, the relative abundances of MBGA and MCG were highest in most soil layers. Likewise Bacteria, the distinguished archaeal community structure between soil layers was observed in NMDS plot (Figure 2.24B). Also this pattern was confirmed by a significant ANOSIM value (Global R = 0.19, $p < 0.001$) between horizons.

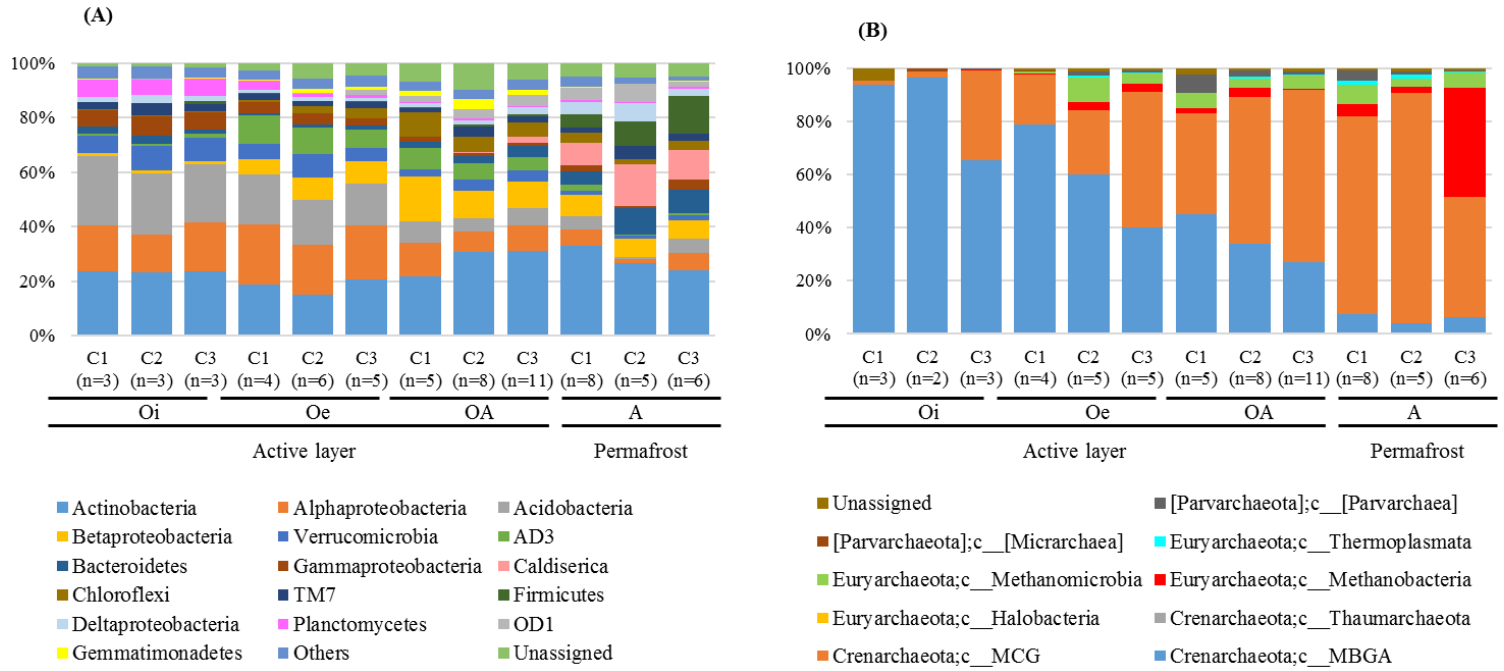


Figure 2.23. Relative abundances of bacterial taxa at phylum level (A) and archaeal taxa at class level (B).

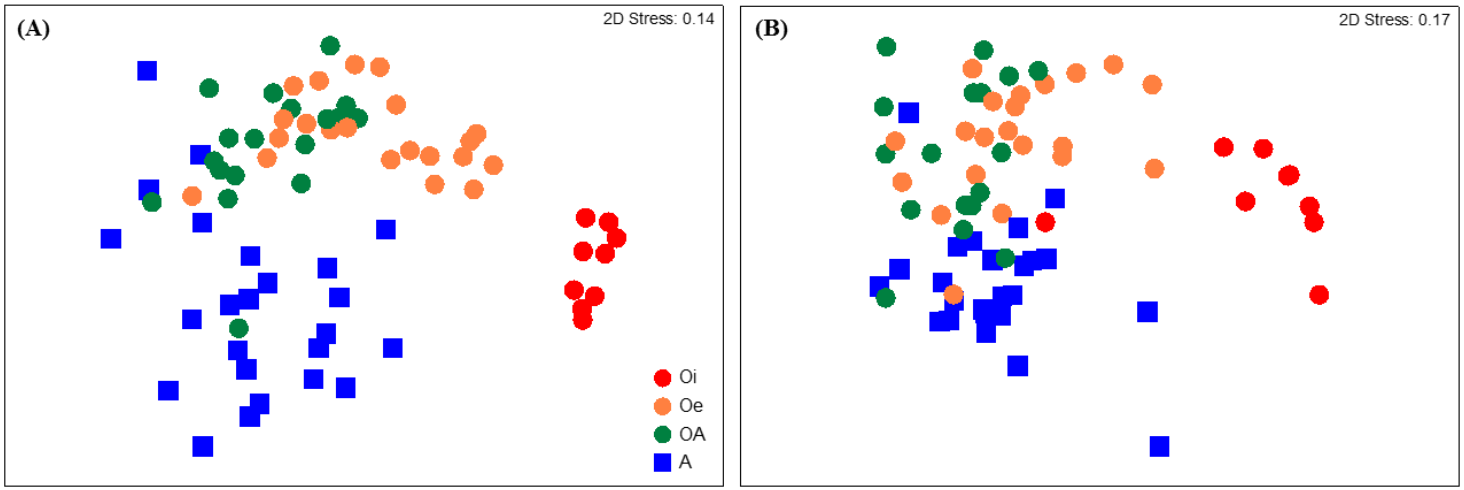


Figure 2.24. Bacterial (A) and archaeal (B) community compositional structure between soil horizons as indicated by NMDS plot based on the Bray-Curtis dissimilarity. Closed circle, active layer; closed square, permafrost.

Distinct microbial communities between active layer and permafrost

Linear Discriminant Analysis Effect Size (LEfSe) analysis was applied to the data for relative abundance of microbial OTUs of active layer and permafrost. Among the dominant bacterial OTUs accounted for over 0.1% from total bacterial abundances, 39 OTUs were identified as being significantly different between active layer and permafrost (Figure 2.25). From the soil layers, 14 OTUs were significantly more abundant in active layer, while 25 OTUs were more abundant in permafrost layer. As to archaeal OTUs, 12 OTUs were differentially represented, with 5 OTUs were more abundant in active layer and 7 OTUs were more abundant in permafrost (Figure 2.26). The lineage of the microbial OTUs with *p*-value were listed in Table 9 and 10.

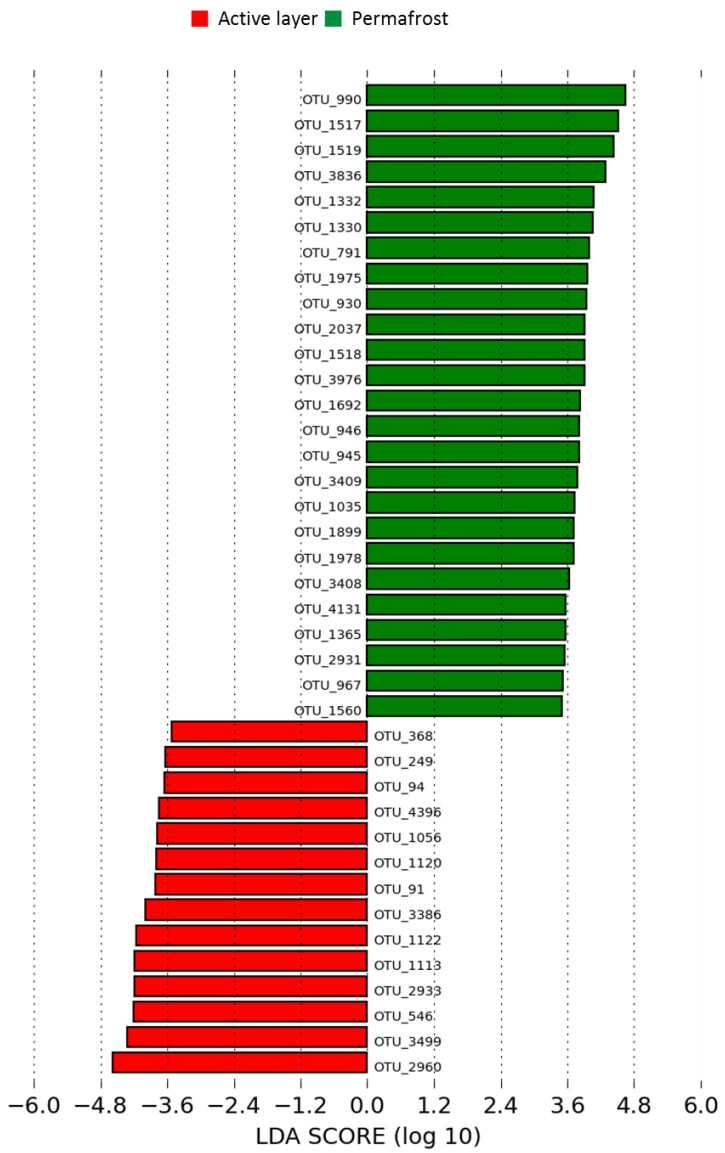


Figure 2.25. Linear Discriminant Analysis Effect Size (LEfSe) analysis showing bacterial OTUs that were significantly differentially abundant between active layer and permafrost, ranked by effect size (all LDA scores >3.5).

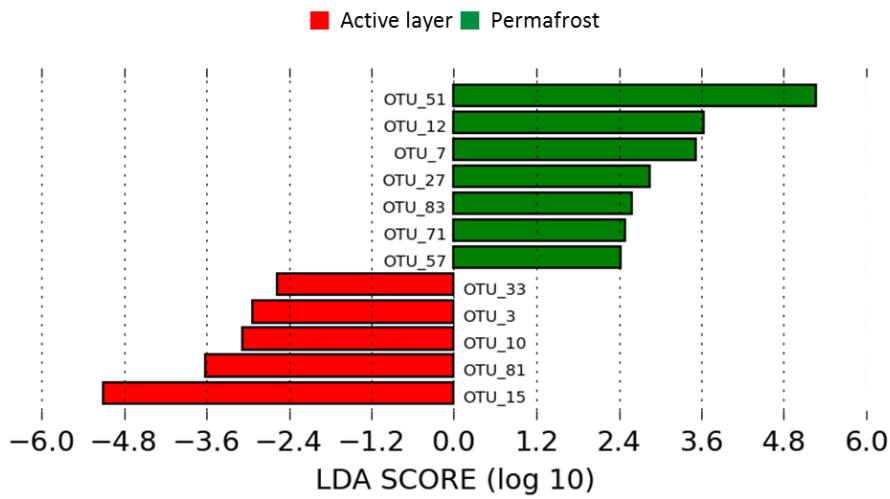


Figure 2.26. Linear Discriminant Analysis Effect Size (LEfSe) analysis showing archaeal OTUs that were significantly differentially abundant between active layer and permafrost, ranked by effect size (all LDA scores >2.0).

Table 2.9. Significantly different relative abundances of dominant bacterial OTUs between active layer and permafrost with *p*-value

OTU no.	Hierarchical taxonomic level *	*p*-value
Permafrost		
OTU_990	*Actinobacteria*; c_*Actinobacteria*; o_*Actinomycetales*; f_*Nocardiaceae*	$p < 0.001$
OTU_1517	*Caldiserica*; c_WCHB1-03; o_; f_; g_; s_	$p < 0.001$
OTU_1519	*Caldiserica*; c_WCHB1-03; o_; f_; g_; s_	$p < 0.001$
OTU_3836	*Deltaproteobacteria*; o_*Syntrophobacterales*; f_*Syntrophaceae*; g_*Syntrophus*; s_	$p < 0.001$
OTU_1332	*Bacteroidetes*; c_*Bacteroidia*; o_*Bacteroidales*; f_; g_; s_	$p < 0.001$
OTU_1330	*Bacteroidetes*; c_*Bacteroidia*; o_*Bacteroidales*; f_; g_; s_	$p < 0.001$
OTU_791	*Actinobacteria*; c_*Actinobacteria*; o_*Actinomycetales*; f_; g_; s_	$p < 0.001$
OTU_1975	*Firmicutes*; c_*Clostridia*; o_*Clostridiales*; f_*Peptococcaceae*; g_*Desulfosporosinus*; s_*meridiei*	$p < 0.001$
OTU_930	*Actinobacteria*; c_*Actinobacteria*; o_*Actinomycetales*; f_*Nocardiaceae*	$p < 0.01$
OTU_2037	*Firmicutes*; c_*Clostridia*; o_*Clostridiales*; f_*Ruminococcaceae*; g_; s_	$p < 0.001$
OTU_1518	*Caldiserica*; c_WCHB1-03; o_; f_; g_; s_	$p < 0.001$
OTU_3976	*Gammaproteobacteria*; o_*Oceanospirillales*; f_*Halomonadaceae*; g_*Halomonas*; s_	$p < 0.05$
OTU_1692	*Chloroflexi*; c_TK10; o_; f_; g_; s_	$p < 0.001$
OTU_946	*Actinobacteria*; c_*Actinobacteria*; o_WCHB1-81; f_At425_EubF1; g_; s_	$p < 0.001$
OTU_945	*Actinobacteria*; c_*Actinobacteria*; o_WCHB1-81; f_At425_EubF1; g_; s_	$p < 0.001$
OTU_3409	*Betaproteobacteria*; o_*Burkholderiales*	$p < 0.001$
OTU_1035	*Actinobacteria*; c_*Thermoleophilia*; o_*Gaiellales*; f_*Gaiellaceae*; g_; s_	$p < 0.001$
OTU_1899	*Firmicutes*; c_*Bacilli*; o_*Bacillales*; f_*Bacillaceae*; g_*Anoxybacillus*; s_*kestanbolensis*	$p < 0.01$
OTU_1978	*Firmicutes*; c_*Clostridia*; o_*Clostridiales*; f_*Peptococcaceae*; g_*Desulfosporosinus*; s_*meridiei*	$p < 0.001$
OTU_3408	*Betaproteobacteria*; o_*Burkholderiales*	$p < 0.05$
OTU_4131	TM7; c_TM7-1; o_; f_; g_; s_	$p < 0.05$
OTU_1365	*Bacteroidetes*; c_*Bacteroidia*; o_*Bacteroidales*; f_; g_; s_	$p < 0.001$
OTU_2931	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Bradyrhizobiaceae*; g_; s_	$p < 0.001$
OTU_967	*Actinobacteria*; c_OPB41; o_; f_; g_; s_	$p < 0.001$
OTU_1560	*Chloroflexi*	$p < 0.001$

Active layer

OTU_368	*Acidobacteria*; c_iii1-8; o_SJA-36; f_; g_; s_	$p < 0.05$
OTU_249	*Acidobacteria*; c_DA052; o_Ellin6513; f_; g_; s_	$p < 0.01$
OTU_94	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Acidobacteriaceae*; g_; s_	$p < 0.001$
OTU_4396	*Verrucomicrobia*; c_[Pedosphaerae]; o_[Pedosphaerales]; f_auto67_4W; g_; s_	$p < 0.05$
OTU_1056	*Actinobacteria*; c_*Thermoleophilia*; o_*Solirubrobacterales*; f_; g_; s_	$p < 0.01$
OTU_1120	AD3; c_JG37-AG-4; o_; f_; g_; s_	$p < 0.01$
OTU_91	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Acidobacteriaceae*; g_; s_	$p < 0.05$
OTU_3386	*Betaproteobacteria*; o_; f_; g_; s_	$p < 0.001$
OTU_1122	AD3; c_JG37-AG-4; o_; f_; g_; s_	$p < 0.05$
OTU_1113	AD3; c_ABS-6; o_; f_; g_; s_	$p < 0.05$
OTU_2933	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Bradyrhizobiaceae*; g_; s_	$p < 0.001$
OTU_546	*Actinobacteria*; c_*Acidimicrobiia*; o_*Acidimicrobiales*; f_; g_; s_	$p < 0.001$
OTU_3499	*Betaproteobacteria*; o_*Gallionellales*; f_*Gallionellaceae*; g_*Gallionella*; s_	$p < 0.05$
OTU_2960	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Hyphomicrobiaceae*; g_*Rhodoplanes*; s_	$p < 0.001$

*c_; o_; f_; g_; s_ means class_;order_;family_;genus_;species_

Table 2.10. Significantly different relative abundances of dominant archaeal OTUs between active layer and permafrost with *p*-value

OTU no.	Hierarchical taxonomic level *	*p*-value
Permafrost		
OTU_51	*Crenarchaeota*; c_MCG; o_pGrfC26; f_; g_; s_	$p < 0.001$
OTU_12	[Parvarchaeota]; c_[Parvarchaea]; o_YLA114; f_; g_; s_	$p < 0.05$
OTU_7	[Parvarchaeota]; c_[Parvarchaea]; o_WCHD3-30; f_; g_; s_	$p < 0.05$
OTU_27	*Crenarchaeota*; c_MBGA; o_NRP-J; f_; g_; s_	$p < 0.05$
OTU_83	*Euryarchaeota*; c_*Methanomicrobia*; o_*Methanosarcinales*; f_ANME-2D; g_; s_	$p < 0.05$
OTU_71	*Crenarchaeota*; c_*Thaumarchaeota*; o_*Cenarchaeales*; f_*Cenarchaeaceae*; g_; s_	$p < 0.05$
OTU_57	*Crenarchaeota*; c_MCG; o_pGrfC26; f_; g_; s_	$p < 0.001$
Active layer		
OTU_33	*Crenarchaeota*; c_MBGA; o_NRP-J; f_; g_; s_	$p < 0.05$
OTU_3	[Parvarchaeota]; c_[Parvarchaea]; o_WCHD3-30; f_; g_; s_	$p < 0.001$
OTU_10	[Parvarchaeota]; c_[Parvarchaea]; o_WCHD3-30; f_; g_; s_	$p < 0.05$
OTU_81	*Euryarchaeota*; c_*Methanomicrobia*; o_*Methanocellales*; f_; g_; s_	$p < 0.05$
OTU_15	*Crenarchaeota*; c_MBGA; o_NRP-J; f_; g_; s_	$p < 0.05$

*c_; o_; f_; g_; s_ means class_;order_;family_;genus_;species_

Relationships between microbial communities and soil properties between active layer and permafrost

To identify the distinctiveness of the correlations between microbial communities and soil layers, ANOSIM was performed. The ANOSIM results showed a significant difference in microbial composition along soil layers: active layer and permafrost (Global R = 0.704, $p < 0.001$) in bacterial communities, and active layer and permafrost (Global R = 0.457, $p < 0.001$) in archaeal communities. To identify the relationship between microbial community and soil property along soil layers, Mantel test was analyzed. The results of Mantel test showed that soil microbial communities were significantly correlated with soil properties (Table 2.11). Bacterial communities were significantly correlated with soil pH, TC, TN, C/N ratio, and MC in active layer. However, no correlation was observed in permafrost. Archaeal communities were significantly correlated with soil pH, TC, C/N ratio, and MC in active layer, and soil pH in permafrost, respectively (Table 2.11). Generally, bacterial and archaeal communities were closely related with various soil properties in active layer. Perhaps, other soil properties might relate with microbial community in permafrost.

Table 2.11. The correlations between soil property and microbial community along soil layers. The Pearson correlation (*r*) and significance (*p*) determined by Mantel tests

Domain	Soil properties	Active layer		Permafrost	
		r	*p*	*r*	*p*
Bacteria	pH	0.458	**0.001**	0.139	0.166
	TC	0.573	**0.001**	-0.079	0.663
	TN	0.274	**0.001**	-0.163	0.919
	C/N ratio	0.447	**0.001**	0.187	0.099
	MC	0.534	**0.001**	-0.027	0.624
Archaea	pH	0.233	**0.006**	0.494	**0.002**
	TC	0.281	**0.001**	0.180	0.123
	TN	0.071	0.144	0.095	0.232
	C/N ratio	0.420	**0.001**	0.261	0.085
	MC	0.209	**0.006**	-0.060	0.717

TC, total carbon content; TN, total nitrogen content, C/N, a ratio of carbon and nitrogen content; MC, moisture content.

2.2.4 Discussion

Microbial community structures are different along soil layers

The microbial community structure changed along soil depth and their vertical distribution was clustered by soil layers. The bacterial richness and diversity were highest in active layer and decreased toward permafrost, and these results corresponded with other depth profile studies in Arctic soils (Yergeau *et al.*, 2010; Deng *et al.*, 2015). However, archaeal richness and diversity were highest in permafrost. This might be explained by the relationships between microbial community and soil properties. Although both bacterial and archaeal communities significantly correlated with various soil properties (e.g. pH, TC, TN, C/N ratio, and MC) in active layer, other factors which are not measured in this study such as O_2 concentration may influence the diversity in Archaea. Our results showed increasing methanogens (*Methanomicrobia* and *Methanobacteria*) in deeper layer. Deeper soil, where anoxic conditions are prevalent, is a relatively appropriate environment for methanogens (Whalen & Reeburgh, 1992). Top surface soil (Oi horizon) was considered aerobic environment, showing the archaeal community structure comprising almost *Crenarchaeota*.

If the soil microbial communities inhabiting the surface simply diluted along soil depth, subsurface soil microbial communities may be quite similar with surface soil microbial communities. However, we found the microbial community structures were significantly different along soil layers. In other words, the relative abundance of microbial community compositions did not change gradually along soil depth. For example, the relative abundance of *Betaproteobacteria*, *Chloroflexi*, and *Gemmatimonadetes* were highest Oe and OA horizons in active layer (Figure 2.23A). Perhaps, the

dominant groups in Oe and OA horizons contain various genera which have abilities to adapt to change of active layer to permafrost seasonally. Then, the physiological characteristics of microbial community may differ along soil layers. This assumption will be discussed in Chapter 4 through metagenome analysis.

The physiological characteristics of microbial community in active layer and permafrost

In the microbial communities between active layer and permafrost, we found that each layer showed dominant microbial groups (Figure 2.25 and 2.26). The dominant OTUs were significantly differentiated between soil layers (Table 2.9 and 2.10). From the investigation of physiological characteristics of the dominant microbial OTUs from literature review, we could speculate the soil layer's metabolic functions. For example, dominant bacterial OTUs which belonged to *Acidimicrobiales* and *Solirubrobacterales* (*Actinobacteria*), *Bradyrhizobiaceae* and *Rhodoplanes* (*Alphaproteobacteria*), or *Gallionella* (*Betaproteobacteria*) in active layer showed characteristics of aerobic, non-spore forming, psycrotolerant, and/or various heterotrophic utilizing diverse materials. When we compared the environmental conditions simply at the sampling time, active layer is a relatively extreme environment compared to other soil layers due to temperature fluctuation and daylight. So the bacterial OTUs which have characteristics of psychrotolerant (members of *Solirubrobacterales* and *Acidimirobiales*) or photoheterotroph (*Acidimicrobiales* and *Rhodoplanes*) were predominant in active layer (Hiraishi & Ueda, 1994; Reddy & Garcia-Pichel, 2009; Mizuno *et al.*, 2015). Nutrient (carbon and nitrogen) cycling

may be activated in active layer. Family *Bradyrhizobiaceae* (*Alphaproteobacteria*) has been known to contribute to the significant involvement nitrogen cycling, nitrate (NO_3^-) denitrification through to N_2 (Anderson *et al.*, 2011). Recently, some study revealed that the order *Acidimirobiales* (*Actinobacteria*) contains glycolate oxidase genes, indicating the ability to use the complex carbon sources like glucose (Mizuno *et al.*, 2015). If the active layer is waterlogged seasonally, bacterial OTUs belonging to *Rhodoplanes* can grow in anaerobic condition by nitrate respiration (Hiraishi & Ueda, 1994).

In contrast to the active layer's dominant OTUs which can utilize various materials, dominant bacterial OTUs in permafrost were associated with degradation of various multi-carbon compounds. The dominant OTUs in permafrost belong to *Burkholderiales* (*Betaproteobacteria*), *Caldiserica*, *Gaiellaceae* (*Actinobacteria*), *Halomonas* (*Gammaproteobacteria*), *Rumirococcaceae*, *Anoxybacillus kestanbolensis* and *Desulfosporosinus meridiei* (*Firmicutes*), or *Syntrophus* (*Deltaproteobacteria*) (Table 2.9). Among the OTUs, the members *Burkholderiales* are phenotypically and metabolically methylotrophic bacteria, capable of growing on C1 compounds such as methanol, methylamine, and formaldehyde, and may play an important role in global cycling of single-carbon compounds (Kalyuzhnaya *et al.*, 2008). In addition, *Burkholderiales* are known as the main player in microbial ecology of bioremediation treatments because they have the most impressive potential for aromatic compound catabolisms (Pérez-Pantoja *et al.*, 2012). The genus *Syntrophus* (*Deltaproteobacteria*) has been known to be associated with hydrogen/formate-using microorganisms (Jackson *et al.*, 1999). The genus *Syntrophus* is strictly anaerobic bacteria and possesses hygrogenase and formate dehydrogenase, utilizing various organic

compounds including methane, formic acid and hydrogen (McInerney *et al.*, 2007). Syntrophic consortia between *Syntrophus* like bacteria and methanogens plays an important role in controlling the flux of methane (McInerney *et al.*, 2009). The member of *Ruminococcaceae* which known as acetogen may closely relate with methanogens (Gagen *et al.*, 2015). Acetogens and methanogens can compete for H_2-CO_2 in anaerobic environments. From the literature review, it was found that bacterial physiological characteristics were quite different between active layer and permafrost. This will be checked in Chapter 4 through metagenomic analysis.

Generally, *Crenarchaeota* group dominated in all soil layers. However, their relative abundance changed along soil depth. The most dominant OTUs were marine benthic group A (MBGA, belonging *Crenarchaeota*) in active layer and miscellaneous crenarchaeota group (MCG) in permafrost. Unfortunately, no isolates of MBGA and MCG have been cultivated or characterized to date. However, these groups are the dominant archaeal groups in anoxic environments and may have significant roles in the global biogeochemical cycles (Meng *et al.*, 2014). The *Euryarchaeota* including methanogenic community in subsoil was relatively diverse compared to surface in active layer, and included members of the order *Methanomicrobiales* and *Methanosarcinales*, the family *Methanomassiliicoccaceae*, and the genus *Methanpbacterium* and *Methanosarcina* in this study. Methanogenesis is the terminal step in the anaerobic decomposition of organic matter in anaerobic environments (Ganzert *et al.*, 2007). Although the relative abundances of the methanogenic communities started to increase in subsoil, the relative abundances were similar in all deeper depth (except the relative abundance of *Methanobacteria* showed abruptly increased in permafrost from Core 3). This indicated that the

environmental conditions from deeper active layer to permafrost were similar in *Euryarchaeota* with methanogenic group to propagate.

In conclusion, this study revealed the microbial community structure between active layer and permafrost, and the microbial communities have different metabolic functions between active layer and permafrost. Most microbial communities interact with various soil properties in active layer, while they did not show the relations with soil properties in permafrost. To predict the change of microbial community compositions in permafrost, further study will be needed to analyze various edaphic factors such as O_2 concentration, dissolved organic matter (DOM), salinity, and various soil elements (e.g. P, K, Ca, Mg, Na, S, Mn, Cu and Zn). This study might give a hint that salinity may be higher in permafrost because one (OTU_3976) of dominant bacterial OTUs were assigned *Halomonas* which is known to be salt-tolerant bacterial genera (Vreeland *et al.*, 1980).

|CHAPTER 3|

Bacterial Community Structure in Arctic Tundra and non-Arctic Soils

3.1 Comparison of bacterial community structure between Arctic and non-Arctic Soil

3.1.1 Introduction

Microorganisms are one of the abundant and diverse organisms on Earth, and most of them remain unidentified (Amann *et al.*, 1995). Ecologists have described the existence of microorganisms following ‘‘Everything is everywhere, but the environment selects (Baas-Becking, 1934)’’. For example, the soil bacterial community structures were distinctive across climatic zones such as temperate and tropical soil (Kim *et al.*, 2014b). This indicated that latitudinal geographic distance might be an important factor to lead the genetic divergence between microbial communities and habitats (Chu *et al.*, 2010). However, microbial diversity based on geographical distance is still under debate, as many studies revealed that microbial community composition is strongly influenced by site-specific environmental conditions (Fierer & Jackson, 2006; Lozupone & Knight, 2007). Besides, determination of microbial diversity may be intractable due to pervasive horizontal gene transfer, which complicates the value of microbial community studies in a given environment (Meyer *et al.*, 2004; Nemergut *et al.*, 2004). Thus, the distribution of microorganisms at various scales (local to global) is a controversial issue and poorly understood.

Arctic tundra soil is one of the extreme environments, and the inhabiting microorganisms are exposed to harsh environmental conditions, such as low temperature, high seasonal temperature fluctuation, high UV radiation exposure, and nutrient limitations. Perhaps, Arctic soil provide

unique ecological niche for microorganisms, especially cold-adapted microorganisms (Jansson & Tas, 2014). When we observed the bacterial community structure between sites with geographical distance through literature review, for example, the bacterial phylum *Bacteroidetes* was one of the dominant groups in Canadian High Arctic soils (80°N), and *Chloroflexi* and AD3 dominated in Alaskan soils (65°N) (Steven *et al.*, 2008; Yergeau *et al.*, 2010; Mackelprang *et al.*, 2011; Tas *et al.*, 2014). Although the previous studies used different techniques (Jansson & Tas, 2014), I could find differences and similarities in microbial community structure across latitudinal distances.

Microorganisms play an important role in nutrient cycles. Microbial community structure and diversity in Arctic soil have received scientific attention due to climate change, and many scientists focus on microbial contribution to carbon cycles because Arctic soil contains considerable amount of soil organic carbon (Tarnocai, 2009). As was discussed earlier on in our study (Chapter 2), microbial community structure and diversity differed along soil depth, and abundant microorganisms in upper soil (e.g. surface soil or active layer) was related to various nutrient cycles, such as carbon and nitrogen. The distinct community diversity and structure are significantly correlated with soil properties. Then, how different is Arctic soil compared to other climatic zones? If the soil environmental factors are different from Arctic soil and non-Arctic soil, can we see the differences of microbial community structure and diversity, and presume the distinct microbial functions?

In this study, we attempted to describe the distribution of soil bacterial community along latitudinal geographical distance embracing Arctic, temperate, and tropical region. I identified the overall bacterial

community structure and diversity between biomes, and assessed relationships with soil properties. To avoid technical bias based on sequencing of 16S rRNA gene, I compared the data from same primer region and sample sequencing technique.

3.1.2 Material and Methods

Sampling locality

To compare the bacterial community structure between Arctic and non-Arctic soil, representative two latitudinal sampling sites were chosen from published data (Tripathi *et al.*, 2012; Kim *et al.*, 2014a; Singh *et al.*, 2014). Temperate soil sites were chosen in Mt. Halla, Korea and tropical soil sites were chosen in FRIM rain forest, Malaysia (Figure 2.27). In all sampling sites, soil samples were collected from surface soils (0~10 cm).

Data for Arctic soil sites were collected in surface soil samples from previous study (Kim *et al.*, 2014a). Among the 36 sampling points, ten sampling points were randomly selected to compare with other latitudinal data. In general characteristics of the sampling sites, the annual mean air temperature and precipitation are –3.1 ± 1.4℃ and 258 mm, respectively. The dominant vegetation was cotton grass (*Eriophorum vaginatum*) or tussock, blueberry (*Vaccinium uliginosum*), and lichen and moss (*Sphagnum* spp.) beds.

For temperate soil sites, eight soil samples were chosen in lower parts of Mt. Halla. In general characteristics of Jeju island, the annual mean air temperature and precipitation are 14.7℃ and 1,900 mm, respectively. Mt. Halla is a shield volcano, consisting of alkaline lavas, and is almost

completely covered with vegetation. The dominant vegetation of lower part of Mt. Halla was pine tree, *Castanopsis cuspidate* var. *sieboldii* and *Quercus salicina* (Singh *et al.*, 2014).

For tropical soil sites, nine soil sites were chosen in tropical rain forest of Forest Research Institute Malaysia (FRIM). In general characteristics of FRIM forest, the mean annual temperature and precipitation are approximately 26.5℃ and 2,620 mm, respectively. Major tree species were *Chukrasia tabularis, Intsia palembanica, Artocarpus elasticus,* and *Shorea pauciflora* (Tripathi *et al.*, 2012).

Soil physicochemical properties

To analysis the soil properties for Arctic soil, soil pH was determined in a 1:10 soil:water (w/v) solution (Thomas *et al.*, 1996). Soil was ground and passed through a 53 μm sieve to determine total carbon (TC) and total nitrogen (TN) content. TC and TN contents were measured by combustion (950℃) (FlashEA 1112; Thermo Fisher Scientific)(Kim *et al.*, 2014a).

To analysis the soil properties for temperate soil samples, soil pH, TC, and TN were measured at National Instrumentation Center for Environmental Management (NICEM, Korea) based on the standard protocol of SSSA (Soil Science Society of America) (Singh *et al.*, 2014).

To analysis the soil properties for tropical soil samples, soil pH was measured in water at the soil to solution ratio of 1:2 using pH meter. TC was determined by the Carbon Analyzer Leco CR-412 (Leco Corporation, MI, USA) with 1 g of soil. TN was determined by sulfuric acid digestion using Se, $CuSO_4$, and K_2SO_4 as catalysts, with 1 g of soil, and determined by the regular Kjeldahl distillation method (Tripathi *et al.*, 2012).

Next Generation Sequencing (NGS) and data processing

Genomic DNA (gDNA) was extracted by using DNA extraction kits. For Arctic soils, gDNA was extracted from 0.5 g of the homogenized soil samples using a FastDNA® SPIN kit for soil (MP Biomedicals) and a QuickPrep adapter (MP Biomedicals), according to the manufacturer's recommended protocol (Kim *et al.*, 2014a). For temperate and tropical soils, gDNA was extracted using MOBIO Power Soil DNA extraction kit (MOBIO Laboratories, CA, USA) as directed by the manufacturer (Tripathi *et al.*, 2012;

Singh *et al.*, 2014). DNA from all soil samples was amplified using primers targeting the V1 to V3 regions (27F–518R) of the bacterial 16S rRNA gene (Chun *et al.*, 2010). PCR reactions were carried out as described in previous study for Arctic soil (Kim *et al*., 2014), for temperate soil (Singh *et al.*, 2014), and for tropical soil (Tripathi *et al.*, 2012). DNA sequencing was performed using a GS-FLX 454 pyrosequencer (Roche).

PCR amplicon pyrosequencing data from all soil sampels (Arctic, Temperate, and Tropical soils) were processed using the QIIME software package, ver. 1.7 (Caporaso *et al.*, 2010a). Briefly, raw flowgrams (sff files) were filtered and noise and chimeras were removed using AmpliconNoise. Sequences were clustered based on operational taxonomic units (OTUs) at 97% similarity using UCLUST. OTUs were assigned to taxa using the RDP Classifier method. Sequence alignments were generated using PyNAST software and the Greengenes database. Details described in Chapter 2.1.

Statistical analysis

Statistical analyses were performed using R (version 3.0.0; The R Foundation for Statistical Computing) and PRIMER-E V6 (Clarke & Gorley, 2006). Analysis of similarity (ANOSIM) with 999 permutations and non-metric multidimensional scaling (NMDS) were conducted to compare bacterial community structure. A Mantel test was used to determine which physical and chemical properties of soil were significantly correlated with the bacterial community. Linear discriminant analysis coupled with effect size (LEfSe) was performed to identify the microbial taxa differentially represented between soil layers at OTU level (https://huttenhower.sph.harvard.edu/galaxy).

Figure 3.1. Sampling locations of Arctic and non-Arctic soil. Soil sites for Arctic soil were chosen in Alaska (USA), temperate soil were chosen in Mt. Halla (Korea), and tropical soil were in FRIM rainforest (Malaysia).

3.1.3 Results

General characteristics of soil properties

The soil properties varied with sampling locations, and were significantly differentiated between biomes (Table 2.13 and Figure 2.26). All soil pH was slightly acidic (3.50 ~ 5.76) without TR2, TR3, and TR4 in Malaysia (average 6.41). The highest contents of carbon (42.25 ± 3.36) and nitrogen (1.56 ± 0.5) were observed in Arctic soil and the contents were lowest in tropical soil (carbon, 2.07 ± 0.57; nitrogen, 0.28 ± 0.06). C/N ratio was higher in temperate soil and lower in tropical soil. This result indicated that Arctic soil is relatively nutrient rich environment, and tropical soil is low nutrient environment.

General characteristics of bacterial communities between Arctic and non-Arctic soils

Quality checked 35,003 bacterial reads from all soil sampling sites were finally processed to next OTUs and other statistical analyses. The number of OTUs was generated at the 97% sequence similarity cutoff. On average 1,107 reads (591 to 1,543) for Arctic soil, 1,168 reads (594 to 1,893) for temperate soil, and 1,621 reads (881 to 3,853) for tropical soil were obtained. To facilitate diversity comparisons among microbial communities, we estimated diversity indices, including the Chao1, Shannon, phylogenetic diversity and observed OTUs, for a randomly selected subset of 591 sequences from each soil sample to avoid effects of different sample sizes.

There were significant differences in bacterial diversity indices in global scale (Figure 2.27). The number of observed bacterial OTUs in the

temperate soil was greater than that of other biomes. This result was supported by bacterial richness (Chao 1) and diversity (Shannon's *H′*) which were randomly selected subset of 591 sequences from each soil sample. However, the richness (Chao 1) of bacterial abundance was not significantly different between Arctic and tropical soil (p = 0.082; Figure 2.27). Phylogenetic diversity in temperate soil was also significantly higher than any other biomes. In accordance with the diversity indices, temperate soil which contains highest richness and diversity has a large number of unique OTUs (1,667 OTUs) (Figure 2.28). And Arctic soil showed the lowest number of unique OTUs (707 OTUs). Although the geographical distance is similarly far away (approximately latitudinal 30 degree) between Arctic and temperate, and temperate and tropical biome, the number of shared OTUs decreased toward Arctic soil; tropical and temperate soils shared 383 OTUs, temperate and Arctic soil shared 152 OTUs. Arctic soil and tropical soil just shared 50 OTUs (Figure 2.28). The results indicated that the bacterial community structures were different along biome, and may have different ecological functions.

Table 3.1. Sample descriptions and soil properties

Ecosystems	Sample	Latitude	Longitude	pH	TC (%)	TN (%)	C/N ratio	Dominant vegetation
Arctic	AKC-1	64°51′ N	163°39′ W	4.04	39.45	1.00	39.56	*Eriophorum vaginatum*, *Vaccinium uliginosum*, *Sphagnum* spp., Lichen
	AKC-3			4.55	42.76	2.22	19.31	
	AKC-7			4.29	43.64	1.73	25.29	
	AKC-10			4.10	40.61	1.67	24.27	
	AKC-18			4.04	45.85	1.79	24.53	
	AKC-19			4.07	48.07	1.86	22.97	
	AKC-23			4.03	41.73	1.23	33.90	
	AKC-27			4.57	43.02	2.10	20.54	
	AKC-34			4.61	35.88	1.34	26.88	
	AKC-36			3.95	41.44	0.63	66.30	
Temperate	YS1-1	33°14′ N	126°23′ E	5.76	6.14	0.47	13.08	Pine, *Castanopsis cuspidate* var. *sieboldii*, *Quercus salicina*
	YS1-2			5.19	10.27	0.53	19.30	
	YS1-4			5.45	8.75	0.65	13.51	
	YS1-5			5.68	9.00	0.56	16.02	
	YS3-1	33, 16′ N	126°27′ E	5.44	15.82	1.05	15.12	
	YS3-2			5.53	18.80	0.64	29.22	
	YS3-3			5.73	20.47	1.03	19.79	
	YS3-4			5.40	13.68	1.07	12.78	
Tropical	FRT02	03°14′ N	101°37′ E	6.43	1.46	0.18	8.11	*Chukrasia tabularis*, *Intsia palembanica*, *Artocarpus elasticus*, *Shorea pauciflora*
	FRT03			6.45	1.55	0.41	3.78	
	FRT 04			6.36	1.56	0.33	4.73	
	FRT 15			3.60	2.94	0.27	10.89	
	FRT 16			4.03	2.56	0.26	9.85	
	FRT 17			4.05	2.12	0.27	7.85	
	FRT 27			4.14	1.56	0.29	5.38	
	FRT 28			4.04	2.70	0.29	9.31	
	FRT 29			3.50	2.17	0.22	9.86	

TC, total carbon; TN, total nitrogen; C/N, a ratio of carbon to nitrogen

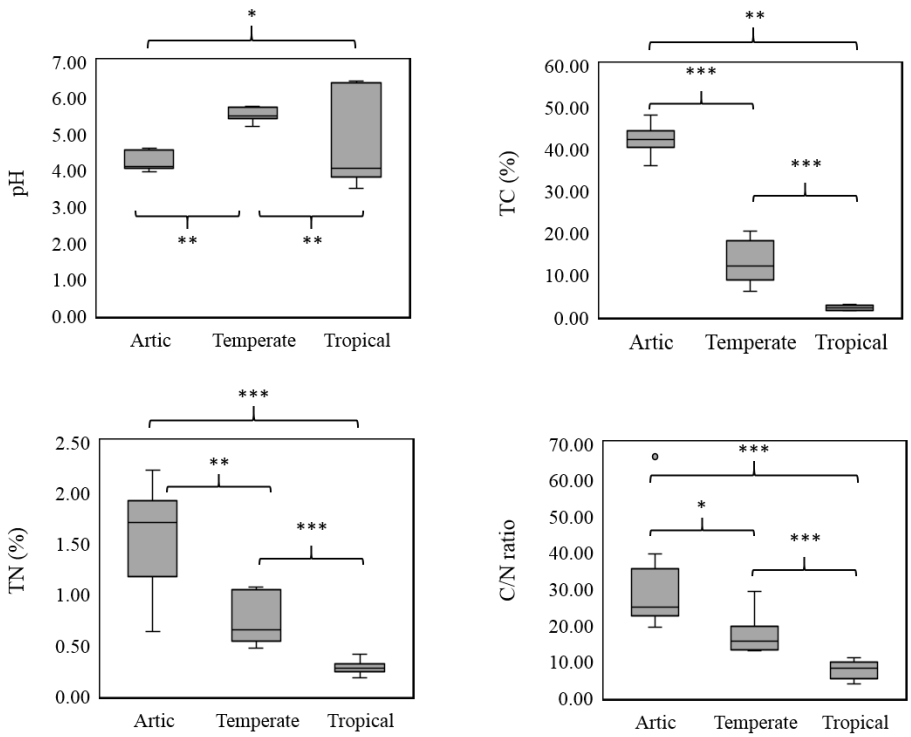


Figure 3.2. Boxplots of soil properties. Asterisks indicate significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

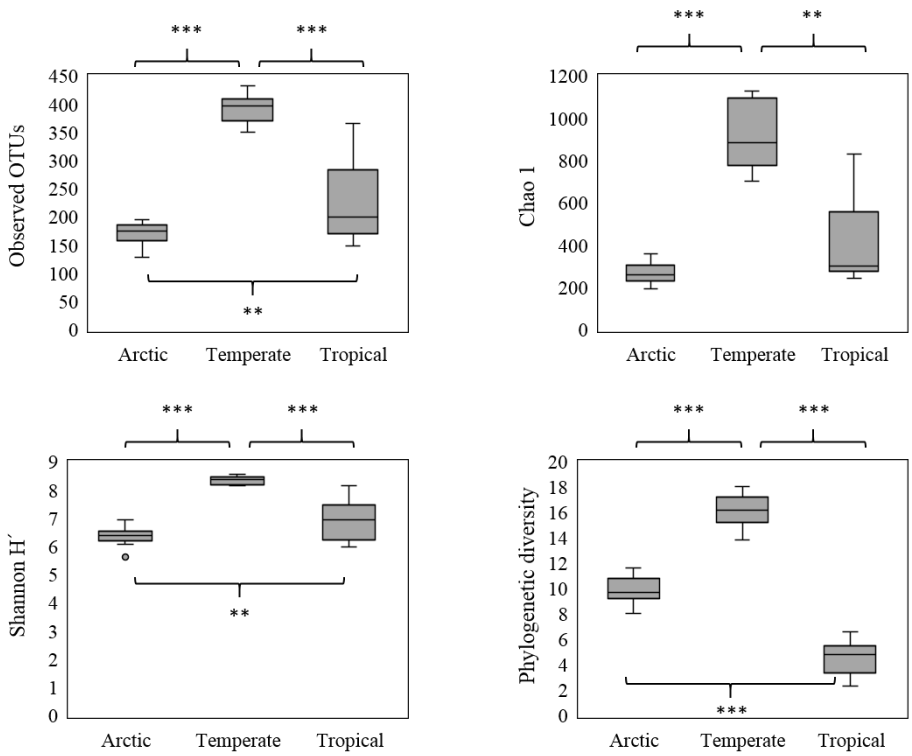


Figure 3.3. Boxplots of alpha diversity indices. Diversity indices represent the randomly selected subsets (n=591) for each sample. Asterisks indicate significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

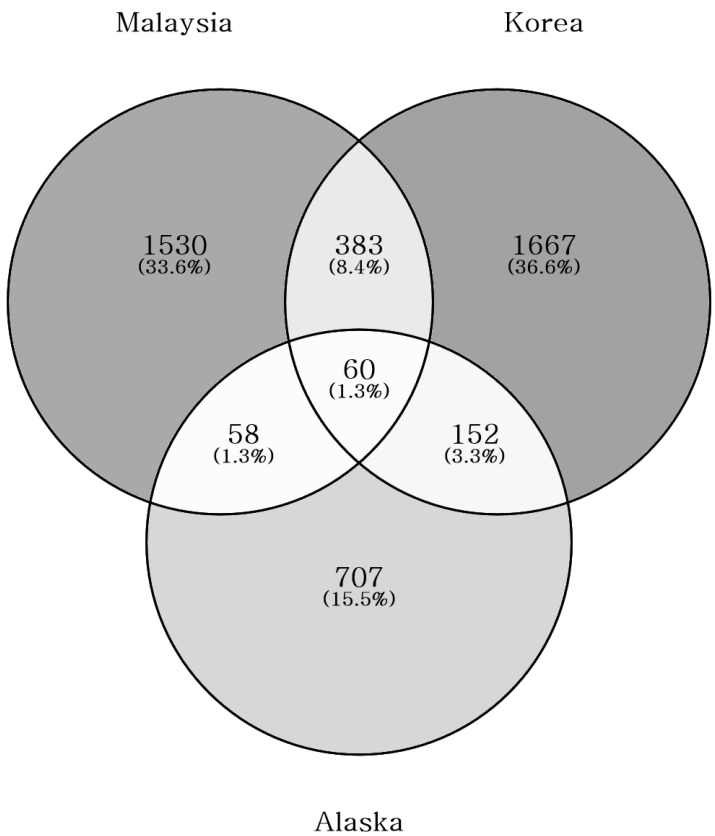
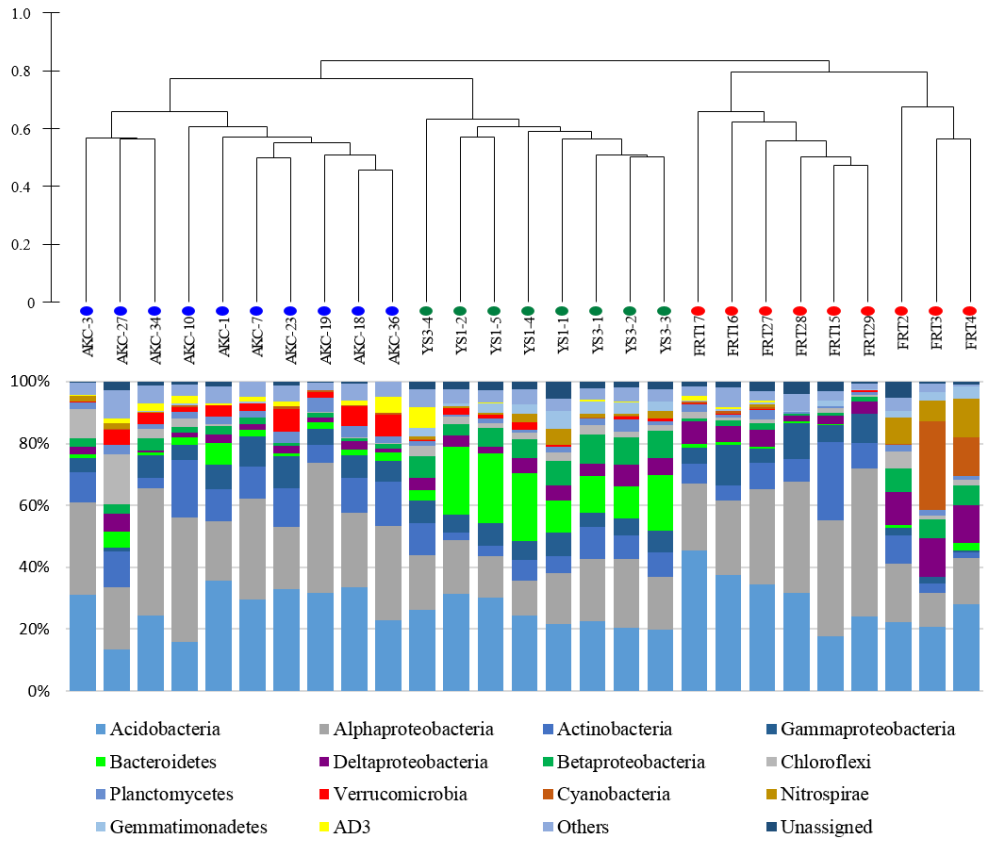


Figure 3.4. Venn diagram illustrating the distribution of OTUs between ecosystems.

Bacterial communities between Arctic and non-Arctic soil

The bacterial community structure was different along latitudinal geographical distance (Figure 3.5). At phylum level, *Acidobacteria* and *Alphaproteobacteria* were predominant in all biomes. However, specific bacterial groups relatively abundant in each biome; the relative abundance of *Verrucomicrobia* and AD3 were dominant in Arctic soil, and *Bacteroidetes* and *Betaproteobacteria* were dominant in Temperate soil, and *Chloroflexi*, *Cyanobacteria*, and *Ntrospirae* were dominant in Tropical soil. This trend was corresponded with the result of NMDS plot for Bacteria based on Bray-Curtis dissimilarities between biomes (Figure 3.6). Bacterial communities were clearly distinguished between biomes. However, three sampling points (TR2, TR3, and TR4) showed distinction within Tropical soil bacterial communities. It might be the influence of soil properties such as soil pH (average 6.41) within FRIM forest (Table 3.1).

I could find that each biome had distinct OTUs (Figure 3.7 and Table 3.2). Although Arctic soil showed relatively low richness and diversity, specific OTUs were relatively dominant (Figure 3.7). Like the structure at phylum level, all soil biomes showed dominant OTUs belonged to *Alphaproteobacteria* and *Acidobacteria*, OTUs belonged *Rhodopanes* was significantly abundant in all biomes. However, some bacterial OTUs significantly abundant in each biome: OTU_1215 (*Sinobacteraceae* of *Gammaproteobaceria*) was much higher in Tropical soil, OTU_658 (*Chitinophagaceae* of *Bacteroidetes*) was much higher in Temperate soil, and OTU_774 (*Sinobacteraceae* of *Gammaproteobaceria*), OTU_106 (AD3), OTU_784 (*Solirubro-bacterales* of *Actinobacteria*) and OTU_1470 (*Acidimicrobiales* of *Actinobacteria*) were much higher in Arctic soil (Figure 3.7).


1.0
0.8
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0.4
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AKC-3
AKC-27
AKC-34
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AKC-1
AKC-7
AKC-23
AKC-19
AKC-18
AKC-36
YS3-4
YS1-2
YS1-5
YS1-4
YS1-1
YS3-1
YS3-2
YS3-3
FRT17
FRT16
FRT27
FRT28
FRT15
FRT29
FRT2
FRT3
FRT4
100%
80%
60%
40%
20%
0%
Acidobacteria
Alphaproteobacteria
Actinobacteria
Gammaproteobacteria
Bacteroidetes
Deltaproteobacteria
Betaproteobacteria
Chloroflexi
Planctomycetes
Verrucomicrobia
Cyanobacteria
Nitrospirae
Gemmatimonadetes
AD3
Others
Unassigned

Figure 3.5. Bacterial phylum distribution between different biomes. The relative abundance of each phylum on different soil samples and all phyla accounting for less 1% in abundance were shown in 'Others'. ● Arctic soils; ● Temperate soils; ● Tropical soils.

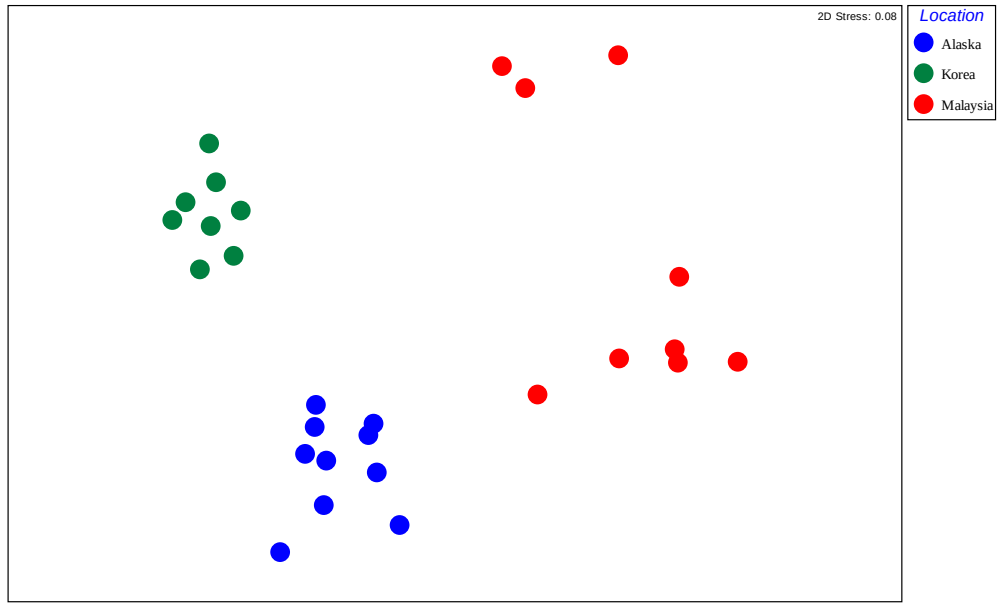

Figure 3.6. A clustering pattern of bacterial communities between sampling locations.

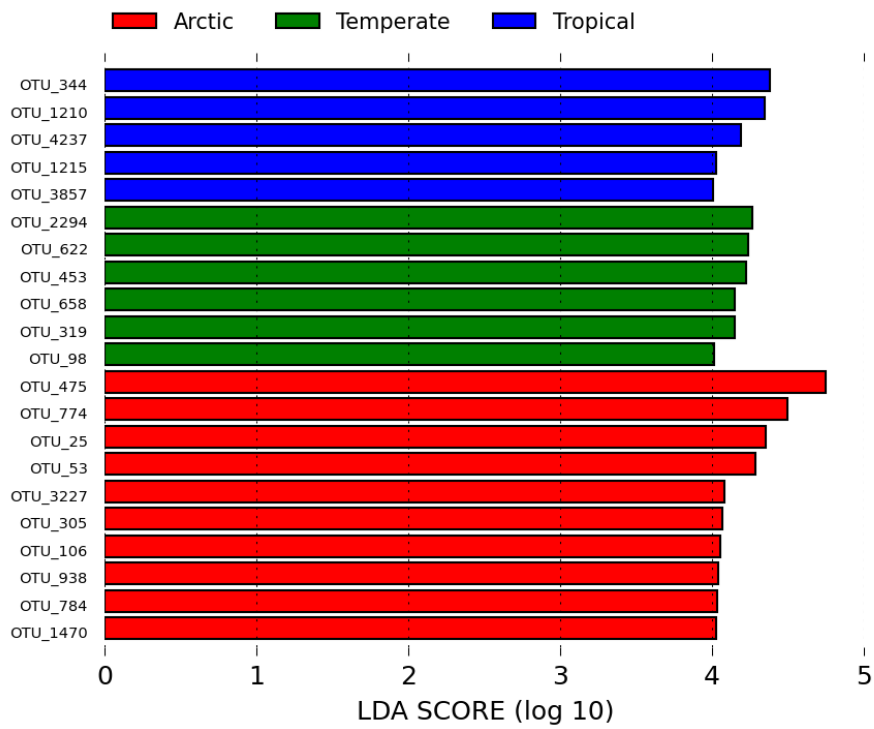

Figure 3.7. Linear Discriminant Analysis Effect Size (LEfSe) analysis showing bacterial OTUs that were significantly differentially abundant between active layer and permafrost, ranked by effect size (all LDA scores >4).

Table 3.2. Significantly different relative abundances of dominant bacterial OTUs from LEfSe analysis between active layer and permafrost with *p*-value

OTU no.	Hierarchical taxonomic level*	*p*-value
Tropical		
OTU_344	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Hyphomicrobiaceae*; g_*Rhodoplanes*; s_	$p < 0.01$
OTU_1210	*Acidobacteria*; c_DA052; o_Ellin6513; f_; g_; s_	$p < 0.01$
OTU_4237	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Koribacteraceae*; g_; s_	$p < 0.01$
OTU_1215	*Gammaproteobacteria*; o_*Xanthomonadales*; f_*Sinobacteraceae*; g_; s_	$p < 0.01$
OTU_3857	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Methylocystaceae*; g_; s_	$p < 0.01$
Temperate		
OTU_2294	*Acidobacteria*; c_Acidobacteria-6; o_iii1-15; f_; g_; s_	$p < 0.001$
OTU_622	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Hyphomicrobiaceae*; g_*Rhodoplanes*; s_	$p < 0.001$
OTU_453	*Alphaproteobacteria*; o_*Rhodospirillales*; f_*Rhodospirillaceae*; g_; s_	$p < 0.001$
OTU_658	*Bacteroidetes*; c_[Saprospirae]; o_[Saprospirales]; f_*Chitinophagaceae*	$p < 0.001$
OTU_319	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Koribacteraceae*; g_Candidatus *Koribacter*; s_	$p < 0.001$
OTU_98	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Koribacteraceae*; g_; s_	$p < 0.001$
Arctic		
OTU_475	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Bradyrhizobiaceae*; g_; s_	$p < 0.001$
OTU_774	*Gammaproteobacteria*; o_*Xanthomonadales*; f_*Sinobacteraceae*; g_; s_	$p < 0.001$
OTU_25	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Hyphomicrobiaceae*; g_*Rhodoplanes*; s_	$p < 0.001$
OTU_53	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Koribacteraceae*; g_Candidatus *Koribacter*; s_	$p < 0.01$
OTU_3227	*Alphaproteobacteria*; o_*Rhizobiales*; f_*Methylocystaceae*; g_; s_	$p < 0.001$
OTU_305	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Acidobacteriaceae*; g_; s_	$p < 0.001$
OTU_106	AD3; c_JG37-AG-4; o_; f_; g_; s_	$p < 0.001$
OTU_938	*Acidobacteria*; c_*Acidobacteriia*; o_*Acidobacteriales*; f_*Koribacteraceae*; g_; s_	$p < 0.01$
OTU_784	*Actinobacteria*; c_*Thermoleophilia*; o_*Solirubrobacterales*; f_; g_; s_	$p < 0.001$
OTU_1470	*Actinobacteria*; c_*Acidimicrobiia*; o_*Acidimicrobiales*; f_; g_; s_	$p < 0.001$

*c_; o_; f_; g_; s_ means class_;order_;family_;genus_;species_

The relationships between bacterial communities and soil properties in Arctic and non-Arctic soil

Canonical correspondence analysis (CCA) was performed to reveal possible relationships between bacterial community composition and environmental parameters (Figure 3.8). CCA results showed that soil properties had the strongest effects on bacterial community. For example, CCA shows a strong positive correlation between Arctic soil bacterial community and soil pH. Temperate soil bacterial community negatively correlated with C/N ratio. And TC, TN and C/N ratio positively correlated with tropical soil bacterial communities, and some bacterial communities negatively correlated with soil pH. The numerical value was confirmed by MANTEL test (Table 3.3). This result indicated that each biome showed different relations between bacterial community and soil properties.

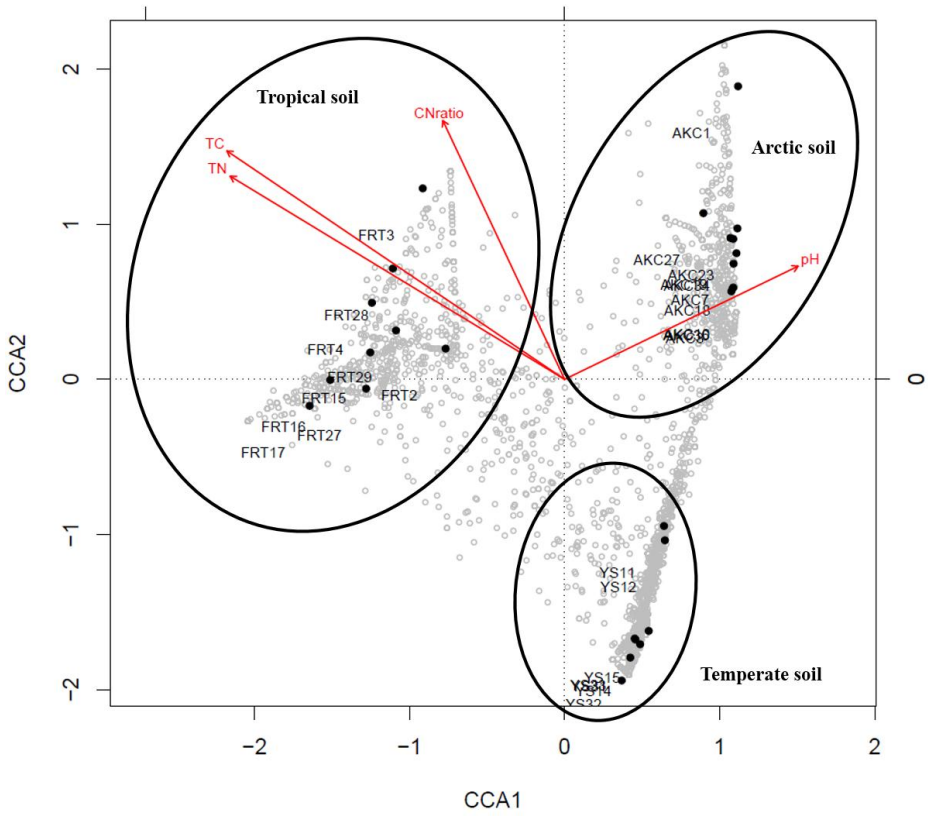

Figure 3.8. CCA showing the correlation of OTU-based bacterial community patterns with soil properties. Arrows indicate the direction and soil properties associated with bacterial community structure between biomes.

Table 3.3. The correlation between soil property and bacterial community in Arctic and non-Arctic soils. The Pearson correlation (*r*) and significance (*p*) determined by Mantel tests

Soil properties	Arctic soil		Temperate soil		Tropical soil	
	r	***p***	***r***	***p***	***r***	***p***
pH	**0.589**	**0.005**	-0.094	0.641	**0.910**	**0.003**
TC	-0.031	0.524	-0.026	0.543	0.264	0.075
TN	0.306	0.068	**0.324**	**0.033**	**0.524**	**0.009**
C/N ratio	0.048	0.376	-0.332	0.922	**0.305**	**0.053**

TC, total carbon content; TN, total nitrogen content; C/N, a ratio of carbon and nitrogen content

3.1.4 Discussion

The bacterial community structure was differentiated with latitudinal geographical distance. This differentiation seems to exert influence on various range of soil properties. Or other unmeasured parameters (e.g. temperature, precipitation, salinity, root exudates, etc.) affect to bacterial community composition. Among the three biomes, anyway, Arctic soil was slightly acidic and a relatively nutrient rich environment compared to the other two biomes, which showed low nutrient conditions.

Nutrient can be a regulator that shapes microbial community structure in resource-poor environments. Temperate and tropical soils were closely related to nutrient content (tropical soil also closely related with soil pH). Both soils were relatively resource-poor environments compared to Arctic soil, with mostly recalcitrant forms of soil carbon (M. Kim, 2013, PhD thesis, Seoul National University). And both temperate and tropical soils showed relatively higher abundance of *Bacteroidetes*, *Betaproteobacteria*, *Nitrospirae*, and *Delta-proteobacteria*, which are well-known for the ability to nutrient cycling such as degradation of complex organic compounds, nitrogen fixing, cycling of iron and sulfur elements (İnceoğlu *et al.*, 2010; Handley *et al.*, 2013; Wang *et al.*, 2013; Lin *et al.*, 2014). Although Arctic tundra soil may be among the most nitrogen (N) limited ecosystems in the world, our study showed no relation between bacterial community and nitrogen content. Rather, soil pH was the main factor that shapes bacterial community structure. However, Arctic soil showed high abundance of *Alphaproteobacteria* and *Gammaproteobacteria*, which prefer higher nutrient conditions, as well as those of other biomes. These results corresponded with other Arctic soil studies (Männistö *et al.*, 2007; Chu *et al.*, 2010). A previous study revealed that the soil pH had greater influence on

bacterial community structure (Männistö *et al.*, 2007). Chu *et al*. (2010) compared bacterial community structure on a global scale, and concluded that bacterial community composition in arctic soil was strongly influenced by local environmental factors associated with soil acidity compared to other factors. However, we still do not know the mechanisms of soil pH that affect microbial community structure in Arctic soil. There is reasonable explanation why soil pH was the main factor of community structure and diversity (Lauber *et al.*, 2009). Soil pH indirectly influences microbial community, which means soil pH is related to a number of soil properties (e.g., nutrient availability, cationic metal solubility, organic C characteristics, soil moisture regimen, and salinity). In other words, the soil properties that are influenced by soil pH can affect and shape microbial community structure and diversity. These results suggested that while bacterial communities in temperate and tropical soils largely contribute to various nutrient cycling, bacterial community in Arctic soil showed high abundance of high nutrient prefer groups such as *Alphaproteobacteria* and *Gammaproteobacteria*, and will actively decompose the nutrients with increasing temperature.

Metabolic functions of bacterial communities were investigated in a given environment by investigating the physiological characteristics of dominant OTUs. Most dominant OTUs in all biomes play an important role in biogeochemical cycle in their habitat. For example, OTU_475 (*Bradyrhizobiaceae* sp.) and OTU_1470 (*Acidimicrobiales* sp.) may contribute to nitrogen cycle (Anderson *et al.*, 2011; Mizuno *et al.*, 2015). OTU_53, 98, 319, 938 and 4237 (*Koribacteraceae* of *Acidobacteria*), which are characterized as acidophilic, may contribute to carbon cycle as degradation of complex plant polymer and CO oxidation (Ward *et al.*, 2009). Although *Actinobacteria* was main bacterial group in all soil biomes,

Actinobacterial OTUs (OTU_784 and 1470) were dominant in Arctic soil. Interestingly, among the dominant OTUs, some bacterial OTUs in Arctic and Tropical soil may contribute to negative feedback in global warming. For example, OTU_3227 and 3857 *Methylocystaceae* (*Alphaproteobacteria*) are identified as methanotroph, which consume methane (Bowman, 2006). These methanotrophs have a symbiotic relationship with methanogen.

In conclusion, this study highlights the different structure of bacterial community and diversity of dominant OTUs between biomes. The bacterial communities correlated differently with soil properties across soil biomes, and perhaps, these relationships make the bacterial community structure of each biome unique. Most dominant OTUs seem to contribute to biogeochemical cycle. In addition, Arctic and Tropical soil contained dominant OTUs, which contribute to the reducing of positive feedback of global warming. Although the sample size was limited, this study might help with advancing an understanding the biogeography of bacterial community at regional scales.

GENERAL CONCLUSIONS

With global warming, Arctic permafrost has been receiving growing scientific attention. There are concerns about the relationship between a considerable amount of carbon deposit in Arctic soil and climate change. Temperature will rise faster in the Arctic, which will consequently cause an intensification in permafrost thawing and the release of long-preserved SOM into the atmosphere via microbial decomposition. Despite the important role of microbes in the decomposition of soil organic matter, their community structure and ecological roles are poorly understood. This study was aimed at investigating the relationship between microbial community structure and environmental factors. The main questions for the study were listed in Chapter 1 and the answers are explained as follows:

How is the microbial community in Arctic soils different from other biomes? The results discussed in Chapter 3 showed a clear difference in bacterial community structure between Arctic soil and other biomes. Generally, *Acidobacteria* and *Alphaproteobacteria* were dominant in Arctic, Temperate, and Tropical soils. However, *Actinobacteria*, *Verrucomicrobia*, and AD3 were relatively higher in Arctic soil. *Actinobacteria* was metabolically active at low temperature and had DNA-repair systems (Johnson *et al.*, 2007), capable of degrading complex organic matter (Yergeau *et al.*, 2010). Perhaps, the physiological characteristics of *Actinobacteria* allows adaptation in Arctic soil. Unfortunately, other relatively abundant groups, *Verrucomicrobia* and candidate phylum AD3, remain undiscovered due to their un-culturability and their lack of physiological characteristics. Thus, further study will be needed using metagenomics analysis.

What are the indigenous microorganisms of Arctic soils? When I observed the dominant OTUs from Arctic soil in comparison with other biomes, I could find the groups including most dominant OTUs distributed across all soil samples. However, I could not clearly identify indigenous bacteria in Arctic soil because the most dominant OTUs were not cultured yet. Although all soil samples shared same bacterial groups at hierarchical high taxonomic level (e. g. family or genus), species may different along biomes. Advancing the culture-dependent techniques, this problem should be solved to identify their physiological and phylogenetical characteristics.

Which soil properties affect microorganisms? The results showed that the soil factors that most influenced microorganisms were the decomposition degree of SOM (substrate availability), soil pH, and total phosphorus. These soil factors showed significant change along soil depth. The decomposition degree of SOM was found to be closely related with the shift of relative abundance of *Chloroflexi*, *Gemmatimonadetes*, *Gammaproteobacteria*, and *Planctomycetes*. The relative abundance of *Acidobacteria* and *Gammaproteobacteria* showed negative relations with increasing soil pH, and *Bacteroidetes* and *Chloroflexi* showed positive relations with increasing soil pH. The relative abundance of *Acidobacteria*, *Alphaproteobacteria*, AD3, *Gammaproteobacteria*, *Bacteroidetes*, *Betaproteobacteria*, and *Chloroflexi* significantly correlated with total phosphorus. However, these relationships were observed in active layer not in permafrost. To identify their relationships in permafrost, more various soil factors such as O_2 content, salinity, temperature, soil elements will be investigated.

How will microbial communities respond to the changing environment? From the results discussed in Chapter 2, the vertical

distribution of microbial community may provide the answer to this question. The relative abundance of *Alphaproteobacteria*, *Gammaproteobacteria*, and MBGA (Marine Benthic Group A) was higher in surface and decreased toward deeper soil layer. The upper soil, which contains a higher amount of nutrients than deeper soil would favor to propagate of copiotrophic groups. Otherwise, *Actinobacteria*, *Bacteroidetes*, *Caldiserica*, *Firmicutes*, and *Methanomicrobia* were dominant in highly decomposed soil, such as permafrost. Other result could support the microbial community structure with soil factors. From the study on the horizontal distribution of bacterial community (Chapter 2.1), the soil properties of site 17 were completely different from those of the other sites. This site composed of mineral layers, and the relative abundance of *Firmicutes* and *Chloroflexi* were relatively higher than other sites. This result indicated that microbes would adapt to substrate availability. For example, the increasing temperature will lead the propagation of copiotrophic groups and allow them to actively decompose the soil organic matter, and make the soil highly mineralized soil. After thriving the copiotrophic microbes, oliotrophic-like microbes may flourish in mineralized soil in active layer as well as permafrost. However, we have to concern about a considerable variable with environmental changes (e. g. vegetation and their exudates, temperature, O_2 condition, hydrological and geological activities, etc.)

This study contributes to advancing understanding of microbial community in moist acidic tundra, Alaska, as well as help in the prediction of their response to warming effect.

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APPENDIX

Table A1. Pearson correlations between soil properties and predicted functional gene categories.

Level 1	Level 3	Depth		pH		TC		TN		TP		C/N ratio	
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
CP	Adherens junction	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
CP	Focal adhesion	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
CP	Tight junction	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
CP	Apoptosis	-0.51	**0.01**	-0.57	**0.002**	0.16	0.42	0.04	0.85	0.24	0.22	0.33	0.09
CP	Cell cycle - Caulobacter	-0.36	0.06	-0.57	**0.002**	0.07	0.71	-0.02	0.92	0.17	0.38	0.21	0.28
CP	Meiosis - yeast	-0.23	0.24	-0.36	0.067	-0.03	0.89	-0.08	0.68	0.06	0.78	0.13	0.52
CP	p53 signaling pathway	-0.51	**0.01**	-0.57	**0.002**	0.17	0.41	0.04	0.84	0.24	0.22	0.33	0.09
CP	Bacterial chemotaxis	-0.36	0.06	-0.56	**0.002**	0.08	0.69	-0.01	0.97	0.20	0.32	0.19	0.34
CP	Bacterial motility proteins	-0.40	**0.04**	-0.60	**0.001**	0.12	0.55	0.03	0.89	0.24	0.23	0.20	0.32
CP	Cytoskeleton proteins	-0.33	0.09	-0.56	**0.002**	0.06	0.76	-0.03	0.86	0.15	0.46	0.21	0.29
CP	Flagellar assembly	-0.47	**0.01**	-0.67	**0.000**	0.18	0.36	0.09	0.66	0.31	0.11	0.22	0.28
CP	Regulation of actin cytoskeleton	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
CP	Endocytosis	0.00	1.00	-0.02	0.912	-0.09	0.65	-0.14	0.48	-0.13	0.53	0.06	0.76
CP	Lysosome	-0.46	**0.01**	-0.71	**0.000**	0.21	0.29	0.12	0.55	0.32	0.10	0.21	0.30
CP	Peroxisome	-0.27	0.17	-0.49	**0.009**	0.01	0.96	-0.07	0.74	0.09	0.65	0.17	0.39
CP	Phagosome	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
EIP	ABC transporters	-0.16	0.42	-0.35	0.072	-0.11	0.58	-0.17	0.39	-0.04	0.84	0.14	0.48

EIP	Bacterial secretion system	-0.41	**0.03**	-0.61	**0.001**	0.12	0.54	0.02	0.91	0.22	0.26	0.23	0.25
EIP	Phosphotransferase system (PTS)	-0.22	0.28	-0.36	0.065	-0.08	0.68	-0.18	0.37	-0.03	0.88	0.23	0.24
EIP	Secretion system	-0.38	**0.05**	-0.58	**0.001**	0.10	0.64	0.00	1.00	0.20	0.31	0.21	0.29
EIP	Transporters	-0.17	0.41	-0.38	**0.051**	-0.10	0.62	-0.16	0.41	-0.02	0.91	0.14	0.50
EIP	Calcium signaling pathway	0.34	0.09	0.12	0.567	-0.27	0.17	-0.22	0.28	-0.26	0.19	-0.13	0.53
EIP	MAPK signaling pathway - yeast	-0.14	0.50	-0.29	0.139	-0.16	0.43	-0.23	0.24	-0.07	0.74	0.16	0.42
EIP	Notch signaling pathway	-0.64	**0.00**	-0.62	**0.001**	0.33	0.10	0.17	0.40	0.36	0.07	0.41	**0.03**
EIP	Phosphatidylinositol signaling system	-0.25	0.20	-0.47	**0.013**	-0.02	0.91	-0.11	0.60	0.07	0.74	0.18	0.37
EIP	Two-component system	-0.35	0.07	-0.57	**0.002**	0.07	0.71	-0.01	0.95	0.18	0.37	0.19	0.34
EIP	VEGF signaling pathway	0.30	0.12	0.30	0.123	-0.31	0.12	-0.29	0.15	-0.23	0.26	-0.10	0.60
EIP	Wnt signaling pathway	-0.64	**0.00**	-0.62	**0.001**	0.33	0.10	0.17	0.40	0.36	0.07	0.41	**0.03**
EIP	Bacterial toxins	-0.41	**0.03**	-0.65	**0.000**	0.15	0.44	0.07	0.75	0.25	0.20	0.20	0.33
EIP	Cellular antigens	-0.39	**0.04**	-0.61	**0.001**	0.15	0.47	0.07	0.75	0.25	0.22	0.18	0.37
EIP	Ion channels	-0.39	**0.04**	-0.61	**0.001**	0.11	0.57	0.02	0.92	0.20	0.31	0.22	0.27
GIP	Chaperones and folding catalysts	-0.31	0.12	-0.54	**0.003**	0.04	0.84	-0.05	0.82	0.14	0.48	0.18	0.36
GIP	Proteasome	0.13	0.52	-0.11	0.597	-0.25	0.20	-0.31	0.12	-0.30	0.13	0.09	0.64
GIP	Protein export	-0.30	0.13	-0.55	**0.003**	0.04	0.84	-0.04	0.84	0.14	0.50	0.18	0.38
GIP	Protein processing in endoplasmic reticulum	-0.17	0.40	-0.38	**0.052**	-0.13	0.51	-0.21	0.28	-0.04	0.84	0.17	0.39
GIP	RNA degradation	-0.33	0.10	-0.56	**0.002**	0.06	0.76	-0.02	0.90	0.16	0.44	0.19	0.35
GIP	Sulfur relay system	-0.25	0.20	-0.49	**0.009**	-0.01	0.98	-0.09	0.67	0.09	0.67	0.17	0.40
GIP	Ubiquitin system	-0.33	0.10	-0.62	**0.001**	0.12	0.55	0.04	0.83	0.21	0.30	0.15	0.45
GIP	Base excision repair	-0.31	0.11	-0.57	**0.002**	0.07	0.73	-0.01	0.96	0.15	0.45	0.18	0.38

GIP	Chromosome	-0.33	0.09	-0.57	**0.002**	0.07	0.73	-0.02	0.92	0.17	0.40	0.19	0.33
GIP	DNA repair and recombination proteins	-0.30	0.12	-0.55	**0.003**	0.05	0.81	-0.03	0.87	0.14	0.49	0.18	0.37
GIP	DNA replication	-0.26	0.20	-0.51	**0.007**	0.00	0.98	-0.07	0.71	0.09	0.65	0.17	0.41
GIP	DNA replication proteins	-0.29	0.14	-0.54	**0.004**	0.04	0.85	-0.05	0.82	0.13	0.52	0.18	0.38
GIP	Homologous recombination	-0.25	0.20	-0.50	**0.008**	0.00	0.99	-0.08	0.68	0.09	0.67	0.17	0.40
GIP	Mismatch repair	-0.31	0.12	-0.55	**0.003**	0.05	0.82	-0.04	0.86	0.14	0.48	0.18	0.37
GIP	Non-homologous end-joining	-0.38	**0.05**	-0.61	**0.001**	0.14	0.49	0.05	0.79	0.22	0.28	0.19	0.34
GIP	Nucleotide excision repair	-0.29	0.14	-0.55	**0.003**	0.05	0.81	-0.03	0.89	0.13	0.51	0.17	0.40
GIP	Basal transcription factors	0.57	**0.00**	0.28	0.160	-0.43	**0.03**	-0.38	**0.05**	-0.56	**0.00**	-0.16	0.43
GIP	RNA polymerase	-0.30	0.13	-0.54	**0.003**	0.04	0.83	-0.04	0.84	0.13	0.51	0.18	0.38
GIP	Transcription factors	-0.37	**0.06**	-0.60	**0.001**	0.09	0.64	0.00	0.98	0.20	0.33	0.20	0.31
GIP	Transcription machinery	-0.35	0.07	-0.59	**0.001**	0.08	0.70	-0.01	0.96	0.18	0.36	0.19	0.33
GIP	Aminoacyl-tRNA biosynthesis	-0.31	0.11	-0.55	**0.003**	0.05	0.81	-0.04	0.85	0.14	0.48	0.18	0.36
GIP	RNA transport	-0.41	**0.04**	-0.63	**0.000**	0.13	0.51	0.04	0.83	0.26	0.19	0.19	0.33
GIP	Ribosome	-0.29	0.14	-0.54	**0.004**	0.04	0.86	-0.05	0.82	0.13	0.52	0.17	0.39
GIP	Ribosome Biogenesis	-0.26	0.19	-0.51	**0.007**	0.01	0.95	-0.07	0.74	0.10	0.61	0.16	0.41
GIP	Ribosome biogenesis in eukaryotes	-0.07	0.71	-0.36	0.068	-0.12	0.56	-0.17	0.38	-0.06	0.77	0.10	0.63
GIP	Translation factors	-0.30	0.12	-0.55	**0.003**	0.04	0.83	-0.04	0.85	0.14	0.48	0.17	0.39
GIP	mRNA surveillance pathway	-0.08	0.70	-0.13	0.504	-0.19	0.36	-0.16	0.42	-0.06	0.76	-0.05	0.80
Metabolism	Alanine, aspartate and glutamate metabolism	0.35	0.07	0.08	0.681	-0.34	0.08	-0.32	0.10	-0.40	**0.04**	-0.09	0.66
Metabolism	Amino acid related enzymes	-0.64	**0.00**	-0.62	**0.001**	0.33	0.10	0.17	0.40	0.36	0.07	0.41	**0.03**
Metabolism	Arginine and proline metabolism	-0.52	0.01	-0.57	**0.002**	0.17	0.39	0.05	0.81	0.25	0.21	0.33	0.09
Metabolism	Histidine metabolism	-0.37	**0.06**	-0.49	**0.010**	0.04	0.83	-0.08	0.70	0.10	0.62	0.31	0.12

Metabolism	Lysine biosynthesis	-0.30	0.13	-0.41	**0.035**	-0.01	0.97	-0.14	0.49	0.04	0.85	0.32	0.11
Metabolism	Lysine degradation	-0.16	0.43	-0.36	0.062	-0.11	0.59	-0.20	0.31	-0.08	0.68	0.22	0.27
Metabolism	Phenylalanine metabolism	-0.52	**0.01**	-0.57	**0.002**	0.17	0.39	0.05	0.81	0.25	0.21	0.33	0.09
Metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
Metabolism	Tryptophan metabolism	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
Metabolism	Tyrosine metabolism	-0.54	**0.00**	-0.76	**0.000**	0.33	0.10	0.25	0.21	0.46	**0.02**	0.17	0.40
Metabolism	Valine, leucine and isoleucine biosynthesis	-0.52	**0.01**	-0.57	**0.002**	0.17	0.39	0.05	0.81	0.25	0.21	0.33	0.09
Metabolism	Valine, leucine and isoleucine degradation	-0.25	0.20	-0.54	**0.003**	0.04	0.83	-0.03	0.89	0.11	0.58	0.15	0.47
Metabolism	Butirosin and neomycin biosynthesis	-0.68	**0.00**	-0.54	**0.004**	0.38	**0.05**	0.21	0.30	0.41	**0.04**	0.46	**0.02**
Metabolism	Caffeine metabolism	-0.51	**0.01**	-0.71	**0.000**	0.24	0.24	0.13	0.52	0.34	0.08	0.25	0.21
Metabolism	Clavulanic acid biosynthesis	-0.42	**0.03**	-0.64	**0.000**	0.16	0.42	0.10	0.64	0.30	0.12	0.15	0.46
Metabolism	Flavone and flavonol biosynthesis	0.45	**0.02**	0.10	0.607	-0.29	0.14	-0.22	0.26	-0.31	0.11	-0.17	0.41
Metabolism	Flavonoid biosynthesis	-0.50	**0.01**	-0.73	**0.000**	0.26	0.18	0.18	0.38	0.38	**0.05**	0.20	0.32
Metabolism	Indole alkaloid biosynthesis	0.13	0.51	-0.02	0.932	-0.33	0.10	-0.34	0.08	-0.25	0.21	0.01	0.95
Metabolism	Isoquinoline alkaloid biosynthesis	-0.52	**0.01**	-0.57	**0.002**	0.17	0.39	0.05	0.81	0.25	0.21	0.33	0.09
Metabolism	Novobiocin biosynthesis	0.30	0.12	0.30	0.123	-0.31	0.12	-0.29	0.15	-0.23	0.26	-0.10	0.60
Metabolism	Phenylpropanoid biosynthesis	-0.31	0.12	-0.52	**0.005**	0.23	0.25	0.25	0.21	0.34	0.08	-0.03	0.87
Metabolism	Stilbenoid, diarylheptanoid and gingerol biosynthesis	-0.43	**0.03**	-0.59	**0.001**	0.14	0.49	0.04	0.86	0.25	0.22	0.24	0.23
Metabolism	Streptomycin biosynthesis	-0.32	0.11	-0.53	**0.004**	0.23	0.26	0.26	0.20	0.34	0.08	-0.06	0.76
Metabolism	Tropane, piperidine and pyridine alkaloid biosynthesis	-0.57	**0.00**	-0.39	**0.044**	0.26	0.18	0.13	0.53	0.35	0.08	0.37	0.06
Metabolism	beta-Lactam resistance	-0.52	**0.01**	-0.57	**0.002**	0.17	0.39	0.05	0.81	0.25	0.21	0.33	0.09
Metabolism	Amino sugar and nucleotide sugar metabolism	-0.40	**0.04**	-0.62	**0.001**	0.12	0.55	0.03	0.89	0.23	0.26	0.21	0.30

Metabolism	Ascorbate and aldarate metabolism	-0.08	0.70	-0.37	0.061	0.12	0.54	0.15	0.46	0.26	0.19	-0.06	0.75
Metabolism	Butanoate metabolism	-0.30	0.13	-0.54	**0.004**	0.05	0.82	-0.03	0.88	0.17	0.39	0.15	0.46
Metabolism	C5-Branched dibasic acid metabolism	-0.22	0.28	-0.44	**0.022**	-0.05	0.82	-0.12	0.54	0.02	0.91	0.17	0.39
Metabolism	Citrate cycle (TCA cycle)	-0.32	0.10	-0.55	**0.003**	0.05	0.80	-0.04	0.85	0.14	0.47	0.20	0.32
Metabolism	Fructose and mannose metabolism	-0.42	**0.03**	-0.59	**0.001**	0.11	0.57	0.01	0.96	0.22	0.28	0.25	0.21
Metabolism	Galactose metabolism	-0.44	**0.02**	-0.56	**0.002**	0.13	0.53	-0.01	0.98	0.17	0.38	0.33	0.09
Metabolism	Glycolysis / Gluconeogenesis	-0.44	**0.02**	-0.60	**0.001**	0.13	0.51	0.02	0.92	0.23	0.24	0.27	0.17
Metabolism	Glyoxylate and dicarboxylate metabolism	-0.48	**0.01**	-0.61	**0.001**	0.16	0.41	0.05	0.82	0.27	0.18	0.29	0.15
Metabolism	Inositol phosphate metabolism	-0.60	**0.00**	-0.72	**0.000**	0.30	0.12	0.14	0.50	0.35	0.07	0.42	**0.03**
Metabolism	Pentose and glucuronate interconversions	-0.31	0.12	-0.55	**0.003**	0.05	0.79	-0.02	0.90	0.15	0.45	0.17	0.41
Metabolism	Pentose phosphate pathway	-0.31	0.11	-0.55	**0.003**	0.05	0.81	-0.04	0.86	0.14	0.47	0.18	0.36
Metabolism	Propanoate metabolism	-0.32	0.10	-0.54	**0.003**	0.05	0.82	-0.04	0.84	0.14	0.49	0.19	0.34
Metabolism	Pyruvate metabolism	-0.35	0.08	-0.58	**0.002**	0.08	0.69	0.00	0.98	0.18	0.37	0.19	0.34
Metabolism	Starch and sucrose metabolism	-0.33	0.10	-0.56	**0.003**	0.06	0.76	-0.02	0.91	0.15	0.44	0.19	0.36
Metabolism	Carbon fixation in photosynthetic organisms	-0.31	0.11	-0.55	**0.003**	0.05	0.81	-0.04	0.86	0.14	0.48	0.18	0.36
Metabolism	Carbon fixation pathways in prokaryotes	-0.30	0.13	-0.54	**0.004**	0.04	0.86	-0.05	0.81	0.13	0.52	0.18	0.37
Metabolism	Methane metabolism	-0.24	0.24	-0.43	**0.024**	-0.02	0.90	-0.10	0.61	0.04	0.86	0.17	0.39
Metabolism	Nitrogen metabolism	-0.36	0.06	-0.56	**0.002**	0.08	0.69	-0.01	0.97	0.17	0.39	0.20	0.31
Metabolism	Oxidative phosphorylation	-0.31	0.12	-0.56	**0.003**	0.05	0.82	-0.04	0.86	0.15	0.47	0.18	0.38
Metabolism	Photosynthesis	-0.31	0.12	-0.50	**0.008**	0.03	0.86	-0.05	0.81	0.11	0.59	0.19	0.34
Metabolism	Photosynthesis - antenna proteins	-0.34	0.09	-0.55	**0.003**	0.07	0.74	-0.02	0.93	0.16	0.43	0.19	0.34
Metabolism	Photosynthesis proteins	-0.24	0.22	-0.48	**0.011**	-0.02	0.92	-0.10	0.63	0.07	0.74	0.16	0.43
Metabolism	Sulfur metabolism	-0.25	0.20	-0.47	**0.013**	0.00	0.98	-0.08	0.69	0.06	0.75	0.17	0.39

Metabolism	Cytochrome P450	-0.60	**0.00**	-0.79	**0.000**	0.34	0.08	0.23	0.25	0.45	**0.02**	0.28	0.16
Metabolism	Peptidases	-0.30	0.13	-0.58	**0.002**	0.06	0.78	-0.02	0.93	0.16	0.43	0.15	0.46
Metabolism	Protein kinases	-0.60	**0.00**	-0.67	**0.000**	0.32	0.10	0.14	0.48	0.32	0.10	0.45	**0.02**
Metabolism	Glycosaminoglycan degradation	-0.52	**0.01**	-0.73	**0.000**	0.26	0.19	0.17	0.40	0.39	**0.05**	0.20	0.31
Metabolism	Glycosphingolipid biosynthesis - ganglio series	-0.44	**0.02**	-0.65	**0.000**	0.16	0.41	0.06	0.76	0.25	0.20	0.23	0.25
Metabolism	Glycosphingolipid biosynthesis - globo series	-0.57	**0.00**	-0.77	**0.000**	0.33	0.09	0.24	0.23	0.45	**0.02**	0.22	0.28
Metabolism	Glycosphingolipid biosynthesis - lacto and neolacto series	0.70	**0.00**	0.52	**0.006**	-0.69	**0.00**	-0.60	**0.00**	-0.64	**0.00**	-0.28	0.16
Metabolism	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	-0.51	**0.01**	-0.69	**0.000**	0.21	0.29	0.10	0.62	0.32	0.10	0.26	0.18
Metabolism	Glycosyltransferases	-0.30	0.13	-0.55	**0.003**	0.04	0.84	-0.04	0.83	0.14	0.48	0.18	0.38
Metabolism	Lipopolysaccharide biosynthesis	-0.48	**0.01**	-0.69	**0.000**	0.22	0.28	0.13	0.52	0.33	0.09	0.20	0.31
Metabolism	Lipopolysaccharide biosynthesis proteins	-0.46	**0.01**	-0.70	**0.000**	0.20	0.31	0.11	0.59	0.31	0.11	0.21	0.28
Metabolism	N-Glycan biosynthesis	-0.13	0.53	-0.27	0.178	-0.16	0.41	-0.24	0.22	-0.12	0.54	0.18	0.36
Metabolism	Other glycan degradation	-0.30	0.13	-0.54	**0.003**	0.04	0.86	-0.05	0.81	0.12	0.55	0.18	0.36
Metabolism	Other types of O-glycan biosynthesis	-0.32	0.10	-0.53	**0.004**	0.04	0.83	-0.05	0.81	0.13	0.52	0.20	0.31
Metabolism	Peptidoglycan biosynthesis	-0.49	**0.01**	-0.70	**0.000**	0.23	0.26	0.14	0.49	0.35	0.08	0.20	0.31
Metabolism	Various types of N-glycan biosynthesis	-0.37	0.06	-0.61	**0.001**	0.11	0.58	0.03	0.88	0.22	0.27	0.18	0.37
Metabolism	Arachidonic acid metabolism	-0.35	0.08	-0.56	**0.003**	0.08	0.70	-0.01	0.96	0.17	0.40	0.20	0.31
Metabolism	Biosynthesis of unsaturated fatty acids	-0.27	0.18	-0.47	**0.013**	0.00	0.99	-0.08	0.70	0.08	0.71	0.17	0.40
Metabolism	Ether lipid metabolism	-0.30	0.13	-0.53	**0.005**	0.02	0.91	-0.06	0.76	0.12	0.55	0.19	0.35
Metabolism	Fatty acid biosynthesis	-0.26	0.19	-0.50	**0.008**	0.00	0.99	-0.08	0.70	0.09	0.66	0.16	0.43
Metabolism	Fatty acid elongation in mitochondria	-0.32	0.10	-0.55	**0.003**	0.05	0.79	-0.03	0.88	0.15	0.46	0.18	0.36
Metabolism	Fatty acid metabolism	-0.40	**0.04**	-0.64	**0.000**	0.14	0.49	0.05	0.80	0.25	0.21	0.19	0.34
Metabolism	Glycerolipid metabolism	-0.27	0.18	-0.50	**0.008**	0.00	1.00	-0.08	0.70	0.09	0.66	0.17	0.40

Metabolism	Glycerophospholipid metabolism	-0.30	0.12	-0.50	**0.008**	0.02	0.93	-0.07	0.74	0.11	0.60	0.20	0.32
Metabolism	Linoleic acid metabolism	-0.37	**0.06**	-0.60	**0.001**	0.09	0.64	0.00	1.00	0.18	0.37	0.21	0.28
Metabolism	Lipid biosynthesis proteins	-0.42	**0.03**	-0.66	**0.000**	0.16	0.43	0.07	0.74	0.27	0.18	0.20	0.31
Metabolism	Primary bile acid biosynthesis	-0.32	0.10	-0.56	**0.003**	0.06	0.78	-0.03	0.89	0.15	0.45	0.18	0.36
Metabolism	Secondary bile acid biosynthesis	-0.29	0.15	-0.50	**0.008**	0.02	0.92	-0.06	0.76	0.10	0.63	0.18	0.36
Metabolism	Sphingolipid metabolism	-0.26	0.18	-0.48	**0.010**	-0.01	0.96	-0.09	0.65	0.07	0.72	0.18	0.37
Metabolism	Steroid biosynthesis	-0.41	**0.03**	-0.65	**0.000**	0.15	0.46	0.06	0.76	0.26	0.20	0.20	0.33
Metabolism	Steroid hormone biosynthesis	-0.28	0.15	-0.50	**0.007**	0.01	0.98	-0.08	0.69	0.10	0.63	0.19	0.34
Metabolism	Synthesis and degradation of ketone bodies	-0.31	0.12	-0.54	**0.004**	0.03	0.87	-0.05	0.81	0.13	0.52	0.18	0.37
Metabolism	alpha-Linolenic acid metabolism	-0.25	0.21	-0.46	**0.015**	-0.03	0.88	-0.11	0.58	0.05	0.79	0.18	0.38
Metabolism	Biotin metabolism	-0.29	0.14	-0.51	**0.006**	0.03	0.87	-0.05	0.82	0.13	0.53	0.17	0.40
Metabolism	Folate biosynthesis	-0.35	0.07	-0.58	**0.002**	0.08	0.68	-0.01	0.98	0.18	0.37	0.20	0.33
Metabolism	Lipoic acid metabolism	-0.39	**0.05**	-0.62	**0.001**	0.11	0.58	0.01	0.94	0.21	0.30	0.21	0.28
Metabolism	Nicotinate and nicotinamide metabolism	-0.16	0.42	-0.23	0.247	-0.11	0.57	-0.14	0.49	-0.08	0.71	0.09	0.65
Metabolism	One carbon pool by folate	-0.40	**0.04**	-0.63	**0.000**	0.11	0.57	0.01	0.94	0.21	0.29	0.23	0.26
Metabolism	Pantothenate and CoA biosynthesis	-0.36	0.07	-0.59	**0.001**	0.10	0.63	0.01	0.97	0.19	0.33	0.20	0.32
Metabolism	Porphyrin and chlorophyll metabolism	0.31	0.11	0.16	0.429	-0.34	0.08	-0.36	0.07	-0.43	**0.03**	0.05	0.81
Metabolism	Retinol metabolism	-0.36	0.07	-0.60	**0.001**	0.10	0.63	0.01	0.96	0.20	0.32	0.19	0.34
Metabolism	Riboflavin metabolism	-0.30	0.13	-0.54	**0.003**	0.03	0.87	-0.05	0.81	0.14	0.49	0.17	0.39
Metabolism	Ubiquinone and other terpenoid-quinone biosynthesis	-0.46	**0.02**	-0.70	**0.000**	0.20	0.33	0.11	0.60	0.31	0.12	0.21	0.30
Metabolism	Vitamin B6 metabolism	-0.46	**0.02**	-0.72	**0.000**	0.23	0.25	0.14	0.48	0.34	0.08	0.19	0.33
Metabolism	Cyanoamino acid metabolism	-0.43	**0.03**	-0.68	**0.000**	0.16	0.43	0.06	0.75	0.27	0.17	0.21	0.30
Metabolism	D-Alanine metabolism	-0.45	**0.02**	-0.51	**0.007**	0.24	0.24	0.14	0.49	0.16	0.41	0.27	0.17

Metabolism	D-Arginine and D-ornithine metabolism	0.00	1.00	0.03	0.894	0.11	0.59	0.07	0.73	-0.17	0.40	0.07	0.71
Metabolism	D-Glutamine and D-glutamate metabolism	-0.39	**0.05**	-0.61	**0.001**	0.11	0.57	0.02	0.91	0.22	0.27	0.20	0.32
Metabolism	Glutathione metabolism	-0.46	**0.02**	-0.68	**0.000**	0.19	0.34	0.09	0.65	0.31	0.11	0.22	0.27
Metabolism	Phosphonate and phosphinate metabolism	-0.44	**0.02**	-0.67	**0.000**	0.18	0.37	0.08	0.68	0.30	0.13	0.21	0.28
Metabolism	Selenocompound metabolism	-0.27	0.18	-0.52	**0.006**	0.01	0.97	-0.07	0.71	0.09	0.66	0.18	0.36
Metabolism	Taurine and hypotaurine metabolism	-0.50	**0.01**	-0.74	**0.000**	0.25	0.20	0.15	0.44	0.37	**0.06**	0.22	0.27
Metabolism	Biosynthesis of 12-, 14- and 16-membered macrolides	-0.29	0.14	-0.54	**0.004**	0.03	0.87	-0.05	0.82	0.13	0.53	0.17	0.41
Metabolism	Biosynthesis of ansamycins	-0.12	0.56	-0.05	0.795	0.25	0.20	0.20	0.33	0.23	0.25	0.17	0.41
Metabolism	Biosynthesis of siderophore group nonribosomal peptides	-0.40	**0.04**	-0.61	**0.001**	0.14	0.50	0.04	0.84	0.23	0.25	0.21	0.28
Metabolism	Biosynthesis of type II polyketide backbone	-0.35	0.07	-0.56	**0.003**	0.07	0.74	-0.03	0.90	0.15	0.44	0.21	0.29
Metabolism	Biosynthesis of type II polyketide products	-0.53	**0.00**	-0.74	**0.000**	0.28	0.16	0.17	0.39	0.38	**0.05**	0.24	0.23
Metabolism	Biosynthesis of vancomycin group antibiotics	-0.34	0.08	-0.57	**0.002**	0.06	0.76	-0.03	0.88	0.16	0.43	0.21	0.30
Metabolism	Carotenoid biosynthesis	-0.59	**0.00**	-0.70	**0.000**	0.38	**0.05**	0.29	0.14	0.55	**0.00**	0.22	0.26
Metabolism	Geraniol degradation	-0.23	0.25	-0.43	**0.024**	-0.03	0.90	-0.10	0.63	0.04	0.85	0.16	0.43
Metabolism	Limonene and pinene degradation	-0.32	0.11	-0.55	**0.003**	0.05	0.82	-0.04	0.84	0.14	0.50	0.19	0.34
Metabolism	Polyketide sugar unit biosynthesis	-0.36	0.06	-0.59	**0.001**	0.10	0.63	0.00	0.98	0.19	0.34	0.20	0.31
Metabolism	Prenyltransferases	-0.30	0.13	-0.44	**0.022**	0.03	0.89	-0.06	0.78	0.09	0.65	0.20	0.31
Metabolism	Sesquiterpenoid biosynthesis	-0.32	0.10	-0.54	**0.004**	0.04	0.82	-0.04	0.83	0.13	0.51	0.20	0.32
Metabolism	Terpenoid backbone biosynthesis	-0.28	0.16	-0.37	**0.055**	-0.01	0.95	-0.11	0.59	-0.02	0.94	0.25	0.21
Metabolism	Tetracycline biosynthesis	-0.64	**0.00**	-0.65	**0.000**	0.27	0.18	0.10	0.63	0.29	0.14	0.47	**0.01**
Metabolism	Zeatin biosynthesis	-0.48	**0.01**	-0.69	**0.000**	0.19	0.35	0.08	0.68	0.30	0.13	0.24	0.22
Metabolism	Purine metabolism	-0.51	**0.01**	-0.69	**0.000**	0.22	0.28	0.10	0.60	0.32	0.10	0.27	0.17

Metabolism	Pyrimidine metabolism	-0.27	0.17	-0.38	**0.052**	-0.07	0.73	-0.16	0.43	0.00	0.99	0.23	0.24
Metabolism	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	-0.32	0.11	-0.52	**0.005**	0.05	0.82	-0.03	0.86	0.12	0.55	0.19	0.35
Metabolism	Aminobenzoate degradation	-0.11	0.60	-0.39	**0.043**	-0.10	0.62	-0.15	0.46	-0.03	0.89	0.09	0.67
Metabolism	Atrazine degradation	-0.25	0.22	-0.45	**0.017**	-0.03	0.88	-0.11	0.58	0.06	0.77	0.17	0.39
Metabolism	Benzoate degradation	-0.31	0.11	-0.55	**0.003**	0.05	0.81	-0.04	0.85	0.14	0.49	0.19	0.35
Metabolism	Bisphenol degradation	-0.27	0.17	-0.52	**0.006**	0.01	0.98	-0.08	0.71	0.10	0.63	0.17	0.39
Metabolism	Caprolactam degradation	-0.29	0.14	-0.53	**0.005**	0.03	0.88	-0.05	0.80	0.12	0.56	0.18	0.37
Metabolism	Chloroalkane and chloroalkene degradation	-0.28	0.16	-0.53	**0.005**	0.03	0.90	-0.05	0.79	0.12	0.56	0.17	0.40
Metabolism	Chlorocyclohexane and chlorobenzene degradation	-0.28	0.15	-0.52	**0.006**	0.02	0.93	-0.07	0.75	0.11	0.59	0.18	0.38
Metabolism	Dioxin degradation	-0.27	0.17	-0.50	**0.008**	0.00	1.00	-0.08	0.68	0.08	0.69	0.18	0.36
Metabolism	Drug metabolism - cytochrome P450	-0.31	0.12	-0.50	**0.008**	0.02	0.93	-0.07	0.74	0.10	0.63	0.20	0.31
Metabolism	Drug metabolism - other enzymes	-0.35	0.07	-0.60	**0.001**	0.10	0.61	0.02	0.91	0.21	0.30	0.18	0.38
Metabolism	Ethylbenzene degradation	-0.28	0.15	-0.53	**0.005**	0.02	0.93	-0.07	0.75	0.11	0.58	0.18	0.37
Metabolism	Fluorobenzoate degradation	-0.21	0.30	-0.45	**0.017**	-0.04	0.84	-0.11	0.58	0.05	0.81	0.14	0.49
Metabolism	Metabolism of xenobiotics by cytochrome P450	-0.24	0.23	-0.47	**0.012**	-0.02	0.91	-0.10	0.61	0.06	0.78	0.17	0.39
Metabolism	Naphthalene degradation	-0.41	**0.03**	-0.63	**0.000**	0.14	0.49	0.05	0.80	0.24	0.22	0.20	0.32
Metabolism	Nitrotoluene degradation	-0.28	0.16	-0.50	**0.008**	0.01	0.97	-0.08	0.71	0.10	0.63	0.18	0.36
Metabolism	Polycyclic aromatic hydrocarbon degradation	-0.40	**0.04**	-0.48	**0.011**	0.08	0.70	-0.05	0.81	0.16	0.42	0.31	0.12
Metabolism	Styrene degradation	-0.32	0.10	-0.57	**0.002**	0.06	0.76	-0.02	0.92	0.16	0.42	0.18	0.38
Metabolism	Toluene degradation	-0.38	**0.05**	-0.56	**0.003**	0.09	0.64	-0.01	0.98	0.18	0.37	0.23	0.25
Metabolism	Xylene degradation	-0.33	0.10	-0.41	**0.034**	0.03	0.88	-0.06	0.75	0.11	0.60	0.23	0.24
OS	Cardiac muscle contraction	-0.34	0.09	-0.58	**0.002**	0.08	0.70	-0.01	0.97	0.17	0.38	0.18	0.36
OS	Vascular smooth muscle contraction	-0.37	**0.05**	-0.58	**0.002**	0.10	0.63	0.01	0.97	0.19	0.35	0.21	0.30

OS	Bile secretion	-0.21	0.29	-0.41	**0.034**	-0.04	0.85	-0.11	0.59	0.02	0.92	0.16	0.43
OS	Carbohydrate digestion and absorption	-0.06	0.76	-0.05	0.795	0.16	0.43	0.10	0.61	0.06	0.76	0.12	0.54
OS	Fat digestion and absorption	-0.22	0.28	-0.43	**0.024**	-0.05	0.79	-0.13	0.53	0.04	0.83	0.15	0.44
OS	Gastric acid secretion	-0.39	**0.04**	-0.60	**0.001**	0.11	0.59	0.02	0.93	0.20	0.31	0.20	0.31
OS	Mineral absorption	-0.56	**0.00**	-0.64	**0.000**	0.27	0.18	0.11	0.59	0.25	0.20	0.42	**0.03**
OS	Pancreatic secretion	-0.43	**0.02**	-0.53	**0.004**	0.09	0.64	-0.04	0.83	0.19	0.35	0.35	0.08
OS	Protein digestion and absorption	-0.39	**0.04**	-0.62	**0.001**	0.12	0.55	0.02	0.92	0.21	0.30	0.22	0.27
OS	Salivary secretion	-0.46	**0.02**	-0.67	**0.000**	0.17	0.39	0.08	0.69	0.29	0.14	0.21	0.29
OS	Adipocytokine signaling pathway	-0.21	0.29	-0.39	**0.044**	-0.04	0.83	-0.11	0.57	0.01	0.97	0.16	0.42
OS	GnRH signaling pathway	-0.18	0.37	-0.34	0.079	-0.08	0.68	-0.16	0.44	-0.04	0.85	0.17	0.40
OS	Insulin signaling pathway	-0.33	0.10	-0.57	**0.002**	0.07	0.72	-0.02	0.93	0.16	0.43	0.20	0.32
OS	Melanogenesis	-0.25	0.21	-0.50	**0.008**	0.00	0.99	-0.08	0.70	0.08	0.67	0.16	0.42
OS	PPAR signaling pathway	-0.30	0.12	-0.15	0.460	0.21	0.29	0.00	0.98	0.13	0.53	0.62	**0.00**
OS	Progesterone-mediated oocyte maturation	-0.24	0.22	-0.49	**0.009**	0.00	0.98	-0.08	0.69	0.08	0.70	0.16	0.43
OS	Renin-angiotensin system	-0.41	**0.03**	-0.63	**0.000**	0.13	0.53	0.02	0.90	0.24	0.23	0.23	0.26
OS	Circadian rhythm - plant	-0.27	0.17	-0.53	**0.005**	0.02	0.92	-0.06	0.78	0.12	0.57	0.16	0.42
OS	Plant-pathogen interaction	-0.30	0.13	-0.54	**0.004**	0.04	0.85	-0.04	0.83	0.13	0.52	0.18	0.37
OS	Aldosterone-regulated sodium reabsorption	-0.29	0.15	-0.54	**0.004**	0.03	0.87	-0.05	0.82	0.13	0.53	0.17	0.39
OS	Endocrine and other factor-regulated calcium reabsorption	-0.05	0.82	-0.02	0.935	0.05	0.82	0.01	0.98	-0.10	0.61	0.10	0.63
OS	Proximal tubule bicarbonate reclamation	-0.25	0.21	-0.43	**0.025**	-0.02	0.94	-0.09	0.66	0.05	0.79	0.16	0.41
OS	Vasopressin-regulated water reabsorption	-0.19	0.33	-0.30	0.124	-0.10	0.60	-0.17	0.39	-0.03	0.87	0.17	0.38
OS	Antigen processing and presentation	-0.24	0.23	-0.42	**0.030**	-0.03	0.89	-0.10	0.63	0.04	0.84	0.16	0.42
OS	Complement and coagulation cascades	-0.26	0.19	-0.38	**0.053**	-0.03	0.88	-0.12	0.56	0.02	0.90	0.21	0.29

OS	Cytosolic DNA-sensing pathway	-0.23	0.25	-0.39	**0.044**	-0.03	0.87	-0.11	0.59	0.02	0.94	0.18	0.38
OS	Fc epsilon RI signaling pathway	-0.34	0.08	-0.52	**0.006**	0.05	0.80	-0.04	0.84	0.14	0.50	0.22	0.28
OS	Fc gamma R-mediated phagocytosis	-0.35	0.07	-0.51	**0.007**	0.06	0.78	-0.03	0.89	0.16	0.44	0.20	0.32
OS	Hematopoietic cell lineage	-0.41	**0.04**	-0.56	**0.002**	0.11	0.60	0.03	0.88	0.22	0.26	0.18	0.37
OS	Leukocyte transendothelial migration	-0.38	**0.05**	-0.51	**0.006**	0.07	0.73	-0.02	0.90	0.16	0.43	0.23	0.24
OS	NOD-like receptor signaling pathway	-0.32	0.11	-0.57	**0.002**	0.08	0.71	0.00	0.98	0.16	0.41	0.17	0.40
OS	RIG-I-like receptor signaling pathway	-0.11	0.57	-0.31	0.113	-0.14	0.49	-0.20	0.32	-0.09	0.66	0.13	0.52
OS	Cholinergic synapse	-0.36	0.06	-0.55	**0.003**	0.07	0.71	-0.01	0.95	0.17	0.39	0.20	0.31
OS	Glutamatergic synapse	-0.34	0.08	-0.47	**0.013**	0.03	0.87	-0.06	0.77	0.12	0.56	0.22	0.27
OS	Long-term depression	-0.25	0.21	-0.42	**0.030**	-0.02	0.91	-0.10	0.62	0.04	0.85	0.18	0.38
OS	Long-term potentiation	-0.29	0.14	-0.47	**0.013**	0.00	1.00	-0.07	0.72	0.10	0.62	0.16	0.43
OS	Neurotrophin signaling pathway	-0.23	0.25	-0.37	0.060	-0.07	0.72	-0.16	0.43	-0.01	0.95	0.20	0.32
OS	Olfactory transduction	-0.37	**0.06**	-0.54	**0.004**	0.09	0.65	0.01	0.94	0.19	0.34	0.18	0.37
OS	Phototransduction	-0.34	0.09	-0.55	**0.003**	0.05	0.79	-0.03	0.87	0.15	0.45	0.19	0.33
OS	Phototransduction - fly	-0.37	0.06	-0.51	**0.006**	0.03	0.90	-0.04	0.84	0.14	0.49	0.17	0.38

CP, Cellular Processes; EIP, Environmental Information Processing; GIP, Genetic Information Processing; OS, Organismal System.

Human disease and Unclassified were omitted.

국문초록(Abstract in Korean)

기후변화 예측 모델에 의하면 지구온난화의 영향은 북극 지역에서 더욱 크게 나타난다고 한다. 이에 따라 현재 북극 지역을 대상으로 다학제적 연구가 활발히 이루어지고 있다. 북극 지역의 토양은 상당량의 탄소가 유기물의 형태로 매장되어 있는데, 지구 온난화의 영향으로 인하여 탄소의 대기 중으로의 유실이 큰 우려가 되고 있다. 왜냐하면 온난화로 동토의 유기물이 미생물 활성에 의해 분해돼 이산화탄소와 같은 가스 형태로 대기 중으로 방출되기 때문이며, 이 기체가 온실기체로서 지구 온난화의 진행 속도를 가속화 시키기 때문이다. 지구 온난화에 의한 동토 환경의 변화에 과학적 관심이 증가함에 따라, 많은 과학자들이 미생물과 탄소 순환에 초점을 맞춘 연구를 진행하고 있다. 그러나 북극 토양을 대상으로 미생물 군집 및 다양성 연구는 타 기후대 지역의 연구에 비해 미비한 실정이다. 따라서 본 연구는 알래스카의 습지 산성 툰드라 토양을 대상으로 미생물 군집 및 이들의 생태학적 역할을 연구하고자 하였다. 뿐만 아니라, 주변 토양 환경 요인과의 관계도 밝히고자 하였다.

제 1 장에서는 북극 환경에 대한 배경지식 및 연구의 필요성을 기술하였다.

제 2 장에서는 전반적인 알래스카 습지 산성 툰드라 토양의 미생물 군집과 이들 군집에 영향을 미치는 환경 요인을 파악하기 위하여 샘플링을 수평 및 수직적으로 접근하여 분석하였다. 알래스카 툰드라 토양은 다양한 식생이 분포함에도

불구하고 표층을 대상으로 박테리아 군집의 수평 분포를 살펴보았을 때, 식생에 따른 군집의 변화는 나타나지 않았으며 오히려 토양의 깊이 및 pH와 밀접한 관련이 있었다. 앞선 수평 분포 연구에서 더 나아가 더 깊은 토양을 세부적으로 살펴보고자 표층에서 동토층까지의 박테리아와 고세균의 군집 구조를 살펴보았다. 박테리아 군집의 수직 분포에 있어 토양의 유기물 분해 정도(decomposition status of soil organic matter (SOM) or substrate availability)가 가장 큰 영향을 미치는 요인으로 분석되었다. 전반적으로 *Acidobacteria*, *Gammaproteobacteria*, *Planctomycetes* 및 WPS-2는 Oi 층에 상대적으로 높은 abundance를 보였고, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, *Verrucomicrobia* 및 AD3는 Oe 층에서 상대적으로 높은 비율로 출현하였다. 그리고 *Actinobacteria*, *Bacteroidetes*, *Caldiserica* 및 *Firmicutes*는 동토의 A층에 상대적으로 높은 비율로 분포하였다. 고세균의 경우, *Crenarchaeota*가 모든 깊이에서 약 80% 이상을 차지하며 우점 그룹으로 나타났다. *Euryarchaeota*는 전체 고세균 군집에서 차지하는 비율이 적었으며 *Euryarchaeota*에 속하는 메탄생성균인 *Methanobacteria*과 *Methanomicrobia*의 abundance가 Oi 층 이하에서 증가하는 경향을 보였다. 이러한 깊이에 따른 미생물 군집의 변화를 살펴봄으로써 활동층과 동토층간의 미생물의 군집의 차이를 확인할 수 있었다. 토양의 유기물 분해 정도의 영향뿐만 아니라 미생물 그룹에 따라 토양의 다양한 요인(e. g. pH, TC, TN, C/N ratio, TP, MC, etc.) 과도 높은 상관관계를 보였다.

제 3장에서는 북극 토양과 타 기후대 토양을 대상으로 박테리아 군집 구조 및 토양 환경 요인과의 관계를 비교

분석하였다. 북극 토양은 타 기후대 토양에 비해 산성을 띠었으며 매우 높은 탄소 함량을 보였다. 온대 및 열대기후 토양은 탄소 및 질소 함량이 매우 적은 환경이었으며 온대 기후대 토양의 pH는 타 기후대에 비해 중성에 가까웠다. 온대 기후의 토양에서 가장 높은 종 풍부도 및 다양성이 분석되었으며, 이와는 반대로 북극 토양에서 가장 낮은 종 다양성 지수를 보였다. 전반적으로 *Acidobacteria*와 *Alphaproteobacteria*는 모든 토양에서 우점하는 양상을 보였으나 *Verrucomicrobia*와 AD3가 북극 토양에서 상대적으로 더 높은 비율로 출현하였고, *Bacteroidetes*와 *Betaproteobacteria*는 온대기후 토양에서, *Chloroflexi*, *Cyanobacteria* 그리고 *Ntrospirae*는 열대기후 토양에서 상대적으로 높은 비율로 출현하였다. 우점하는 OTUs를 살펴보았을 때, 대부분의 우점 OTUs는 biogeochemical cycle에 중요한 역할을 하는 것으로 파악되었으며, 이 중 지구 온난화의 negative feedback 역할을 하는 즉, 메탄을 소비하는 OUT가 북극 토양과 열대 토양에서 높은 비율로 출현하는 것을 확인하였다. 비록 샘플의 수가 제한적이지만, 본 연구는 regional scale의 박테리아 군집의 biogeography를 이해하는데 도움을 줄 수 있을 것으로 여겨진다.

앞서 설명한 연구 결과들은 알래스카 습지 산성 툰드라 토양의 미생물 군집을 보다 더 이해할 수 있도록 정보 제공에 기여할 수 있으며, 아울러 온난화에 대한 미생물 군집의 변화를 예측하는데 도움을 줄 수 있을 것이다.

주요어: 활동층, 동토, 미생물 군집, 토양 층위, soil pH, next generation sequencing (NGS)

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