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SOIL MICROBIOLOGY

Temporal Changes in Soil Bacterial Diversity and Humic Substances Degradation in Subarctic Tundra Soil

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Abstract Humic substances (HS), primarily humic acids (HA) and fulvic acids (FA), are the largest constituent of soil organic matter. In microcosm systems with subarctic HS-rich tundra soil (site AK 1-75; approximately 5.6 °C during the thawing period) from Council, Alaska, the HA content significantly decreased to 48 % after a 99-day incubation at 5 °C as part of a biologically mediated process. Accordingly, levels of FA, a putative byproduct of HA degradation, consistently increased to 172 % during an identical incubation process. Culture-independent microbial community analysis showed that during the microcosm experiments, the relative abundance of phyla *Proteobacteria* (bacteria) and *Euryarchaeota* (archaea) largely increased, indicating their involvement in HS degradation. When the indigenous bacteria in AK 1-75 were enriched in an artificial mineral medium spiked with HA, the changes in relative abundance were most conspicuous in *Proteobacteria* (from 60.2 to 79.0 %), specifically *Betaproteobacteria*-related bacteria. One hundred twenty-two HA-degrading bacterial strains, primarily from the genera *Paenibacillus* (phylum *Firmicutes*) and *Pseudomonas* (class *Gammaproteobacteria*), were cultivated from AK 1-75 and nearby sites. Through culture-dependent analysis with these bacterial isolates, we observed increasing HS-degradation rates in parallel with rising temperatures in a range of 0 °C to 20 °C, with the most notable increase occurring at 8 °C compared to 6 °C. Our results indicate that, although microbial-mediated HS degradation occurs at temperature as low as 5 °C in tundra ecosystems, increasing soil temperature caused by global climate change could enhance HS degradation rates. Extending the thawing period could also increase degradation activity, thereby directly affecting nearby microbial communities and rhizosphere environments.

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Introduction

Arctic tundra is found in Alaska, Greenland, Canada, Europe, and Siberia and is characterized by an extremely cold climate, low precipitation, a short growing season, and a limited supply of nutrients. This region has long, cold winters and short, cool summers, with temperatures varying between −34 °C and + 12 °C. Freezing temperatures result in most soils of Arctic tundra regions to consist of permafrost, which is overlain by an active layer (a hotspot of microbial activity and abundance) that is exposed to seasonal freeze–thaw cycles. Increased temperatures will increase the thickness of this active layer, promoting the availability of large pools of soil organic matter (SOM) for microbial degradation. This would lead to increased emissions of greenhouse gases such as CO_2 and CH_4, thus, accelerating climate change. Therefore, recent studies on Arctic tundra soils have been focused on the microbial communities and their functional potential relating to SOM decomposition in the active layer [1–3].

Humic substances (HS) are ubiquitous natural organic compounds found throughout the environment and are the largest constituent (60–80 %) of SOM; thus, they are considered a key component of terrestrial ecosystems [4, 5]. These substances are resistant to microbial degradation because their macromolecular structures consist of high-molecular-weight compounds [6]. Accordingly, a large amount of HS is stored in cold regions, including the Arctic and Antarctic tundra, due to long-term low levels of microbial degradative activities.

Cold-adapted microbes indigenous to soil are directly and/or indirectly involved in *in situ* biodegradation in cold environments. Their degradative activities and shifts in their community composition have been well reported [7–9], and many bacteria are known to degrade HS under various physiological conditions [10–12]. Most of these bacteria have been isolated by selecting for their degrading abilities *in vitro*, but it is uncertain if these bacteria were actively predominant in cold environments. Moreover, little attention has been given to the ecological impact of HS degradation on the soil microbial community in cold environments.

We hypothesized that indigenous cold-adapted bacteria play a critical role in the HS degradation process in soil because they are both abundant and diverse within this environment, and they presumably possess versatile catabolic pathways for such degradation. Thus, we investigated the soil bacterial community composition by 16S rRNA gene amplicon pyrosequencing and the respective HS degradative capacity of cultivable bacteria isolated from subarctic Alaskan tundra. Our results illustrate the potential microbial-mediated HS degradation in cold terrestrial ecosystems with relation to temperature and phylogenetic diversity.

Materials and Methods

Study Site and Measurement of Soil Temperature

A site for tundra soil sampling (Council, Alaska, USA) was chosen and designated as AK 1-75 (64°50.68′ N, 163°42.69′ W; pH 4.6; total organic carbon, 40.5 %; total nitrogen, 1.9 %; C/N ratio, 21.5). The soil temperature (Ts) was measured at a depth of approximately 20 cm in one site (64°50.63′ N, 163°42.64′ W), which was 75 m away from AK 1-75, using thermocouple probes (T-type, Weather Tech., Korea), a data logger (CR1000, Campbell Scientific, Inc., USA), and a soil power system. Temperatures were recorded from late June to late December in 2011 and from late June to mid-September in 2012.

Soil Sampling and Microcosm Design

Top layer (0–20 cm) AK 1-75 tundra soil containing decaying plant and moss debris was collected on June 29, 2012. The soil was homogenized and stored at−20 °C until used for microcosm experiments. At the Korea Polar Research Institute, the frozen samples were slowly thawed at 5 °C for 4 days, and a small fraction (200 g) was then incubated in a 500-ml beaker at 5 °C for up to 99 days. The beaker was wrapped in plastic to maintain the initial water content of the soil but homogenized with a spatula for aeration every 2 weeks. At 33-day intervals during the incubation, the soil was subjected to HS component extraction and pyrosequencing of bacterial and archaeal 16S rRNA genes.

Extraction and Structural Analysis of HS Components

AK 1-75 soil was completely dried at 45 °C overnight and passed through a 230 mesh-testing sieve (63-μm sieve pore diameter) to remove coarse plant debris and small stones. One gram of the soil was treated with 25 ml of 0.5 N NaOH for 3 h with continuous shaking and then incubated at 4 °C for 12 h. The NaOH extract was separated by centrifugation at 5,500×*g* for 15 min and acidified to pH 2.0 with 5.0 N HCl. The insoluble fraction containing humic acids (HA) was separated from the fulvic acids (FA) fraction by centrifugation and redissolved in 0.1 N NaOH. The final HA and FA solutions were prepared by centrifugation (4,000×*g* for 7 min), from which solid HA or FA was obtained by lyophilization. Gel permeation chromatography was used to detect the changes in molecular mass distribution of HA and FA. The HA and FA solutions (10 mg/ml each) were each filtered through 0.2-μm membrane filters, and 10 μl of filtrate was loaded onto Ultrahydrogel-500 columns (7.8 mm ID×300 mm, Waters, Inc.) linked to a ShodexOHpak SB-804 HQ column (8.0 mm ID×300 mm, Showa Denko America, Inc.) attached to a Hewlett Packard 1100 HPLC apparatus. The flow rate of the mobile phase (degassed water) was 0.5 ml/min, and the absorbance was monitored at OD_{254} with a diode array detector.

Enrichment of HA-Degrading Bacteria

A small fraction (50 g) of refrigerated AK 1-75 soil was mixed with 100 ml mineral salts basal medium (MSB) [13], strongly homogenized by vortexing, and placed at 5 °C overnight. After two low-speed centrifugations (123×*g*, 2 min, 5 °C), 50 ml supernatant was used as the soil indigenous microbe suspension for enrichment culturing with HA that was previously extracted from AK 1-75 soil. The cell suspension (3 ml) was inoculated into a 250 ml Erlenmeyer flask containing 50 ml MSB. Subsequently, HA (approximately 16.2 mg in 0.1 N NaOH) was added, and the solution was incubated at 5 °C for 21 days with shaking. As a control, an additional flask prepared with the same procedure was immediately frozen at −20 °C without incubation. After centrifugation (13,400×*g*, 10 min, 5 °C), the supernatant was filtered through a 0.4-μm cellulose filter, and the filter membrane was mixed together

with the pellet that contained more fine soil particles and enriched microbes. The mixture was subjected to soil DNA extraction and pyrosequencing of PCR-amplified bacterial 16S rRNA genes.

Pyrosequencing and Analysis of Bacterial 16S rRNA Genes

AK 1-75 soil metagenomic DNA was extracted using a soil DNA extraction kit (MO Bio, USA) according to the manufacturer's instructions, followed by PCR amplification using primers targeting the V1 to V3 hypervariable regions of the prokaryotic 16S rRNA genes. The primers used for bacteria were V1-9F (5′-CCTATCCCCTGTGTGCCTTGGCAGTC-TCAG-AC-GAGTTTGATCMTGGCTCAG-3′; underlining indicates the gene-specific section) and V3-541R (5′-CCAT CTCATCCCTGCGTGTCTCCGAC-TCAG-X-AC-WTTAC CGCGGCTGCTGG-3′; the X barcode is uniquely designed for each sample, followed by a common linker). The primers used for archaeal organisms were AV1-21F (5′-CCTATCCC CTGTGTGCCTTGGCAGTC-TCAG-AG-TCCGGTTGAT CCYGCCGG-3′) and AV3-519R (5′-CCATCTCATCCCTG CGTGTCTCCGAC-TCAG-X-GA-GGTDTTACCGCG GCKGCTG-3′). The PCR conditions used for 16S rRNA gene amplification were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and elongation at 72 °C for 90 s. The amplified products were purified using resin columns and subjected to pyrosequencing by Chunlab Inc. (Korea) with a 454 GS FLX Titanium Sequencing System (Roche, USA).

Original sequencing reads were separated by the unique barcodes, from which the barcode, linker, and PCR primer sequences were removed. Individual collections of sequences were depleted of non-16S rRNA sequences and chimeras using HMMER 3.0 and BLAST. The trimmed sequences were assigned to taxonomic categories via alignment with the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net/). For calculation of alpha diversity measures, sequences were clustered and assigned to operational taxonomic units (OTUs) using the CD-HIT algorithm. The OTUs were input to the MOTHUR software platform [14] to generate diversity indices such as rarefaction curves. Taxonomic categories derived from EzTaxon-e were used to calculate the relative abundance (expressed as a percentage) of bacteria at the phylum and class taxa level in the samples.

Temperature-Dependent HA-Degradative Ability of Cultivable Bacteria

One hundred twenty-two cold-adapted bacterial strains, previously isolated for their viability on HA-supplemented MSB plates [12], were tested for their ability to degrade HA during increases in temperature. Bacterial cells from glycerol stocks in 96-well plates were transferred onto MSB agar plates supplemented with HA (extracted from AK 1-75 soil) dissolved in 0.1 N NaOH as the sole carbon source (0.1 % HA final concentration). During incubation at a varying temperature range of 0 °C to 37 °C, the strains were scored for their growth rate.

Results

Measurement of Soil Temperature

The sampling site (AK 1-75) for tundra soil was chosen as a model site to characterize the degradative capacity of HS-degrading bacteria and to examine the changes in their community structure at both low temperatures and during a rise in temperature. According to the meteorological data of the International Arctic Research Center of the University of Alaska-Fairbanks, the annual air temperature at the AK 1-75 site ranges from −30 °C to 20 °C, the ground is snow-covered from the end of November to the beginning of May, and the growing season lasts from middle of June to early September. The Ts at a depth of approximately 20 cm in one location near AK 1-75 ranged from 8 °C to −6 °C from mid-June to late December. During the thawing period, usually from mid-June to early September, Ts ranged from 4 °C to 8 °C in 2011 and 2012, with an average Ts of 5.5 °C (±1.5 °C) and 5.8 °C (±0.9 °C) in 2011 and 2012, respectively (Fig. 1).

HS Contents (HA and FA) in Tundra Soil

Twenty grams of AK 1-75 tundra top layer soil (0 to 20 cm) containing HS was completely dried and treated with 0.5 N NaOH for HS extraction. From the NaOH extracts, amounts of insoluble fraction containing HA and soluble fraction containing FA were 9.5 (±0.8) and 7.8 (±0.5)g which comprised approximately 47 and 39 % (86 % collectively) of the dried soil sample, respectively. These data confirmed that HS are the largest constituent of soil organic matter and that HA content is often higher than FA content within the surface layer of various types of soils [5, 15].

Fate of Soil HS at Low Temperatures

Microcosms were created in a beaker containing 200 g AK 1-75 soil, and the experiment was performed at 5 °C up to 99 days to approximate the annual and thawing period temperatures at the sampling site. HA and FA were extracted from AK 1-75 microcosms and analyzed every 33 days during the incubation (Fig. 2a), and the amount of extracted HA consistently decreased in a time-dependent manner. After 99 days of incubation, initial HA levels (0.86 g/2 g dried soil, *w*/*w*)

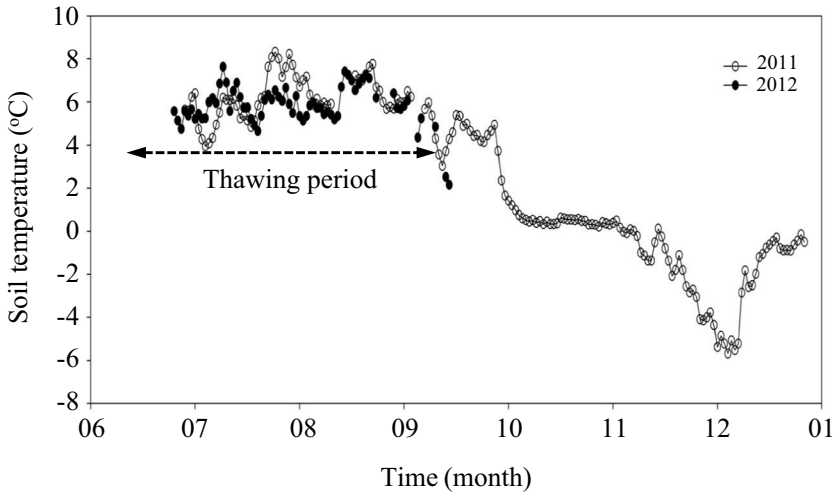

Fig. 1 Temporal variation of soil temperature at a depth of approximately 20 cm. The site for temperature measurement was 75 m away from the sampling site (AK 1-75) for tundra soil

decreased to 48 % (0.41 g), which indicates that some HA were removed through a biologically mediated process; although, it is also possible that HA were degraded in a strictly physical or chemical manner. In contrast, FA content (initially 0.72 g/2 g dried soil) consistently increased until the end of the incubation period, resulting in an overall increase of 172 % (1.24 g), indicative of an accumulation of FA. These data suggest that high-molecular HA are being degraded by indigenous microbes in AK 1-75 soil at low temperatures, resulting in an accumulation of low-molecular FA byproducts that are soluble in water under all pH conditions. Previously, Wunderwald et al. [16] reported that synthetic HA were depolymerized by fungal manganese peroxidase to produce lower molecular weight FA-like substances. Both of these studies indicate microbes degrade HA into FA metabolites.

In addition, gel permeation chromatography was used to detect changes in levels of HA and FA, each of which was separately extracted from the microcosm soil incubated for 99 days at 5 °C (Fig. 2b). When compared with initial HA levels on day 0 (T=0) as a control, HA levels significantly decreased by T=99, with the higher molecular weight peak (15.4–26.4 min at T=0) being almost removed (via degradation). In its place, a lower molecular weight peak (37.0 min at T=99) appeared. In contrast, FA levels increased during the incubation period, resulting in the production of a new, lower molecular weight metabolite peak observed at 42.7 min at T=99.

Phylogenetic Analysis of Microbial Communities at Low Temperatures During Microcosm Experiments

Because microorganisms play a key role in soil organic matter decomposition, the information on their diversity and community composition is of great importance in predicting their associated functions (i.e., the degradation rates and routes for HS). Pyrosequencing of bacterial and archaeal 16S rRNA genes was used to determine the microbial community composition in AK 1-75 tundra soil during the microcosm experiment and the response of the community to low temperatures. Bacterial sequences in samples incubated for 33, 66, and 99 days were clustered into 1,450, 1,151, and 538 OTUs, respectively.

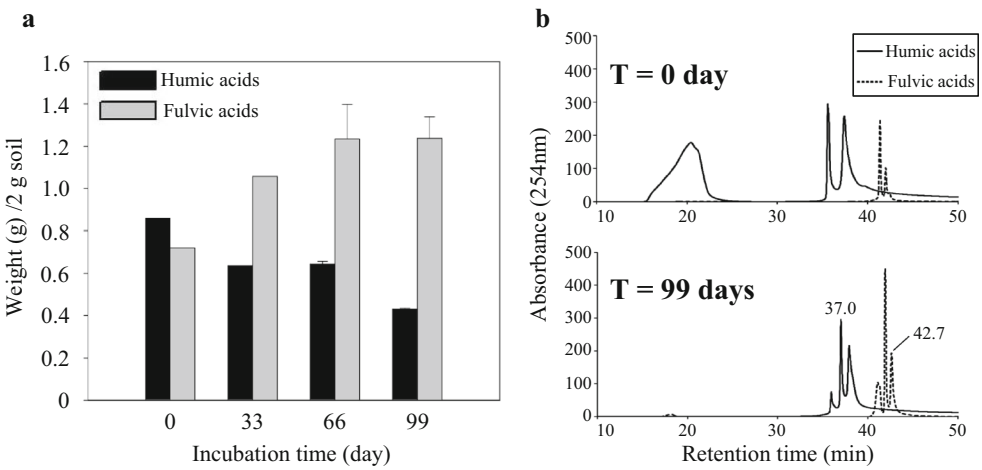

Fig. 2 Time-course changes in HA and FA content determined by direct weighing (**a**) and gel permeation chromatography (**b**), during microcosm experiments at 5 °C. The microcosm was composed of AK 1-75 soil rich in HS. Mean and standard errors are derived from n=1 (0–33 days) or n=3 (66–99 days)

Archaeal sequences were clustered into 596, 1,804, and 769 OTUs, respectively. Generally, there was a rapid decrease in the number of OTUs (especially bacterial community) as the incubation proceeded. Although rarefaction analysis revealed that plateau levels were not reached in the samples, it showed the microbial community response to progression of HS degradation. We observed obvious changes in the bacterial and archaeal community at the phylum level during short-term incubation at temperatures as low as 5 °C. Among *Bacteria* taxa (Fig. 3a), members of the phylum *Proteobacteria* increased in abundance during incubation within soil containing high levels of organic matter, such as HS, which verifies the observation that *Proteobacteria* are more abundant in soils with higher carbon availability [17]. Within the *Proteobacteria*, the abundance of class *Betaproteobacteria* changed most significantly during the incubation period. At the genus level within *Betaproteobacteria*, the abundance of chemolithotrophic iron-oxidizing *Gallionella* increased up to fivefold as incubation time progressed. Obligate methylamine-utilizers *Methylotenera* were first detected after 66 days of incubation. In contrast, other main phyla (oligotrophic *Acidobacteria*, metabolically versatile *Actinobacteria*, and widely distributed *Bacteroidetes*), whose dominant presence and ecological functions in tundra soils have been described [1, 2], responded negatively to an increase in incubation time. Because oligotrophic microorganisms normally thrive in low-nutrient environments, the decreased proportions of *Acidobacteria* in this microbial community might be explained by the consistent increase of low-molecular weight compounds, which act as available substrates for surrounding bacteria. These results show that shifts in taxonomic composition of microbial communities occurred with HS degradation.

In *Archaea* (Fig. 3b), the most abundant phylum after 33 days of incubation was *Euryarchaeota*, followed by *Crenarchaeota*. During incubation at 5 °C, the abundance of the former group consistently increased, but that of the latter group consistently decreased. Most *Euryarchaeota* in this sample were categorized as methanogens. Within *Euryarchaeota*, the abundance of class *Methanomicrobia* changed most significantly. At the genus level within *Methanomicrobia*, the abundance of yet uncultivated AY175392_g increased from 31 % (33 days) up to 55 % (99 days) as the incubation time progressed, while the abundance of anaerobic methanogen *Methanosarcina* and AY175392_f_uc slightly increased. Some results have been previously reported that are in agreement with this observation. Most archaeal sequences from Alaskan permafrost metagenomes were identified as methanogens in *Euryarchaeota* (62–95 %). As the permafrost thawed, the methanogens in metabolically versatile *Methanomicrobia* increased in relative abundance [18]. From the active layer and 2 m-permafrost in the Canadian high Arctic, most of the archaeal communities were composed of *Methanomicrobia* (30 % and 38 %, respectively) and *Methanobacteria* (3 % and 5 %) [19]. Considering methanogenesis from low-molecular-weight compound degradation is an important form of archaeal metabolism, these data indicate that *Euryarchaeota* may also have its own role in HS degradation and mineralization within soil environments. Because there are few reports describing archaeal diversity and ecological functions in the organic layer of tundra regions, our results warrant further study on this group of prokaryotes in the context of HS degradation and their phylogenetic diversity.

Enrichment and Diversity Analysis of HS-Degrading Bacteria

Viable enrichment cultures capable of degrading HS were obtained using MSB artificial mineral medium and AK1-75 soil during a 21-day incubation at 5 °C. Some changes in bacterial diversity were obvious at the phylum level following in the initial enrichment (Fig. 4a). When compared with a control soil (immediately frozen without culturing), the changes in relative abundance were most conspicuous in phylum *Proteobacteria* (from 60.2 to 79.0 %), followed by *Bacteroidetes* (from 10.1 to 19.3 %), indicating a greater abundance of these phyla when compared to the control. Among *Proteobacteria*, the *Betaproteobacteria* were highly

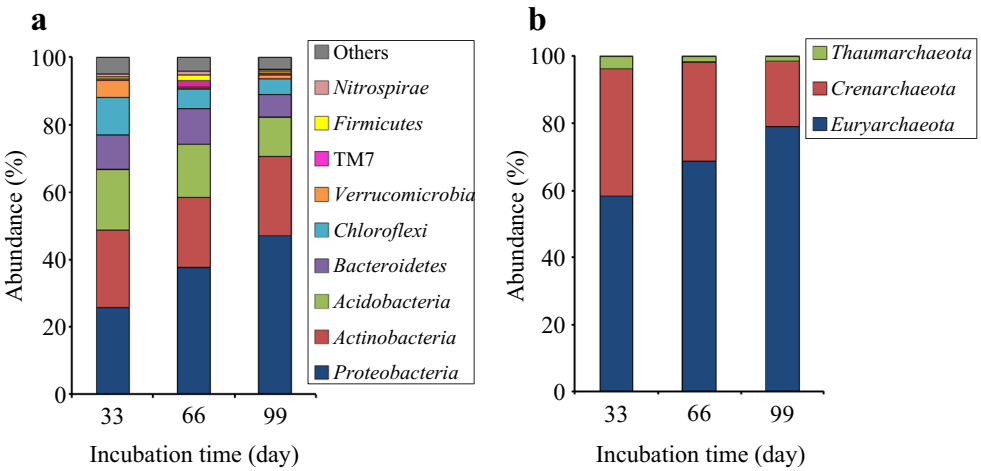

Fig. 3 Time–course changes in the relative abundances of dominant bacterial (**a**) and archaeal (**b**) phyla during the microcosm experiments. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified at the phylum level

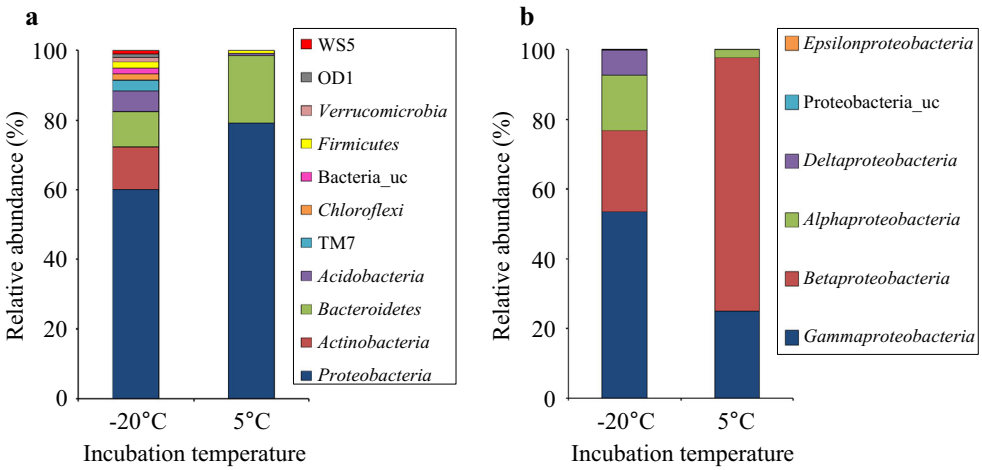

Fig. 4 Relative abundances of bacterial phyla (**a**) and different classes within *Proteobacteria* (**b**). Bacteria were enriched in an artificial minimal medium containing a small fraction of AK 1-75 soil and its HA extract. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified at the phylum or class level

enriched to over 72.7 % relative abundance, suggesting that a few bacterial populations in *Betaproteobacteria* (which could be active in HS degradation) became dominant (Fig. 4b). At the genus level within *Betaproteobacteria*, the abundances of genus *Janthinobacterium* (soil-dwelling bacteria producing a dark-violet color), *Collimonas* (chitinolytic bacteria found in slightly acidic dune soils), and *Glaciimonas* (psychrophile isolated from alpine glacier cryoconite) increased by 287-, 116-, and 54-fold, respectively, when compared to the control sample.

Temperature-Dependent HA Degradative Ability of Cultivable Bacteria

To examine the predominant bacteria involved in HS degradation and subsequent changes in HA degradation rates relative to temperature, 122 cold-adapted bacterial strains were scored for their ability to grow on HA-supplemented MSB plates. They were previously isolated for their ability to degrade HA at 15 °C from AK 1-75 and 32 nearby sites and were identified as primarily *Paenibacillus* spp. and *Pseudomonas* spp. [12] (Fig. 5a). Overall, the number of bacterial strains showing a higher score for their growth rate rapidly increased as the temperature rose to 20 °C; at this temperature and above, the growth rates lowly or sharply decreased (Fig. 5b). More specifically, bacteria with scores of 4 at 4 °C only accounted for 0.8 % of the 122 bacterial strains, yet the number at 20 °C sharply increased to 59.8 % and then slowly decreased again in the range of 25 °C to 30 °C. At 37 °C, 21.3 % of bacteria showed low HA degradative activities, while the remaining 78.7 % of bacterial strains were unable to grow at all. Although this experiment was performed with available isolated bacteria, we predict that an increase in temperature within cold tundra ecosystems could enhance the degradation rate of HS by bacteria and, in turn, affect the diversity of surrounding microbial communities.

Discussion

The total amount of organic carbon in permafrost has been estimated to be 1,672 Pg, which is approximately equal to that in land plants and the atmosphere. Most soils of Arctic tundra regions consist of permafrost, which is overlain by an active layer that has been exposed to seasonal freeze–thaw cycles. The temperature rise will increase the thickness of active layer [3], which will make the tundra regions become more highly responsive and vulnerable to climate change. Tundra ecosystems are important for their high accumulation of organic carbon attributed to low microbial degradative activity at low temperatures. Thus, future global warming may accelerate decomposition of organic matter in the tundra environment [1] by alleviating unfavorable conditions for microbial growth and metabolic activity. Consequently, soil microorganisms could mineralize more organic carbon, such as HS, and significantly affect the carbon dynamics of the tundra ecosystem.

A subarctic tundra soil (AK 1-75) from Council, Alaska was rich in HS (86 % of dried soil) including both higher molecular weight HA and lower molecular weight FA. In microcosm systems with AK 1-75 soil, HA content significantly decreased due to microbial degradation processes for 99 days, even at 5 °C. Fulvic acids, considered a metabolic byproduct of HA degradation, consistently increased during the incubation period (Fig. 2a). The growth rates of 122 cold-adapted HA-degrading bacterial strains isolated from AK 1-75 soil and nearby sites on HA-supplemented medium increased in parallel with temperature changes from a range of 0 to20°C. Interestingly, when examined at a lower temperature range, the HA degradation rate sharply increased at 8 °C compared to 6 °C, which was very similar to the temperature of AK 1-75 soil (Fig. 5b). These data indicate that the temperature rise in cold tundra ecosystems could drastically enhance the degradation rates of HS by bacteria. In addition, the extension of the thawing period could also increase microbial degradation of

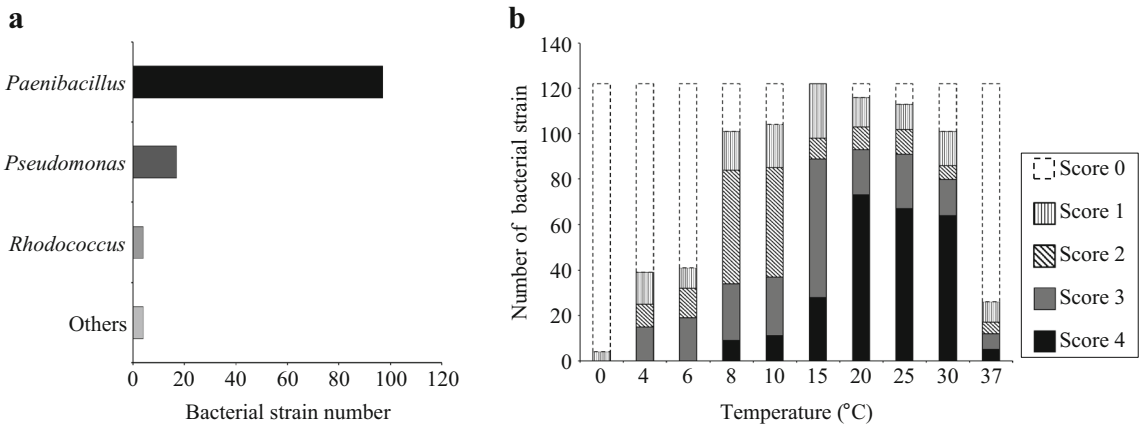

Fig. 5 Taxonomic distribution of HA-degrading indigenous bacteria (**a**) and their HA-degradation rates (**b**) at various temperatures. Bacterial strains were isolated from AK 1-75 soil for the ability to degrade HA and were scored for their degradation abilities in HA-supplemented medium at 0 °C to 37 °C as follows: scores 4, 3, 2, and 1 indicate the formation of a dense colony of 5.0-mm diameter within 3, 6, 9, and 12 days, respectively. A score of 0 indicates no cell growth after 12 days of incubation

HS, resulting in the direct effects on surrounding microbial and rhizosphere communities.

Although soil microbial diversity and metabolic activity in cold environments have gained scientific attention, it is not yet clear exactly how the diversity and activity of cold-adapted microbes are related specifically to HS degradation and overall ecosystem function. Moreover, before our current study, little attention has been paid to the ecological impact of HS on the soil microbial community in natural environments at low temperatures. Further research should be conducted to predict the response of the microbial community and the ecological impact of these responses in the warming global climate. The specific population changes in bacterial communities present in AK 1-75 soil at low temperatures were assessed, and the relative abundance of the predominant bacterial populations was determined. As revealed by pyrosequencing and phylogenetic analyses, community shifts in the microcosm systems showed phylum *Proteobacteria* populations were predominant in the HS-rich microcosm and increased in abundance with increase of incubation time. This indicates that *Proteobacteria* are involved in HS depolymerization at low temperatures *in situ* in Alaskan tundra soil (Fig. 3), which may occur through direct initial HS degradation reactions using extracellular enzymes and/or further metabolism of the HS degradative intermediates from fungi or other soil bacteria. This hypothesis is partially based on many ecological data that fungi, in particular saprotrophic basidiomycetes, produce various extracellular enzymes, such as laccase and peroxidases, which catalyze the breakdown of biopolymers to low-molecular weight organic matter that can then be utilized by other soil microorganisms [2]. Indeed, the abundance of *Proteobacteria*, specifically class *Betaproteobacteria*, was positively affected during HA-degrading enrichment culturing at 5 °C (Fig. 4).

The cultivable bacterial collection, previously isolated for their abilities to degrade HA, was composed of mainly genus *Paenibacillus* (79.5 %) from the class *Bacilli* and genus *Pseudomonas* (13.9 %) from the class *Gammaproteobacteria* [12] (Fig. 5a). We observed a distinct difference between the culture-independent and culture-dependent experiments; for example, *Paenibacillus* strains were barely detected from the microcosm metagenomes, despite their abundance in the enriched cultures (abundance of 0.3 %) and predominance in the culture collection (abundance of 79.5 %). It seems likely that the MSB medium and other conditions used for bacterial enrichment and isolation may have been preferential for *Paenibacillus* species.

In addition, HA and/or HA-derived metabolites may exert a selective pressure on the metabolic activity of the overall soil microbial community, as HA are known to inhibit the enzymatic activities of many microbes [20]. However, the suppression imposed on the microbial community could be overcome by certain populations that possess an arsenal of enzymes with different modes of action, leading to a predominance of *Proteobacteria* in the environment, including classes *Betaproteobacteria* and *Gammaproteobacteria*. Additional experiments could determine how the presence HS and its byproducts may affect microbial diversity and catabolic potential. Further work is needed to evaluate the influence of HS *in situ*, especially at low temperatures. In conclusion, our data regarding the microbial-mediated degradation of HS could allow us to predict the effects of external conditions, such as temperature changes due to global climate change, on the phylogenetic and functional diversity of microbial communities in polar ecosystems.

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