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Diversity and community structure of soil bacterial communities in polygonal tundra soils from Samoylov Island, Lena Delta, Siberia

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Summary. During the Arctic summer, bacteria are active above the permafrost in an environment with sharp temperature and oxygen gradients. The present study addressed the diversity and abundance of bacteria in soil layers near the surface and above the permafrost of the rim and center of a low-centered polygon in the Lena Delta, Siberia. 16S rRNA gene clone libraries revealed the presence of all major soil bacterial groups and of the candidate divisions OD1, OP5, and OP11, and indicated a small-scale heterogeneity of these polygonal tundra soils. The diversity at the top of the elevated polygon rim was significantly different from that of the bottom and from both water-saturated sites of the polygon's center. The overall species-level diversity was very high (Shannon index of 5.3) but varied within the sites and decreased towards the permafrost table, coinciding with decreasing dissolved organic carbon (DOC) and phosphate concentrations. According to the number of operational taxonomical units (OTUs) and cells visualized by fluorescence in-situ hybridization, Bacteroidetes and Actinobacteria were the dominant members of the bacterial community in all sites. Bacteroidetes contributed almost 50% to all Bacteria cells while sequences affiliated with Bacteroidetes/Chlorobi represented on average 23% of all OTUs. Our results provide evidence of the extremely diverse bacterial communities present in permafrost soils and of the influence on diversity of nutrient concentrations, oxygen, and DOC. [**Int Microbiol** 2008; 11(3):XXX-XXX]

Key words: bacterial diversity · clone libraries · nutrient gradients · active layer · tundra · Siberian Arctic

Introduction

Members of the domain Bacteria represent the major group of organisms in soils, with a large number of cells (typically 10^9 bacteria/g soil) and level of diversity [8,48]. According to DNA reassociation kinetics and 16S rRNA gene sequence similarities, the number of bacterial species (per 100 g of soil) is estimated to be in the range of 0.5 to 1 × 10^6 [7,9,47]. Together with archaea and viruses, bacteria are important drivers of biogeochemical processes, such as C, N, and S cycles, and directly influence a wide range of ecosystem functions [40]. Numerous studies have therefore analyzed the composition and diversity of the bacterial community in soils from different environments. Based on more than 30 clone libraries and the results of several other studies using cultivation-independent methods, Alpha-, Beta-, and Gammaproteobacteria, Actinobacteria, Acidobacteria, and, to a lesser extent, Firmicutes, Bacteroidetes, and Planctomycetes have been recently identified as major soil bacterial phyla, although their relative abundances have been found to vary significantly depending on the study site [6,19]. While soil bacterial diversity is influenced by several biotic and abiotic factors [42], it remains unclear whether there are general pat-

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terns of bacterial diversity. Some studies suggest that patterns of bacterial diversity are determined by a few environmental variables. Fierer and Jackson [13], for example, reported that bacterial diversity is related to pH only, and not to temperature or latitude. In contrast, Lozupone and Knight [26] found a strong correlation between bacterial diversity and salinity, but no correlation with pH. These discrepancies stress the importance of studying soil bacterial communities in ecologically significant environments.

The Artic permafrost-affected tundra stores an immense quantity of organic carbon [37,57]. The recent increases in the temperature of the Siberian permafrost have made it susceptible to degradation [35]. The Lena Delta is part of the Siberian Arctic and is located in the zone of continuous permafrost. It is a representative site of wet tundra and is therefore useful in the study of global climate change [The Expedition LENA 2005. Report. http://epic.awi.de/ Publications/Wet2007c.pdf]. Microbial diversity and its influence on carbon dynamics and ecosystem stability in Arctic permafrost-affected soils remain poorly understood [49]. Although the diversity of particular functional microbial groups in Arctic regions, for example, methanogenic archaea [14,16,17] and methanotrophic bacteria has been investigated [20,25,51], analyses of the entire bacterial community are rare. Studies on permafrost communities have evidenced the presence of the major soil bacterial groups, which, at the phylum level, display diversities similar to those of communities from lower-latitude soils [23,45,56]. Investigations focused on species diversities in high Arctic permafrost and tundra soils have thus far been restricted to samples from Norway and Canada [15,35]. In this study, we characterized the diversity of the bacterial community in a low-centered polygon of the Lena Delta, Siberia. These high Arctic tundra wetland polygons feature an exposed rim and a depressed, water-saturated center. To determine microbial heterogeneity and its impact on the soil bacterial community, samples were obtained in the summer from horizons near the surface and at depths above the permafrost representative of extreme habitats.

Material and methods

Study site and soil samples. Samoylov Island (72° 22′ N, 126° 28′ E) is located in the Lena Delta, northeast Siberia, in the zone of continuous permafrost. The morphology of Samoylov is dominated by the typical patterned ground of polygonal tundra, which covers at least 70% of the island's area. Representative of these polygonal tundra environments are low-centered polygons, usually not exceeding 20 m in diameter, which emerge from annual freeze-thaw processes [52]. The low-centered polygon is characterized by steep temperature gradients [24], with temperatures at the surface's upper 5 cm reaching 8–12°C but decreasing to around 0°C near the permafrost table (20–40 cm soil depth). The elevated polygon rim is dominated by sandy material, the depressed center by dead and living biomass (mostly roots of the sedge, *Carex aquatilis*). The organic carbon content in the rim generally does not exceed 3.0% and varies only slightly with depth. Due to high accumulation rates, the organic carbon content is about 16% near the surface of the polygon center but decreases with depth by two thirds [24]. The polygon rim is characterized by oxic conditions (positive redox potentials) near the surface and anoxic conditions (negative redox potentials) near the permafrost table [11]. The center of the polygon is water-saturated throughout the entire active layer [24]. Vertical concentrations of methane vary between the polygon rim and the polygon center. Compared to the rim, methane concentrations are significantly higher within the active layer of the polygon center, where they vertically also fluctuate less than in the rim [24]. Although these polygons display very heterogeneous environmental conditions, they are highly similar among themselves [11].

One such low-centered polygon was sampled in the summer of 2005 (July 7 to September 1) during the expedition LENA 2005 [42, http://epic.awi.de/Publications/Wet2007c.pdf]. Cores (Ø 56 mm) from the low-centered polygon were sampled every 3–4 days. The cores were frozen immediately and were kept frozen until further processing. In this study, two cores, sampled on September 1 from the rim and the center of the polygon, were used for molecular analysis. Two additional cores were used for pore water analysis.

Pore-water extraction and analysis. Cores were thawed and sectioned in slices of 1.5 cm thickness. To prevent the loss of pore water, the slices were placed in Petri dishes which were then sealed airtight, leaving only a tiny access for the insertion of a Rhizon (soil moisture samplers, Rhizosphere Research Products, Wageningen, Netherlands). Pore water extraction with Rhizons was carried out according to the method of Seeberg-Elverfeldt and colleagues [43]. Conductivity and pH were determined with the MultiLab 540 (WTW, Weilheim, Germany). Concentrations of anions and cations were measured with an ion chromatograph (DX320, Dionex, Sunnyvale, CA, USA) and an emission spectrometer (ICP-OES Optima 3000 XL, Perkin-Elmer, Waltham, MA, USA).

Extraction of total DNA. Cores were sectioned under sterile conditions in 1-cm-thick slices. Near-surface and near-permafrost samples of both the polygon rim (6–10 cm and 28–32 cm soil depth) and the polygon center (6–10 cm and 24–28 cm soil depth) were thoroughly homogenized and dispersed into subsamples of 6 × 0.5 g. Total DNA was extracted from four subsamples of each depth. The remaining subsamples were used for fluorescence in-situ hybridization (FISH). Total genomic DNA was extracted with the BIO 101 Fast DNA SPIN Kit for Soil (Qbiogene, Carlsbad, CA, USA), combining heat, detergents, and mechanical force against beads to lyse microbial cells. The large size of the genomic DNA was controlled by electrophoresis on a 1% agarose gel against a Lambda *Eco*RI/*Hin*dIII marker (ABgene, Epsom, UK) with ethidium bromide staining.

PCR amplification. PCR amplification reactions were done in a thermal cycler (iCycler, Bio-Rad, Hercules, CA, USA). PCR reaction mixes (25 µl) contained 1× PCR reaction buffer, 0.2 µM primer, 0.25 µM dNTPs, 1.25 U MasterTaq Polymerase (Eppendorf, Hamburg, Germany) and 0.6–160 ng template. The universal bacterial primers GM3 (5′-AG AGTTTGATCMTGGC-3′), targeting *Escherichia coli* position 8–24, and GM4 (5′-TACCTTGTTACGACTT-3′), targeting *E. coli* position 1492-1507, were used to amplify nearly the entire bacterial 16S rRNA gene [33]. PCR conditions were: 5 min at 94°C (initial denaturation), followed by 20 cycles of 1 min at 94°C, 1 min at 42°C (annealing), and 3 min at 72°C, and by a final elongation at 60°C for 60 min. PCR products (in 96-well plates, ABgene) were purified by centrifugation (980 ×*g* at 4°C) through Multi Screen 96-well plates (Millipore, Billerica, MA, USA) on a column of Sephadex G-50 Superfine powder (GE Healthcare Bio-Science, Göteborg, Sweden).

Construction of clone libraries and sequencing. Purified PCR products were ligated into the linear plasmid vector pCR4 supplied with the TOPO TA Cloning kit for sequencing (Invitrogen, Karlsruhe, Germany) and subsequently transformed via heat-shock into chemically competent *E. coli* cells following the manufacturer's protocol. Cells were incubated overnight at 37°C on agar plates containing 0.05% ampicillin. Colonies were screened by PCR with vector primers for correct size of the insert and the amplicons were directly sequenced. Sequencing was performed with the BigDye Terminator cycle sequencing kit, version 3.1 (Applied Biosystems, Foster City, CA, USA) with vector primers.

Sequence analysis. Sequences were edited with Sequencing Analysis 5.2 (Applied Biosystems) and checked for chimeras with Bellerophon [18] and with the Chimera-Check of the Ribosomal Database Project (Michigan State University). Contigs were assembled with Sequencher 4.7 (Gene Codes, Ann Arbor, MI, USA). Sequences were imported into ARB [www.arb-home.de; www.arb-silva.de] and phylogenetically analyzed. Rarefaction analysis and estimation of diversity indices (Shannon diversity index, Chao1 and ACE richness estimators, and the Simpson evenness index) were performed with DOTUR. Only sequences with > 700 nucleotides were used for diversity analyses. Species-level diversities were based on a definition of species according to operational taxonomic units (OTUs). 16S rRNA gene sequences with ≥ 97% similarity were considered as one OTU. The phylogenetic relatedness between the different sites was determined using the Cluster environment and principal component analysis tools of the UniFrac software package [http://bmf2.colorado.edu/unifrac/index.psp].

Nucleotide accession numbers. The 16S rRNA gene sequences were deposited in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession numbers EU644091 through EU644168 (Rim_Bottom), EU644169 through 644279 (Rim_Top), EU644280 through EU644346 (Centre_Bottom), and EU644347 through EU644445 (Centre_Top).

Fluorescence in-situ hybridization (FISH). FISH was performed on subsamples taken from depths of the polygon rim and center to obtain a quantitative complementation of the clone library analysis. Probes (Cy3-labeled) targeting the main soil bacterial groups were used (Table 1). Fixation of soil samples, hybridization, DAPI staining, and determination of cell counts were carried out as described elsewhere [24].

Results

Habitat description and pore water geochemistry. Pore-water extracts from the polygon rim and center were analyzed to obtain additional habitat information. In the uppermost 5 cm of the polygon rim, concentrations of nitrate, sulfate, and aluminum were constantly <2 mg/l.) In contrast, phosphate concentrations were between 0.85 and 3 mg/l in the uppermost 5 cm of both the polygon rim and the polygon center. With increasing soil depth, phosphate became depleted. In the polygon rim, pH values varied little, between 6.0 and 6.5 (slightly acidic), with depth whereas in the center they increased from 5.6 (slightly acidic) in the

* please check

Table 1. rRNA-targeting oligonucleotide probes used for FISH

Probe	Target group	Sequence (5′-3′) of probe	Target site[a]	FA[b] (%)	Ref.
EUB338	Domain Bacteria	GCTGCCTCCCGTAGGAGT	16S rRNA (338)	0-35	[3]
EUB338 II	Domain Bacteria	GCAGCCACCCGTAGGTGT	16 S rRNA(338)	0-35	[5]
EUB338 III	Domain Bacteria	GCTGCCACCCGTAGGTGT	16 S rRNA(338)	0-35	[5]
NON338	Complementary to EUB338	ACTCCTACGGGAGGCAGC	16 S rRNA	ND	[50]
ALF968	Alphaproteobacteria (except of Ricketsiales)	GGTAAGGTTCTGCGCGTT	16S rRNA(968)	35	[Neef]*
Bet42a	Betaproteobacteria	GCCTTCCCACTTCGTTT	23S rRNA (1027)	35	[28]
Gam42a	Gammaproteobacteria	GCCTTCCCACATCGTT	23S rRNA (1027)	35	[28]
CF319a[c]	Most Flavobacteria, some Bacteroidetes and Sphingobacteria	TGGTCCGTGTCTCAGTAC	16S rRNA (319)	35	[29]
CF319b[c]	Same as CF319a	TGGTCCGTATCTCAGTAC	16S rRNA (319)	35	[29]
CFB719[c]	Most Bacteroidetes, some Flavobacteria and Sphingobacteria	AGCTGCCTTCGCAATCGG	16S rRNA (719)	30	[54]
HGC69a	Actinobacteria (gram-positive bacteria with high G+C content)	TATAGTTACCACCGCCGT	23S rRNA (1901)	25	[38]
LGC354a	Firmicutes (gram-positive bacteria with low G+C content)	TGGAAGATTCCCTACTGC	16S rRNA (354)	35	[32]
LGC354b	Same as LGC354a	CGGAAGATTCCCTACTGC	16S rRNA (354)	35	[32]
LGC354c	Same as LGC354a	CCGAAGATTCCCTACTGC	16S rRNA (354)	35	[32]

ND not determined.
[a]*Escherichia coli* numbering
[b]Percentage (vol/vol) of formamide in the hybridisation buffer
[c]A combination of these probes detected 71.2 % of all Bacteroidetes-Chlorobi cells of this study (according to the 'probe match' function in the ARB software)

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Table 2. Relative abundance and affiliation of bacterial operational taxonomic units (OTUs) detected in a low-centered polygon on Samoylov Island, Lena Delta, Siberia

Phylum	Ratio of OTUs [%]			
	Rim, top (133 seq, 93 OTUs)	Rim, bottom (119 seq, 67 OTUs)	Centre, top (104 seq, 78 OTUs)	Center, bottom (74 seq, 59 OTUs)
Alpha-, Beta-, and Gammaproteobacteria	25.8		9.0	
Deltaproteobacteria	5.3	7.5	11.5	13.6
Bacteroidetes-Chlorobi	20.4	32.8	17.9	18.6
Actinobacteria	16.1	23.9	10.3	16.9
Thermomicrobia		14.9	25.6	27.1
Acidobacteria	9.7			
Verrucomicrobia	5.4			
Firmicutes		6.0		13.6
Others (<5%)	17.3 (OD1, OP11, Planctomycetes, Gemmatimonadetes, Cyanobacteria, Thermomicrobia)	14.9 (OP5, OP11, Verrucomicrobia)	25.6 (OD1, OP11, Firmicutes, Acidobacteria, Planctomycetes, Verrucomicrobia)	10.2 *(Acidobacteria, Verrucomicrobia)*

uppermost centimeters to 7 (neutral) close to the permafrost table. Concentrations of dissolved iron showed a gradient, with increased iron(II) in anoxic habitats. DOC concentrations ranged from 57 to 288 mg carbon/l and decreased drastically below the uppermost 3 cm at both sites.

Diversity of the soil-bacterial community. For the analysis of soil bacterial communities in a low-centered polygon, 430 partial and full-length sequences of >700 nucleotides were obtained. Ten recognized phyla were detected, including members of Proteobacteria (including the sub-

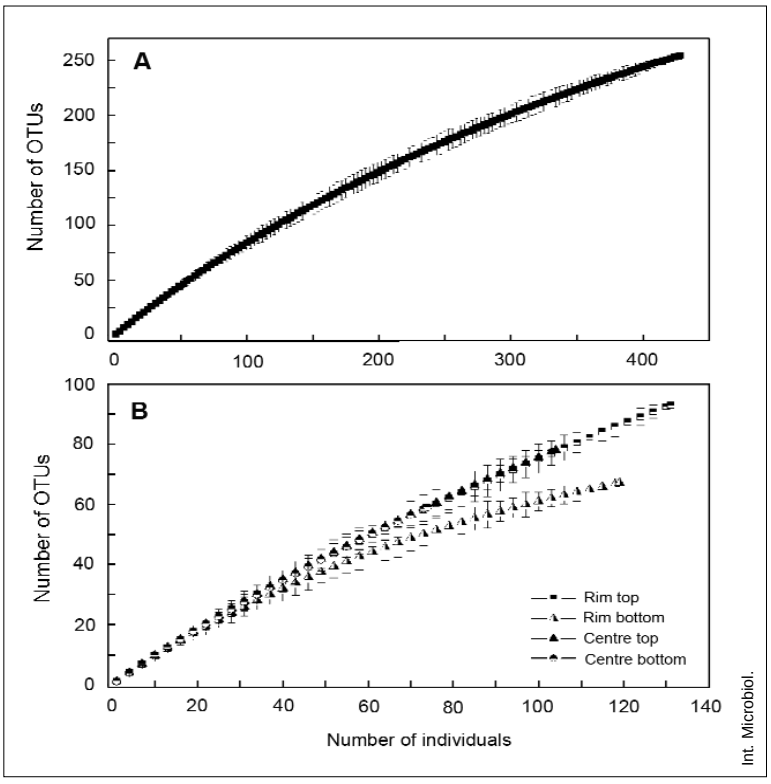

Fig. 1. Rarefaction curves of 16S rRNA gene sequences based on a 97% sequence similarity of the soil bacterial community in a low-centered polygon on Samoylov Island, Lena Delta. Rarefaction curves of (**A**) the total number of sequences and (**B**) the top and bottom of the polygon rim and the polygon center. Only sequences >700 bp were used for rarefaction analysis. Error bars reflect a confidence interval of 95%.

Table 3. Diversity indices of bacterial 16S rRNA gene sequences in active layer samples from Samoylov Island, Lena Delta, Siberia

	Sequence similarity of 97%				
DI[a]	Total	Rim, top	Rim, bottom	Centre, top	Center, bottom
Shannon, min, max[b]	5.3, 5.2, 5.4	4.3, 4.2, 4.5	4.0, 3.9, 4.1	4.2, 4.0, 4.4	3.9, 3.7, 4.1
Simpson	0.005	0.011	0.014	0.009	0.011
Chao1, min, max[b]	427, 368, 515	239, 169, 375	94, 79, 129	209, 142, 344	177, 112, 321
ACE, min, max[b]	465, 397, 567	247, 175, 385	113, 90, 163	301, 176, 325	311, 161, 381

[a]Diversity Index, calculated with DOTUR [41], Neighbor Joining algorithm (distance matrix) with Felsenstein correction [10].
[b]Confidence interval of 95%.

phyla Alpha-, Beta-, Gamma-, and Deltaproteobacteria), Bacteroidetes/Chlorobi, Verrucomicrobia, Planctomycetes, Firmicutes, Cyanobacteria, Thermomicrobia, and the new bacterial phyla Gemmatimonadetes and Acidobacteria [27]. Members of the candidate divisions OD1, OP5, and OP11 were also present. The soil bacterial community was analyzed with respect to the extremes of horizontal and vertical gradients. The top and bottom of both the polygon rim and the polygon center hosted different communities (Table 2). The number of bacterial phyla and subphyla decreased with depth in the polygon rim (12 vs. 8) and in its center (11 vs. 7). Cells affiliated with Bacteroidetes/Chlorobi accounted for on average 22% to the total number of OTUs. Thereby, the majority of OTUs (~60 %) belonged to the Sphingobacteriaceae. Actinobacteria and Thermomicrobia, in particular Chloroflexi, made up on average 17% of all OTUs.

Except for the top of the polygon rim, members of the genera *Clostridium* (phylum Firmicutes), *Intrasporangium*, *Propionibacterium*, and *Rubrobacter* (all Actinobacteria) were universally abundant. Sequences of the ammonia-oxidizing *Nitrosomonas* (Betaproteobacteria), the nitrogen-fixing *Azospirillum* as well as members of the Rhizobiaceae, and Bradyrhizobiacea (all Alphaproteobacteria) were only detected near the oxic surface of the polygon, primarily in the rim.

The overall Shannon index of the soil bacterial community within the low-centered polygon (based on the sum of all

Table 4. Total and cell counts of various soil bacterial groups in a low-centered polygon on Samoylov Island, Lena Delta, Siberia, obtained through DAPI staining and FISH (cell counts are presented per gram wet soil weight)

			Relative to Bacteria [%]				
			CFB group[c](cells/g [10^7]) (mean ± SD)				
Site	Total[a] (cells/g [10^7]) (mean ± SD)	Bacteria[b] (cells/g [10^7]) (mean ± SD)	CF319a/b	CFB719	*Proteobacteria*[d] (cells/g [10^7]) (mean ± SD)	High GC (cells/g [10^7]) (mean ± SD)	Low GC (cells/g [10^7]) (mean ± SD)
Rim							
6–10 cm	51.7 ± 6.21	18.0 ± 0.48	0.76 ± 0.29	4.58 ± 0.95	2.49 ± 0.77	3.14 ± 0.38	0.85 ± 0.23
			4.2	25.5	13.8	17.5	4.7
28–32 cm	11.5 ± 6.21	6.07 ± 2.24	2.97 ± 0.63	2.04 ± 0.06	0.08 ± 0.04	0.78 ± 0	1.65 ± 0.08
			48.9	33.6	1.4	12.9	27.2
Centre							
6–10 cm	26.4 ± 7.00	9.19 ± 1.24	0.25 ± 0.06	0.69 ± 0.35	0.48 ± 0.01	1.8 ± 0.04	1.8 ± 0.01
			4.6	7.5	5.3	19.6	12.7
24–28 cm	8.28 ± 3.35	3.9 ± 0.98	0.82 ± 0.12	0.5 ± 0.04	0.04 ± 0.008	0.96 ± 0.13	0.76 ± 0.15
			22.9	13.9	1.2	26.7	20.9

[a]Obtained through DAPI staining.
[b]Probe mix of EUB 338 I, II, III.
[c]*Cytophaga-Flavobacterium-Bacteroides* group.
[d]Probe mix of ALF968, Bet42a, Gam42a.

sequences) was 5.3 (Table 3). According to the rarefaction curve (Fig. 1A), we under-sampled the low-centered polygon and at least twice as many clones would have been necessary to approach the plateau of the curve (data for extrapolation not shown). Separate comparisons of the four sites showed that the Shannon index varied between 4.3 (top of the polygon rim) and 3.9 (bottom of the polygon rim). Consistent with the phylum level, bacterial diversity at the species level also decreased towards the permafrost table at both sites and rarefaction curves showed a higher species level diversity near the surface than near the permafrost table. This trend, however, was more evident between the near-surface and near-permafrost levels of the polygon rim than at the polygon center (Fig. 1B).

Analyses with the UniFrac software showed that the bacterial communities at the top and bottom of the polygon center were highly similar whereas those of the top of the polygon rim clustered far from the other sites and were significantly different from those of either the center or the bottom of the rim (data of the environmental matrix not shown).

Composition of the bacterial communities. Total (DAPI-stained cells) and bacterial (EUB338-stained cells) cell counts were significantly higher in the rim than in the center of the polygon, in particular in the near-surface horizons. Total cell counts (TCC) in the rim were about five times higher near the surface than near the permafrost table. In the center, TCC near the permafrost table made up around one third of all cells near the surface (Table 4). Members of the Bacteroidetes phylum contributed between 4.2 and 48.9% to all Bacteria-affiliated cells and were particularly abundant near the permafrost tables of the polygon rim and center. According to the probe match tool of the ARB software, a combination of the probes CF319a/b and CFB719 detected 71% of all sequences included in this study and affiliated with the group Bacteroidetes/Chlorobi. Gram-positive bacteria with high and low GC-content were also very abundant and made up between 4.7 and 26.7%, respectively, of all Bacteria cells. Members of the Alpha-, Beta-, and Gammaproteobacteria were highly abundant near the surface of the polygon rim, but their cell counts decreased significantly with depth. While they contributed up to 14% to all bacterial cells near the surface, they made up less than 2% of all Bacteria near the permafrost table.

Discussion

Our study of a low-centered polygon of the Lena Delta, Siberia, showed that the bacterial community in permafrost soils is represented by all major soil bacterial groups, including the new phyla Acidobacteria and Gemmatimonadetes. The bacterial community at the study site was dominated by members of the Bacteroidetes, in particular Sphingobacteriaceae, and to a lesser extent by Actinobacteria. The outstanding abundance and diversity detected here is not surprising, as Sphingobacteriaceae have been predominantly isolated from soils of various environments [22,46] yielding many cultivates that are cold-adapted, psychrophiles, or psychrotrophs [44]. Members of the Sphingobacteriaceae are able to utilize a large number of carbohydrates. Also, relative to other strains, cold-adapted Sphingobacteriaceae are often enriched in either anteiso- or unsaturated cellular fatty acids [30,46], compounds known to enhance the ability of microbial cells to adapt to low temperatures [21,36,53]. Similar features in terms of substrate utilization and cellular fatty-acid composition have been noted in members of the genus *Bacteroides* , for example, in *Bacteroides distasonis* [39], which is closely related to the sequences of our study. We conclude that members of the Bacteroidetes, in particular the Sphingobacteriaceae, are especially capable of surviving under extreme conditions such as those that characterize the active layer of Siberian permafrost soils. The physiological and chemical properties of these bacteria allow them to successfully compete with other soil bacterial groups. The high abundance and diversity of the Bacteroidetes indicate the important contribution of this group to the carbon cycle within the low-centered polygon. Through the delivery of a wide range of low-molecular-mass fatty acids, they may well be of importance for the anaerobic food chain; for example, for fermenting bacteria such as *Clostridium, Intrasporangium,* and *Propionibacterium*, which were almost universally abundant in the sites investigated.

On the species level, the bacterial community in permafrost soils was found to be highly diverse (overall Shannon index of 5.3). This observation is supported by related studies on high Arctic soils from Norway and Canada, in which bacterial diversities that partly even exceeded those of boreal forest soils were reported [15,35]. Our results also point to species-level diversities higher than those of boreal forests and tundra, habitats that are similar to that studied in this work (Shannon indices between 3.0 and 3.2). The considerable distance of Shannon indices found in this work compared to those obtained by Fierer and Jackson, who used T-RFLP instead of clone libraries, most likely not only reflects real variations in diversity but certainly also result from the different methods used for diversity analysis [4].

In addition to a general description of bacterial diversity and composition in high Arctic permafrost soils, we also sought to understand how these variables are influenced by the pronounced gradients of polygonal tundra environments. Several studies have dealt with the influence of depth gradients or landscape (surface soil) patterns on the soil bacterial

community and their results suggested that its composition and biomass are influenced by the amount of nutrients [1,2,12]. In our study, bacterial diversity decreased towards the permafrost table, in particular within the polygon rim. Other studies have reported that broad-scale patterns of bacterial diversity are determined either by salinity [26], soil pH [13], or by sediment mineral chemistry and temperature [31]. The salinity, conductivity, and pH values determined here varied only slightly vertically and thus do not explain the different levels of bacterial diversity observed on the small biogeographical scale used in our study. More-diverse sites near the surface can be distinguished from the less diverse ones near the permafrost table by the presence of oxygen, higher nutrient concentrations, in particular phosphate, higher DOC concentrations, and by large temperature fluctuations over the year, including pronounced freeze-thaw cycles. Also, near-surface horizons are younger than deep active-layer zones. This suggests that, within the low-centered polygon investigated, the availability of nutrients, oxygen, and dissolved organic matter rather than single biotic or abiotic factors regulate soil bacterial diversity. Time might be an additional factor influencing bacterial diversity. It is feasible that due to the external, primarily eolian input of new species the diversity near the surface is higher than at depth while in older active-layer zones successful bacterial groups have been selected over time while others have disappeared.

Members of the sub-phyla of Alpha-, Beta-, and Gammaproteobacteria were only detected near the surface of the low-centered polygon, especially the polygon rim. In addition, their cell counts decreased significantly towards deep active-layer zones. Representative taxa of these groups such as the genus *Azospirillum,* the families Rhizobiaceae, and Sphingomonadaceae, and the order Nitrosomonadales, obtain energy via respiration and require high redox potentials (E_h). During the Arctic summer, the E_h values within low-centered polygons varied between –90 and 0 mV in the water-saturated zone near the permafrost table but reached up to 300–400 mV in dry sites near the surface [11]. We assume that these variations in redox conditions lead to the formation of a phylogenetically different bacterial community near the surface of the polygon rim than at other sites and, in turn, to similar bacterial communities near the surface and near the permafrost table within the entirely water-saturated polygon center—as was shown by the UniFrac analysis. Given that soil respiration (soil CO_2 production) increases exponentially with increasing E_h [54], the redox regime within soils is thought to influence both the composition of the bacterial community and, as a result, soil respiration rates. Due to the continuously rising surface temperatures and degradation of permafrost, dry/wet site ratios will shift in Arctic terrestrial permafrost environments.

According to our study, the extreme temperature regime and widely inhibited decomposition processes characteristic of permafrost soils do not restrict bacterial diversity but rather seem to potentiate it. Moreover, soil bacterial groups other than those reported for more moderate terrestrial habitats dominated in the low-centered polygon studied here. Members of the Bacteroidetes phylum known to be very abundant in cold marine habitats, for example, seem to be extremely capable of competing and surviving under the harsh environmental conditions of Siberian permafrost soils as well. This group of bacteria might therefore primarily contribute to carbon cycling processes in cold habitats in general.

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