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ORIGINAL ARTICLE

Distinct microbial communities associated with buried soils in the Siberian tundra

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Cryoturbation, the burial of topsoil material into deeper soil horizons by repeated freeze–thaw events, is an important storage mechanism for soil organic matter (SOM) in permafrost-affected soils. Besides abiotic conditions, microbial community structure and the accessibility of SOM to the decomposer community are hypothesized to control SOM decomposition and thus have a crucial role in SOM accumulation in buried soils. We surveyed the microbial community structure in cryoturbated soils from nine soil profiles in the northeastern Siberian tundra using high-throughput sequencing and quantification of bacterial, archaeal and fungal marker genes. We found that bacterial abundances in buried topsoils were as high as in unburied topsoils. In contrast, fungal abundances decreased with depth and were significantly lower in buried than in unburied topsoils resulting in remarkably low fungal to bacterial ratios in buried topsoils. Fungal community profiling revealed an associated decrease in presumably ectomycorrhizal (ECM) fungi. The abiotic conditions (low to subzero temperatures, anoxia) and the reduced abundance of fungi likely provide a niche for bacterial, facultative anaerobic decomposers of SOM such as members of the *Actinobacteria*, which were found in significantly higher relative abundances in buried than in unburied topsoils. Our study expands the knowledge on the microbial community structure in soils of Northern latitude permafrost regions, and attributes the delayed decomposition of SOM in buried soils to specific microbial taxa, and particularly to a decrease in abundance and activity of ECM fungi, and to the extent to which bacterial decomposers are able to act as their functional substitutes.

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Introduction

Northern latitude terrestrial ecosystems are key components in the global carbon (C) cycle (McGuire *et al.*, 2009) with permafrost-affected soils storing more than twice as much C as is currently contained in the atmosphere (IPCC, 2007; Tarnocai *et al.*, 2009). Cryoturbation processes driven by repeated freeze–thaw cycles lead to the subduction of soil organic matter (SOM) from the surface into

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deeper soil layers. Cryoturbated soils (Turbels, according to the Soil Classification Working Group, 1998, or Turbic Cryosols, according to the World Reference Base for Soil Resources (WRB), Deckers *et al.*, 2002) contain more than one-third of the arctic soil organic carbon (SOC) (~581 Gt C; Tarnocai *et al.*, 2009). Although buried soil horizons contain similar amounts (g^{-1} dry weight) of C and nitrogen (N) as topsoils, radiocarbon (^{14}C) dating revealed that the C pool in these soil horizons is much older than in unburied topsoil horizons, indicating that decomposition processes are strongly retarded (Kaiser *et al.*, 2007; Hugelius *et al.*, 2010). Cryoturbation therefore is an important mechanism for long-term C storage in these soils (Davidson and Janssens 2006; Bockheim 2007). Northern latitude permafrost regions are particularly vulnerable to climate change, being exposed to the globally strongest increase in mean annual surface air temperatures (4–8 °C, IPCC, 2007). Longer frost-free vegetation periods and increased active layer depths are predicted to promote the availability of large SOC pools for microbial decomposition, leading to increased greenhouse gas emissions from these soils and thereby accelerating climate change (Schuur *et al.*, 2008; Tarnocai *et al.*, 2009; Schuur and Abbott 2011).

Besides physicochemical parameters like temperature, pH, moisture, oxygen availability, soil mineral assemblage and quality of the SOM, the microbial community structure and the accessibility of SOM to the decomposer community are thought to be crucial factors in the process of SOM accumulation and storage in soils (Schmidt *et al.*, 2011; Dungait *et al.*, 2012). The main gaps in our current understanding of SOM stabilization in buried soil horizons and its vulnerability to decomposition are however the structure and the SOM degradation capacities of the microbial community. Recent studies on arctic soils focused on microbial communities in the active layer and the underlying permafrost and targeted their functional potential (Yergeau *et al.*, 2010; Tveit *et al.*, 2012), their response to permafrost thaw (Mackelprang *et al.*, 2011) and related community dynamics to the availability of SOC (Waldrop *et al.*, 2010; Coolen *et al.*, 2011). We expanded on these studies by specifically addressing the microbial communities in cryoturbated soils, and particularly the ones associated with buried topsoils. We aimed to dissect differences in community composition and associated potential functions to identify microbial indicators for the retarded decomposition of SOM in buried soil horizons. To estimate microbial abundances and assess differences along the soil depth profiles, we quantified bacterial, archaeal and fungal SSU rRNA genes from 85 soil samples that were collected from three different vegetation zones in the Siberian tundra. These samples comprised topsoil, subsoil and buried topsoil as well as mineral soils sampled from frozen ground below the active layer (hereafter called permafrost samples). We further characterized the microbial communities in a subset of 36 samples using prokaryotic SSU rRNA gene amplicon sequencing and pyrosequencing of the fungal Internal Transcribed Spacer (ITS) region. Changes in community structure and phylogenetic variability were linked to changes in abiotic soil properties, thus allowing us to propose potential key microorganisms involved in the degradation of SOM in buried soils.

Materials and Methods

Field site description, soil sampling and sample preparation

Soils were collected in August 2010 in northeast Siberia in a transect along the upper Kolyma river (Cherskii, Republic of Sakha, Russia). The study site covered the bioclimatic subzones E and D (Walker *et al.*, 2005), also called southern and typical tundra subzone in the Russian classification, respectively. Three sampling sites were chosen with respect to their dominant vegetation, classified as shrubby grass tundra, shrubby tussock tundra and shrubby lichen tundra (Table 1). Soils were sampled from triplicate soil pits for each of the sampling sites, resulting in a total of 85 soil samples (Table 1, Supplementary Table S1). Soil pits were 5 m in length and up to 1 m deep depending on the depth of the permafrost table (Supplementary Figure S1). After soil pits were classified into soil horizons, samples were collected using alcohol-sterilized knives. To obtain a representative sample from each soil horizon, at least three samples were pooled horizon-wise along the length of each soil profile. Organic and mineral topsoil horizons (O and A) were taken from the uppermost active layer and mineral subsoil horizons (B and C) and as well as buried topsoils (Ojj and Ajj) were sampled from deeper parts of the profile. Permafrost samples were obtained by coring (1–2 cores per soil profile, length: ~30 cm), using a steel pipe pushed into the frozen ground of the soil pits (Hugelius *et al.*, 2010). Initial soil processing included removal of living roots prior to homogenizing the soil fraction of each sample. Samples for extracting soil nucleic acids were fixed in RNA*later* RNA Stabilization Reagent (Ambion, Life Technologies, Carlsbad, CA, USA) and kept cold until further processing. Samples for the analyses of soil context data (as summarized in Supplementary Table S1) and enzyme activity potentials (cellobiohydrolase, endochitinase, N-acetylglucosaminidase, leucine aminopeptidase, phenoloxidase and peroxidase) were stored in closed polyethylene bags at 4 °C until analyzed. For details on analytical methods and the determination of enzyme activity potentials please refer to Supplementary Material and methods.

Table 1 Overview on sampling sites, replicate soil pits and soil samples

Site	*Dominant vegetation*	*Replicate plots*	*Coordinates*	*Active layer depth (cm)*	*Number of samples*[a]
Shrubby grass tundra	*Betula exilis*, *Salix phenophylla*, *Carex lugens*, *Calamagrostis holmii*, *Aulacomnium turgidum*	A	69.44°N, 161.71°E	55–65	6 (2/2/2/0)
		B	69.44°N, 161.71°E	45–63	10 (3/3/2/2)
		C	69.43°N, 161.72°E	33–63	10 (3/3/3/1)
Shrubby tussock tundra	*Eriophorum vaginatum*, *Carex lugens*, *Betula exilis*, *Salix pulchra*, *Aulacomnium turgidum*	D	69.45°N, 161.75°E	48–68	9 (3/3/2/1)
		E	69.44°N, 161.75°E	38–55	8 (3/3/2/0)
		F	69.44°N, 161.76°E	43–63	11 (4/3/2/2)
Shrubby lichen tundra	*Betula exilis*, *Vaccinium uligonosum*, *Flavocetraria nivalis*, *Flavocetraria cucullata*	G	68.75°N, 161.59°E	35–63	10 (3/3/3/1)
		H	68.75°N, 161.59°E	43–68	8 (3/2/3/0)
		I	68.75°N, 161.60°E	33–58	13 (3/3/5/2)

[a]Total number of soil samples from the respective soil pit. Numbers in brackets indicate soil samples from the four different soil horizons (topsoil/subsoil/buried topsoil/permafrost).

Nucleic acid extraction, purification and quantification
Nucleic acid extractions were conducted according to a modified bead-beating protocol (Urich *et al.*, 2008, see Supplementary Material and Methods) and further purified using the CleanAll DNA/RNA Clean-up and Concentration Micro Kit (Norgen Biotek Corp., Ontario, Canada). Total DNA was quantified using SybrGreen (Applied Biosystems, Life Technologies, Carlsbad, CA, USA; Leininger *et al.*, 2006).

Quantification of bacterial, archaeal and fungal SSU rRNA genes by quantitative PCR
Bacterial and archaeal small subunit (SSU) rRNA genes were amplified with the primer set Eub338Fabc/Eub518R (Daims *et al.*, 1999; Fierer *et al.*, 2005) for *Bacteria* and Arch519F/Arch908R (Jurgens *et al.*, 1997; Teske and Sorensen 2007) for *Archaea*. Fungal SSU genes were amplified using the primers nu-SSU-0817-5′ and nu-SSU1196-3′ (Borneman and Hartin, 2000). Product specificity was confirmed by melting point analysis and amplicon size was verified with agarose gel electrophoresis. Detection limits for the various assays (that is, lowest standard concentration that is significantly different from the non-template controls) were <100 gene copies for each of the genes. Samples, standards and non-template controls were run in triplicates. Please refer to Supplementary Materials and methods for details. Throughout this paper, the abundance of SSU rRNA genes will be used instead of the abundance of organisms, because such a calculation is based on a theoretical average number of functional gene copies per microorganism.

Barcoded amplicon sequencing of bacterial and archaeal SSU rRNA genes on the Illumina GAIIx platform and sequence analysis
Sample preparation was performed as described by Caporaso *et al.* (2011). Each sample was amplified in triplicate, combined and cleaned using the Ultra Clean htp 96-well PCR clean up kit (MO BIO Laboratories, Solana Beach, CA, USA). The PCR primers (515F/806R) targeted the V4 region of the SSU rRNA, previously shown to yield accurate phylogenetic information and to have only few biases against any bacterial taxa (Liu *et al.*, 2007; Bates *et al.*, 2011; Bergmann *et al.*, 2011). Amplicons were sequenced on the Illumina GAIIx platform (Illumina Inc., San Diego, CA, USA). Quality filtering of reads was applied as described previously (Caporaso *et al.*, 2011). Reads were assigned to operational taxonomic units (OTUs, cutoff 97% sequence identity) using a closed-reference OTU picking protocol using QIIME (Caporaso *et al.*, 2010). Alpha diversity metrics and community relatedness (principal coordinate analysis, from unweighted Unifrac distances) were calculated after rarifying all samples to the same sequencing depth. For further details please refer to Supplementary Materials and methods.

Pyrosequencing of fungal ITS genes and sequence analysis
The ITS region of the fungal rRNA gene was amplified in triplicates from each sample using bar-coded primers ITS1F (Gardes and Bruns 1993) and ITS2 (White 1990). ITS-amplicons ($\sim$280 base pairs) were sequenced on half a picotiter plate of the GS FLX+ system (Roche, Basel, Switzerland) using Titanium chemistry at GATC Biotech (Konstanz, Germany). Initial read quality filtering was performed using mothur 1.27 (Schloss *et al.*, 2009) and overlapping regions of 18SrDNA, 5S and ITS2 region were trimmed using the ITS1 region extractor of the UNITE online pipeline (Abarenkov *et al.*, 2010). *De novo* and database-based chimerical sequence removal, OTU-picking and taxonomic assignment were performed using the QIIME bioinformatic pipeline (Caporaso *et al.*, 2010). Details on the procedure can be found in Supplementary Materials and methods.

Statistical analyses to evaluate the linkage between variability of soil context data and microbial community structure

Principal component analysis was used to summarize the soil context data (Supplementary Table S1) and the prokaryotic community structure (relative abundance of bacterial and archaeal taxa, Supplementary Table S2). After data standardization (zero mean and unit s.d.) and centered log-ratio transformation with multiplicative zero replacement (Aitchison, 1982; Martín-Fernández *et al.*, 2003; Jørgensen *et al.*, 2012), community structure and soil context data were effectively reduced to a single variable (PC1 score). To test for relationships between community structure and soil context data, Spearman rank-order correlations (ρ) were determined between the first principal component (PC1) scores of the relative abundance and the geochemical data. To assess the correlation between variability of the community structure and soil context data, Spearman's ρ was computed between PC1 scores from the relative abundance data and the original soil context data. To further test the community–environment relationships, we applied a unimodal type of constrained ordination, canonical correspondence analysis (CCA). Soil context parameters (explanatory variables, Supplementary Table S1) were related to differences in prokaryotic and fungal community structure (that is, changes in relative OTU abundances; response variables). The contribution of each explanatory variable was tested using forward selection and the Monte-Carlo permutation test within the CCA framework (Canoco v5; Ter Braak and Šmilauer, 2012).

Further statistical analyses

To determine significant differences in gene abundances, gene abundance ratios, taxa abundances (bacterial/archaeal, phyla and OTUs) and enzyme activity potentials between soil horizons (topsoil, subsoil, buried soil and permafrost), one-way ANOVA followed by Tukey's HSD test was performed. Differences were considered significant at P-values <0.05. Prior to one-way ANOVA, Shapiro–Wilk tests and Bartlett tests were used to examine whether the conditions of normality and homogeneity of variance were met and followed by log-transformation (gene abundances) or square-root transformation (taxa abundances). Pearson's product–moment correlation (R) was used to assess linkages between individual taxa, gene abundances and enzyme activities as well as between geochemical data and diversity measures. All analyses were performed in R 2.15.0 (R Development Core Team; Team RDC, 2012). The STAMP bioinformatic package (Parks and Beiko, 2010) was used to determine significant differences in the relative abundance of fungal taxa between topsoils, subsoils and buried soils (Welch's test, two-sided, P-values <0.05).

Data deposition

The SSU rRNA gene sequence data are available from MG-RAST (project numbers 44668685.3–44668734.3). Fungal raw reads were deposited in NCBI Sequence Read Archive (SRA) under the submission ID SUB282590 and BioProject ID PRJNA209792.

Results

Soil characteristics

Major soil properties varied significantly between soil horizons (Table 2). Total organic carbon (TOC), total nitrogen (TN), carbon-to-nitrogen (C/N) ratios and water content were highest in topsoils and lowest in subsoils, whereas pH was highest in subsoils. Buried topsoils were lower in pH than in subsoils, and showed higher TOC and TN values as well as higher C/N ratios and water content (Table 2).

Table 2 Soil context data and potential enzyme activities in topsoils, buried topsoils and subsoils[a]

	Topsoils	*Subsoils*	*Buried topsoils*
Soil chemical data			
pH	5.3 ± 0.4 (c)	6.1 ± 0.6 (a)	5.8 ± 0.4 (b)
TOC (% dry weight)	15.5 ± 11.7 (a)	1.3 ± 0.8 (c)	10.4 ± 5.7 (b)
TN (% dry weight)	0.7 ± 0.5 (a)	0.1 ± 0.0 (b)	0.6 ± 0.3 (a)
C/N ratio	20.2 ± 6.8 (a)	11.5 ± 1.8 (c)	16.2 ± 2.1 (b)
Water content (%)	49.8 ± 22.2 (a)	17.2 ± 1.3 (b)	40.8 ± 9.3 (a)
Potential activities (nmol h^{-1} g^{-1} dry soil)			
Hydrolytic enzymes[b]	1572.3 ± 867.6 (a)	85.4 ± 61.9 (c)	481.4 ± 352.2 (b)
Oxidative enzymes[c]	9894.5 ± 5548.8 (a)	5092.9 ± 3539.7 (b)	13 480.9 ± 6069.1 (a)

[a]Mean and standard deviations. Small letters indicate significant differences ($P<0.05$) between soil horizons as determined by one-way ANOVA and Tukey's HSD test. Number of samples from topsoils/buried topsoil/subsoils: 27/24/25 (chemical data) and 16/22/18 (enzyme activities). Detailed information on soil chemical data is available in Supplementary Table S1.
[b]Sum of potential activities of all hydrolytic enzymes measured (cellobiohydrolase, endochitinase, N-acetylglucosaminidase, leucine aminopeptidase).
[c]Sum of potential activities of all oxidative enzymes measured (phenolxidase, peroxidase).

Principal component analysis analysis on a comprehensive data set of soil properties (Supplementary Table S1) placed buried topsoil horizons at an intermediate position and supported the finding that buried topsoil horizons were highly variable with regard to their soil properties, resulting in a shared ordination space with unburied topsoil and subsoil horizons (Supplementary Figure S2).

High prokaryotic abundances accompanied by a reduction of fungal abundance in buried soil horizons

Quantitative PCR (qPCR) was used to estimate numbers of bacterial, archaeal and fungal SSU rRNA genes from a total of 85 samples. Figure 1 shows abundances for one representative soil profile for each vegetation zone. Detailed soil horizon profiles and quantification results for individual soil pits can be found in Supplementary Figures S3A–C. Average bacterial SSU gene abundances were highest in topsoil samples (3.5×10^{10} genes g^{-1} soil) and decreased with depth, based on the mean of all vegetation types and biological replicates. However, abundances were much higher in buried topsoil horizons (9.4×10^{9} genes g^{-1} soil) than in the surrounding subsoils (3.7×10^{8} genes g^{-1} soil, one-way ANOVA, $P<0.01$). Average fungal SSU gene copy numbers were significantly higher in topsoils (6.4×10^{10} genes g^{-1} soil) than in buried topsoils and subsoils (8.1×10^{7} and 1.7×10^{8} genes g^{-1} soil, respectively, one-way ANOVA, $P<0.01$). In permafrost samples, bacterial SSU rRNA gene copies ranged from 0.1×10^{6} to 3.8×10^{6} genes g^{-1} dry soil. Fungal SSU genes could only be amplified from a single permafrost sample and accounted for 0.4×10^{6} genes g^{-1} dry soil. The mean ratio between fungal and bacterial SSU rRNA gene copies (fungal:bacterial ratios, FB) was lowest in buried topsoils (FB = 0.03) resulting from a decrease in fungal SSU genes compared with the topsoil samples (FB = 5.5, one-way ANOVA, $P<0.05$).

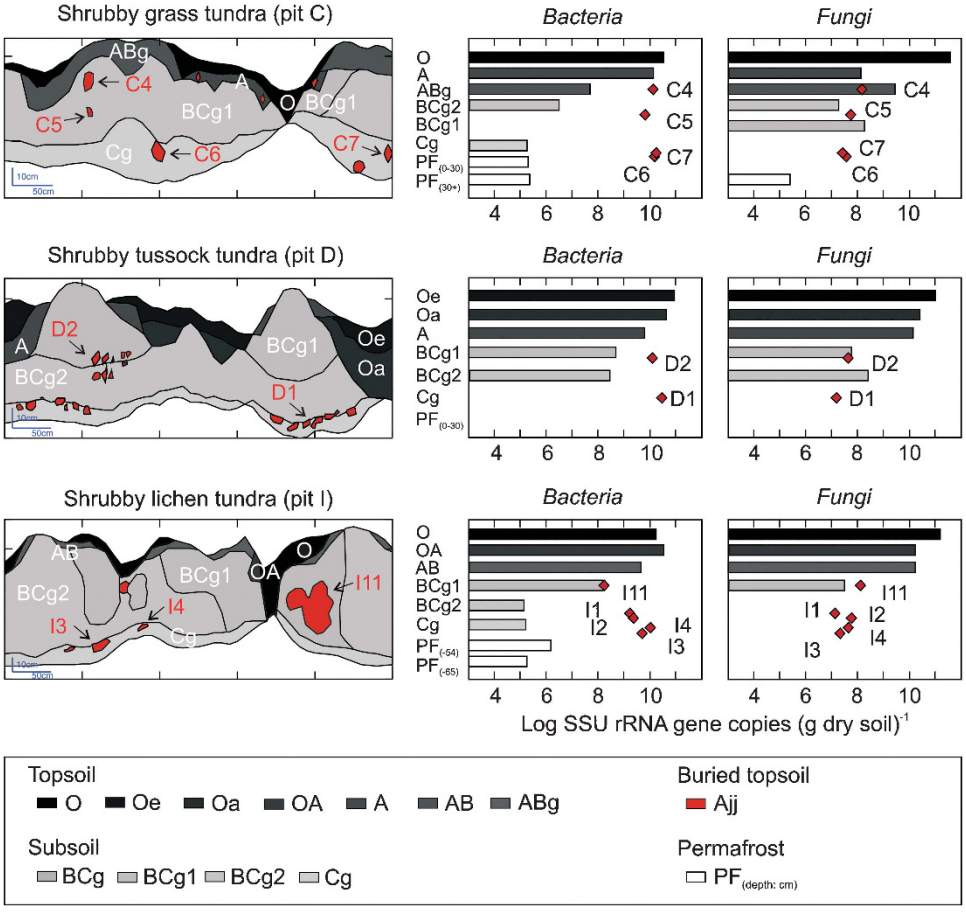

Figure 1 Schematic drawings of one representative soil pit profile for each vegetation type (shrubby grass tundra: pit C, shrubby tussock tundra: pit D and shrubby lichen tundra: pit I). O, Oe, Oa, OA, A, AB: topsoil, BCg, Cg: subsoil. Ajj: buried topsoils, PF: permafrost (depth in cm below surface indicated in brackets). Location of buried topsoils (Ajj) is indicated and labeled with the corresponding sample ID. If sample IDs are missing, location of the soil sample was only reported in the soil pit description, but not included in the drawings. Bar charts show abundances of *Bacteria* and *Fungi* as bacterial and fungal SSU gene copy numbers g^{-1} dry soil (logarithmic scale). Supplementary Figures S3A–C provide information on SSU rRNA gene quantifications for all nine soil pits (three replicates per vegetation type, including the ones presented in this figure).

The observation of a significant decrease in FB ratios in buried topsoils held true for all three vegetation types (Figure 2).

Archaeal SSU rRNA genes accounted for <2% of the total number of prokaryotic SSU rRNA gene copies in buried topsoils and subsoils. On average, archaeal gene copies were one order of magnitude higher in the buried topsoils (1.2×10^8 genes g^{-1} dry soil) than in the subsoils (0.1×10^8 genes g^{-1} dry soil). Amplification of archaeal SSU rRNA genes from topsoil samples resulted in unspecific fragments (Supplementary Figure S4). Sequencing identified these fragments to be of fungal origin. Thus, the co-amplification of fungal DNA resulted in an overestimation of archaeal SSU rRNA genes in these samples (see Supplementary Figures S3A–C).

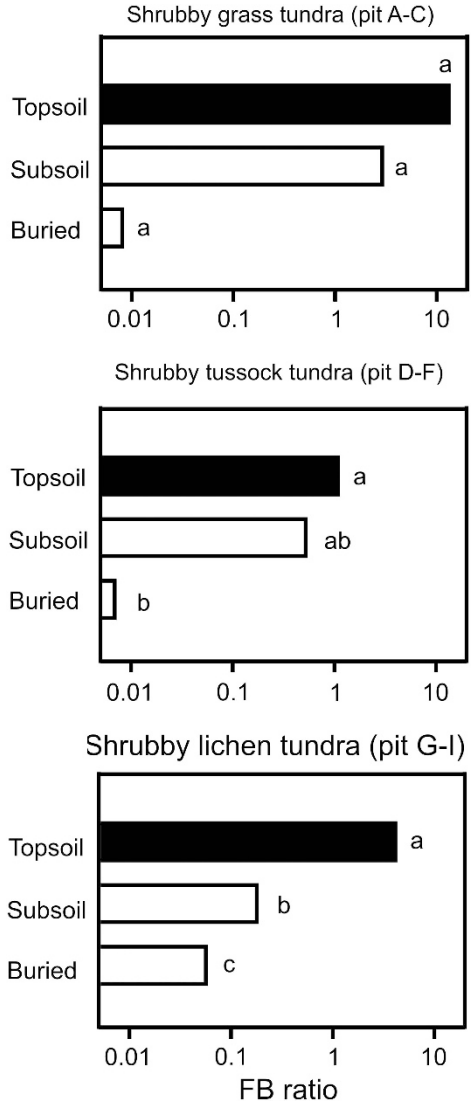

Figure 2 Fungal–bacterial (FB) ratios for the three different sampling sites as calculated from bacterial and fungal SSU rRNA gene copies g^{-1} dry soil. Small letters indicate significant differences between soil horizons as determined by one-way ANOVA and Tukey's HSD test.

Potential hydrolytic and oxidative enzyme activities

Hydrolytic enzymes showed significantly higher potential activities (g^{-1} dry soil) in topsoils than in buried topsoils (one-way ANOVA, $P<0.01$, Table 2). Potential oxidative enzyme activities were higher in buried topsoils than in non-buried topsoils, but this difference was not significant (Table 2). Both potential hydrolytic and oxidative enzyme activities were lowest in subsoils and significantly different from activities in buried and non-buried topsoil horizons (one-way ANOVA, $P<0.05$). Hydrolytic and oxidative enzyme activities were positively correlated to bacterial SSU gene abundances (Pearson's $R>0.5$, $P<0.01$), whereas fungal SSU gene abundances only showed significant correlations to the potential activity of hydrolytic enzymes (Pearson's $R>0.6$, $P<0.01$), but not to oxidative enzyme activities ($P>0.05$).

Sequencing statistics and distribution of microbial taxa across all horizons

Illumina tag sequencing of 36 samples (Supplementary Tables S2 and S3) yielded a total of 1.36×10^6 bacterial and archaeal SSU rRNA gene sequences and 2.81×10^5 fungal ITS sequences after extensive read-quality filtering (see Materials and methods for details). Bacterial and archaeal sequences clustered in 4580 OTUs, representing 98 classes (93 bacterial and 5 archaeal) within 38 phyla (for a detailed list of taxa and their relative abundance, see Supplementary Table S2). The dominant bacterial phyla were *Actinobacteria*, *Proteobacteria* (alpha-, beta- and gamma classes), *Verrucomicrobia*, *Acidobacteria* and *Bacteroidetes*, accounting for ~84% of all sequences. In addition, *Gemmatimonadetes*, *Chloroflexi*, *Deltaproteobacteria*, *Firmicutes* and *Planctomycetes* were present in all soil horizons, but at lower abundances (14% of all sequences), and 29 other rare phyla (< 0.5% each) were identified. Rare phyla included archaeal taxa that represented <0.1% of all sequence reads. Most of them were affiliated with the *Thaumarchaeota* (order *Nitrososphaerales*) and the *Euryarchaeota* (orders *Methanomicrobiales* and *Methanobacteriales*).

Fungal ITS sequences were clustered into 3397 OTUs representing 19 classes within 6 phyla (for a detailed list of taxa and their relative abundance, see Supplementary Table S3). Across all soil horizons, the most abundant phyla were *Ascomycota* (66.8%) and *Basidiomycota* (30.1%). *Ascomycota* were dominated by *Leotimycetes* (42.2%). Within the *Basidiomycota*, the class *Agaricomycetes* accounted for the majority of all sequences (69.9%). Other phyla (including the *Chytridiomycota*, *Glomeromycota* and *Zygomycota*) accounted for only a minor fraction of the fungal community across all horizons (~3%).

Major differences in prokaryotic community composition and diversity among soil horizons and linkage to environmental context data

Bacterial community composition in unburied topsoils and buried topsoils showed significant differences on the phylum level (Figure 3a), including higher relative abundances of *Actinobacteria* and *Gemmatimonadetes* (one-way ANOVA, $P<0.05$) and a decrease of *Acidobacteria*, *Proteobacteria* (alpha-, gamma- and delta-subclasses) and *Planctomycetes* (one-way ANOVA, $P<0.01$) in the buried horizons. The only significant difference between subsoils and buried topsoils was a lower relative abundance of *Deltaproteobacteria* in the latter (one-way ANOVA, $P<0.05$). Community composition of individual buried topsoil horizons was highly variable, in particular with regard to the relative abundance of members of the phyla *Chloroflexi* and *Verrucomicrobia* (Supplementary Figure S5, Supplementary Table S2). A major fraction of OTUs from buried topsoils was shared either with topsoils or subsoils alone or with both topsoil and subsoil horizons (1853 OTUs, 86.5% of all OTUs from buried topsoils; Supplementary Figure S6), reflecting the process of topsoil material being buried into deeper soil and the invasion of microorganisms from the surrounding mineral horizons. A minor fraction of OTUs was exclusively detected in buried topsoils (290 OTUs, 13.5% of all OTUs from buried topsoils, Supplementary Figure S6). None of these OTUs accounted for >0.001% of all sequences recovered from these horizons. Species richness and diversity were highest in topsoil horizons followed by buried topsoils and mineral subsoils (one-way ANOVA, $P<0.001$; Figure 4). Likewise, there were significant positive correlations between alpha diversity metrics (Shannon, Faith's PD) and the measured soil variables. While soil pH was the strongest predictor of alpha diversity in topsoils (Pearson's $R>0.6$, $P<0.001$), strongest correlations in buried topsoils were found for TOC and TN (Pearsons' $R>0.7$, $P<0.05$ each). None of the measured soil parameters were significantly correlated with species richness and diversity in subsoils.

Principal coordinate analysis based on unweighted UniFrac distances supported the distinctness of the communities in buried and unburied soil horizons (Figure 5a), showing a separation of the topsoil communities from those of the buried topsoils and subsoils along the first principal coordinate. While topsoil communities in individual samples were highly similar to each other, communities in buried topsoils and subsoils exhibited a larger between-sample variability. Biplot analysis of unweighted UniFrac distances and the relative abundance of the eight most abundant phyla showed that *Acidobacteria*, *Bacteroidetes* and *Verrucomicrobia* were associated with topsoils, whereas the phyla *Chloroflexi*, *Gemmatimonadetes* and *Firmicutes* were associated with a cluster of subsoil and buried topsoil horizons (Figure 5b). The clustering of soil horizons was broadly congruent with principal component analysis using relative taxa abundance data (Supplementary Figure S2) and CCA of relative OTU abundances (Figure 5c), indicating that the measured soil context parameters explained most of the biological variation.

To further investigate whether the prokaryotic community structure was related to changes in soil characteristics, principal component analysis was performed independently on the soil context data (Supplementary Table S1) and the relative taxa abundance data (Supplementary Table S2). Sample scores on PC1 of the two data sets (soil context and prokaryotic community structure) showed a significant rank-order correlation at the phylum level ($\rho=0.621$, $P<0.001$), (Supplementary Figure S7) demonstrating that changes in the soil properties (PC1 explained 52% of the variation) co-varied with changes in the community structure (PC1 explained 22% of the variance at phylum level). PC1 scores (prokaryotic community structure) were extracted and showed significant correlations with pH ($\rho=-0.754$, $P<0.001$), C/N ratio ($\rho=0.649$, $P<0.001$), TOC ($\rho=0.505$, $P<0.01$), moisture ($\rho=0.436$, $P<0.01$), $\delta^{13}C$ ($\rho=-0.605$, $P<0.001$)

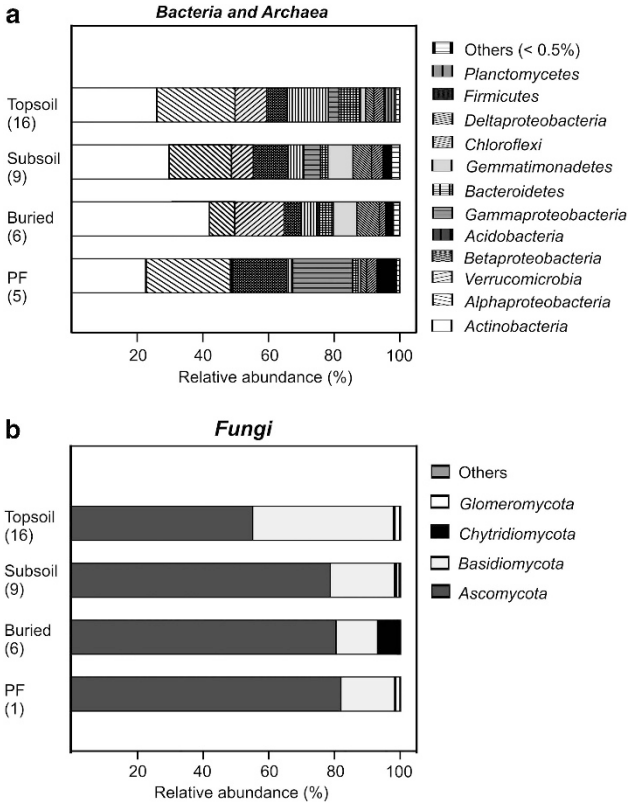

Figure 3 Prokaryotic (**a**) and fungal (**b**) community structure shown as relative abundance on phylum level and based on SSU rRNA gene Illumina tag sequencing and fungal ITS pyrosequencing, respectively. 'Others' include phyla with <0.5% relative abundance (see Supplementary Tables S2 and S3 in the supplementary for detailed information). Number of samples analyzed given in brackets.

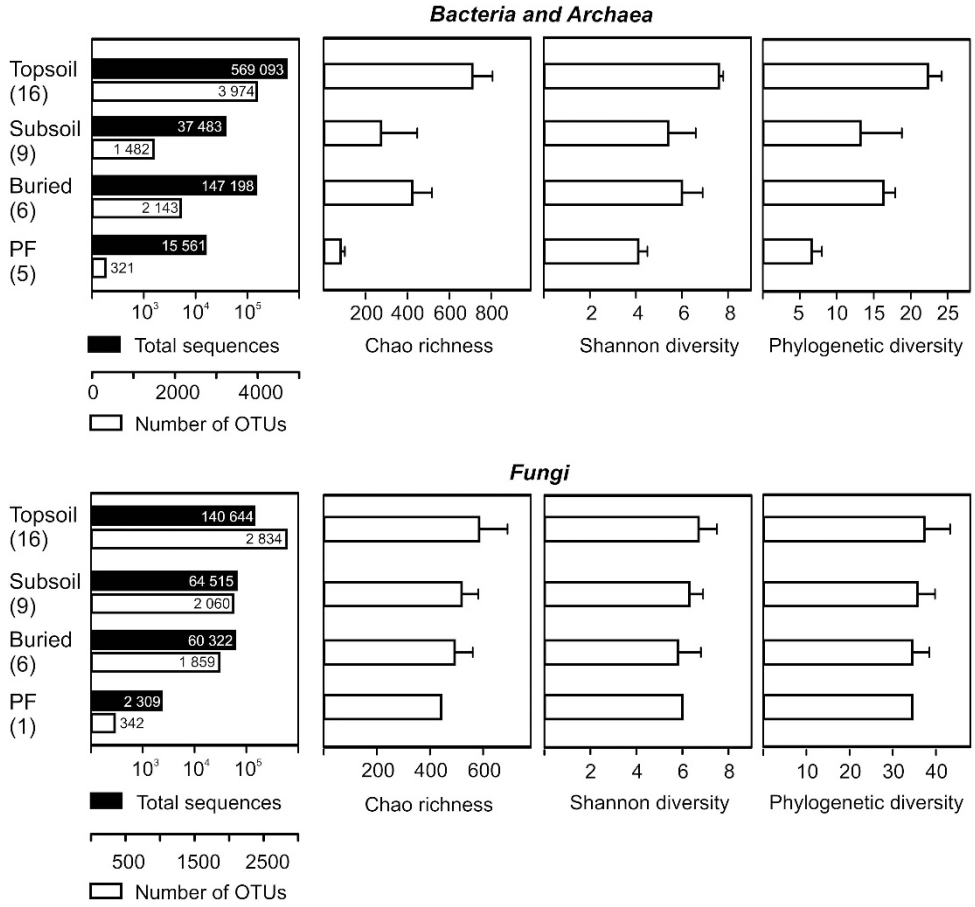

Figure 4 Overview of total sequences, number of OTUs, and microbial diversity topsoils, subsoils, buried topsoils and permafrost samples. Microbial diversity indicated by Chao1 richness, Shannon diversity and Faith's phylogenetic diversity (PD). Calculation of richness and diversity estimators was based on OUT tables rarified to the same sequencing depth (that is, the lowest one of total sequencing reads). Total sequences refer to the total number of taxonomically assigned sequences (see Material and Methods for details). OTUs were defined as <3% nucleotide sequence difference.

and $\delta^{15}N$ ($\rho = -0.543$, $P<0.001$) (Supplementary Figures S8A–F). For buried topsoils, strongest PC1 score correlations were found for TOC ($\rho = 0.886$, $P<0.05$) and TN ($\rho = 0.886$, $P<0.05$) (Supplementary Figure S8G–H). These results were in agreement with the CCA that identified pH, $\delta^{13}C$ and $\delta^{15}N$ having the strongest positive influence on axis 1, while C/N ratio, TOC TN and moisture were acting most negatively (Figure 5c).

Major differences in fungal community composition and diversity among soil horizons and taxa–environment relationships

Fungal community composition was significantly different in topsoils compared with buried topsoils and subsoils (Figure 3b). The relative abundance of *Basidiomycota* was significantly higher (one-way ANOVA, Tukey's HSD test, $P<0.05$) in topsoils (42.6%) than in buried topsoils (12.6%) and subsoils (19.5%). *Ascomycota* dominated in buried topsoils (80.4%) and subsoils (78.7%) with *Leotiomycetes* and *Sordariomycetes* being the most abundant classes. *Chytridiomycota* accounted for 6% of all sequences from buried topsoils, resulting from an exceptionally high relative abundance in one sample (G4, Supplementary Table S3), but accounting for <1% in all other samples. In particular, the ectomycorrhizal (ECM) families *Russulales*, *Thelephorales* and *Sebacinales* showed significantly lower abundance in buried than in unburied topsoils (Welch's *t*-test, two-sided, $P<0.05$; Supplementary Figure S9). A major fraction of fungal OTUs from buried topsoils was shared either with topsoils or subsoils alone or with both topsoil and subsoil horizons (1650 OTUs, 88.8% of all OTUs from buried topsoils; Supplementary Figure S6). A minor fraction of OTUs was exclusively detected in buried topsoils (209 OTUs, 11.2% of all OTUs from buried topsoils, Supplementary Figure S6). Species richness and diversity were slightly, but not significantly higher in topsoils than in subsoils and buried topsoils (one-way ANOVA, $P>0.05$; Figure 4).

To examine species–environment relationships, CCA was applied to fungal OTU abundance and a set of 14 environmental variables (Figure 6). The first two CCA axes (CCA1 and CCA2) explained 72.8% of

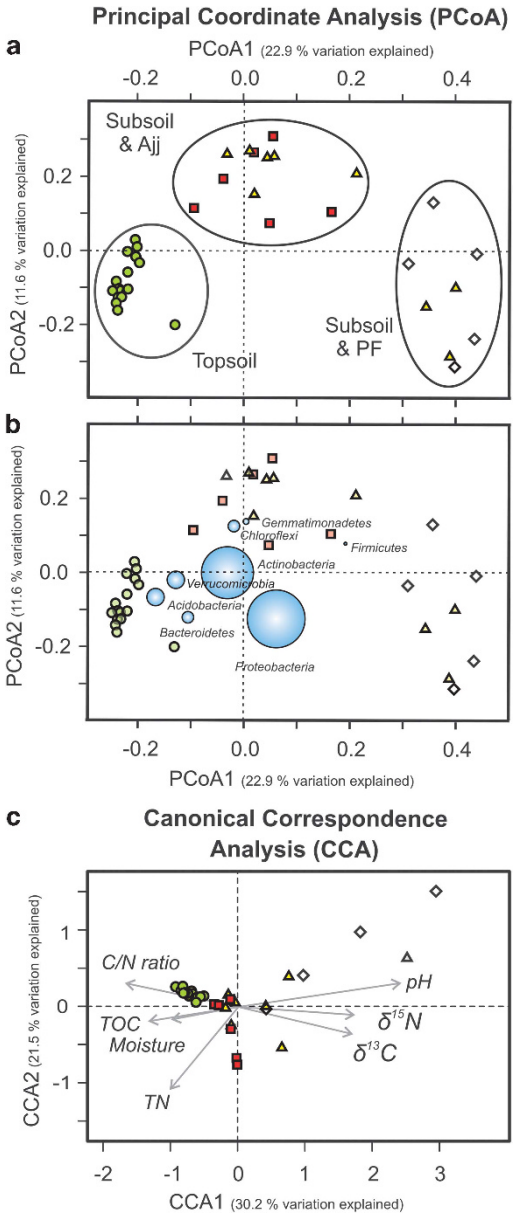

Figure 5 Phylogenetic dissimilarity between soil samples. Topsoils: green circles, subsoils: yellow triangles, buried topsoils: red squares, permafrost: white diamonds. See Supplementary Table S2 in the supplementary for detailed information on samples analyzed. (**a**) Principal coordinate analysis plot illustrating unweighted UniFrac distances between bacterial communities in individual samples. (**b**) The coordinates of the eight most abundant taxa are plotted as a weighted average of the coordinates of all samples, where the weights are the relative abundances of the taxon in the samples. The size of the sphere representing a taxon is proportional to the mean relative abundance of the taxon across all samples. (**c**) CCA plot for the first two dimensions to show the relationship between prokaryotic community structure (relative abundance of bacterial and archaeal OTUs) and environmental parameters. Correlations between environmental variables and CCA axes are represented by the length and angle of arrows (environmental factor vectors).

the total variance in the fungal community composition and 13.7% of the cumulative variance of the fungal species–environment relationship (Figure 6).

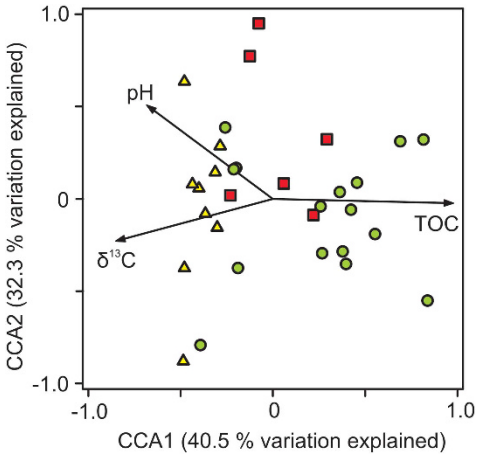

Figure 6 CCA ordination plots for the first two dimensions to show the relationship between fungal community structure (relative abundance of fungal OTUs) and environmental parameters. Topsoils: green circles, subsoils: yellow triangles, buried topsoils: red squares. See Supplementary Table S3 in the supplementary for detailed information on samples analyzed. Correlations between environmental variables and CCA axes are represented by the length and angle of arrows (environmental factor vectors).

CCA1 distinguished fungal topsoil communities from subsoil communities. Exceptions were mixed soil horizons (AB horizons) that were initially classified as topsoil horizons, but clustered with the subsoil communities in CCA analysis. Fungal communities in buried topsoils were placed at an intermediate position. CCA indicated that only TOC, pH and $\delta^{13}C$ contributed significantly to the species–environment relationship ($P<0.05$, 1000 Monte Carlo permutations), providing 13.7% of the total CCA explanatory power. It was furthermore indicated that the spatial distribution of certain genera within the *Basidiomycota* and *Ascomycota* was related to distinct soil environmental parameters (Supplementary Figure S10). The distribution of *Ascomycota* was correlated to changes in pH and $\delta^{13}C$ values, whereas the distribution of *Basidiomycota* was related to changes in TOC content.

Discussion

The low abundance of ECM basidiomycota is a potential key factor in the reduced degradation of SOM in buried soils

Recently, evidence is building up that the stability of organic matter in soil is mainly controlled by its stabilization in soil mineral associations, its accessibility to the decomposer community, the presence and activity of extracellular enzymes, and microbial community structure, rather than being controlled by its molecular structure or chemical recalcitrance (Schmidt *et al.*, 2011; Dungait *et al.*, 2012). Stabilization mechanisms for SOC are of particular interest in Northern high latitude soils as they contain ~50% of the global SOC pool and are particularly

vulnerable to changes in abiotic conditions (for example, temperatures, moisture). Although fungi have a critical role in the transformation of SOM, fungal communities in terrestrial ecosystems of the Northern high latitudes are still poorly studied (Timling and Taylor, 2012). Fungi, and in particular saprotrophic basidiomycetes, are thought to be the dominant producers of extracellular enzymes catalyzing the breakdown of biopolymers to low-molecular weight dissolved organic matter that can then be utilized by other microorganisms (Bailey *et al.*, 2002; Baldrian 2008). We found pronounced differences in the abundance and composition of the fungal communities between unburied and buried topsoil horizons. It was indicated that lower fungal to bacterial ratios in buried than in unburied topsoil horizons resulted from a significant reduction in *Basidiomycota*, specifically of the ECM families *Russulales*, *Thelephorales* and *Sebacinales* (Supplementary Figure S9). Interestingly, there is accumulating evidence that mycorrhizal fungi, besides saprotrophs, may act as potent decomposers of SOM and have a crucial role in SOM transformation especially in arctic and boreal ecosystems (Talbot *et al.*, 2008). Although primarily considered to metabolize low-molecular weight carbon compounds, ECM fungi have been shown to produce extracellular enzymes to break down nutrient and C-rich molecules (Read and Perez-Moreno 2003; Read *et al.*, 2004; Talbot *et al.*, 2008). Recent studies however suggested that ECM fungi are responsible for degrading different C and nutrient fractions of SOM than saprotrophic communities and that the decomposition of SOM by ECM fungi is linked to their depolymerization abilities for organic N and P compounds (Rineau *et al.*, 2013; Talbot *et al.*, 2013). So far, only few ECM fungi were found to produce enzymes that break down C-rich biopolymers like cellulose, pectin or lipids in culture (Talbot and Treseder, 2010). Talbot *et al.*, 2008 suggested several mechanisms by which ECM fungi may contribute to SOM decomposition, one of them described as the 'Coincidental Decomposer' hypothesis. According to this hypothesis, SOM decomposition by ECM fungi is a consequence of exploiting the soil for nutrients. ECM fungi thereby contribute substantially to the mobilization of N-rich SOM and, consequently, to an increase in host plant C-fixation and subsequent input of C to the soil (Lindahl *et al.*, 2007; Orwin *et al.*, 2011; Hobbie *et al.*, 2013). Consequently, the low abundance of ECM fungi in buried topsoils presumably leads to less mobilization of N and results in an even higher N-limitation in arctic soils. In addition, ECM fungi in buried topsoils have lost their connection to the host plant and are therefore not 'primed' by plant-derived carbon ('Priming Effect' hypothesis, Talbot *et al.*, 2008). Thus, the synthesis of extracellular fungal enzymes is probably low and depolymerization of C- and N-rich substrates dramatically decreases. Indeed, Wild *et al.* (2013) described on average 84% reduction in protein depolymerization and 68% reduction in amino acid uptake in buried topsoils. Thus, the reduced abundance of ECM fungi, their reduced activity in the depolymerization of N-rich compounds together with unfavorable abiotic conditions such as subzero temperatures and high moisture presumable lead to a retarded decomposition and thus to the stabilization of OM in buried topsoils in Northern high latitude regions.

The distinctness of the bacterial community in buried topsoils reflects soil properties

As surface soil is rich in labile carbon and nitrogen and represents the primary location of root exudates, the presence of rapidly growing copiotrophic microorganisms is favored (Fierer *et al.*, 2007). In contrast, slow-growing oligotrophic organisms are better adapted to resource-poor locations and complex organic substrates and therefore occur in deeper soil horizons. Together with rather harsh abiotic conditions (low to subzero temperatures, high moisture, anoxia due to water logging), the distribution of microorganisms along the soil depth profile thus follows gradients of C and N availability as well as SOM composition, resulting in distinct community patterns and less microbial biomass in deeper soil horizons (Hartmann *et al.*, 2009; Eilers *et al.*, 2012). However, our analyses showed that pockets of buried topsoil horizons interrupted this continuum by being different from the subsoil in major soil parameters (for example, higher TOC, TN), and harboring a prokaryotic community with significantly higher bacterial and archaeal abundances than found in the surrounding subsoil horizons. As buried topsoils showed similarly low fungal abundances as the surrounding subsoil horizons, the remarkably low FB ratios in the buried topsoils were foremost a consequence of the high bacterial abundances therein. Prokaryotic communities in topsoil horizons were phylogenetically highly similar (Figure 5a) and relatively uniform in composition across all pits regardless of geographic position and landscape cover (Supplementary Figure S5). In contrast, communities in buried topsoils and subsoils were not only distinct from the topsoil communities, but also highly variable (Supplementary Figure S5). This variability in community composition was most likely a result of the greater variability in soil properties, namely the concentration and quality of the SOM. Abundance patterns of prokaryotic OTUs affiliated with potential representatives of distinct metabolic traits (for example, anaerobic respiration, fermentation, methano-/methylotrophy) further supported the hypothesis that community structure reflected differences in soil properties (Supplementary Table S4). The elevated abundance of OTUs assigned to fermentative members of the *Chloroflexi* (*Anaerolineae*) and the *Firmicutes* (*Clostridia*) as well as members of the anaerobic, sulfur- and

metal-reducing *Desulfuromonadales* (*Geobacteraceae*) indicated the potential for anaerobic degradation processes to occur in these deeper soil horizons.

Actinobacteria, in particular the order *Actinomycetales*, dominated the prokaryotic community in buried topsoils and were found in significantly higher relative abundance than in non-buried topsoils (Figure 3a). *Actinobacteria* were shown to maintain metabolic activity and DNA repair mechanisms at subzero temperatures (Johnson *et al.*, 2007). As buried soils experience longer freezing periods than topsoil horizons, this adaptation might select for *Actinobacteria* and disfavor the growth and/or maintenance of other taxa. This hypothesis is in line with a cross-seasonal study on arctic tundra soils that indicated that *Actinobacteria* might be persistent in the active fraction of the soil community over seasons and are thus ecologically relevant in frozen soils (McMahon *et al.*, 2011). More importantly, members of this phylum have been described to be adapted to low carbon availability (Fierer *et al.*, 2003) as well as to be metabolically versatile, including specialists that are able to solubilize and modify lignin and lignocelluloses and thereby gain access to the associated polysaccharides (McCarthy 1987; Roes-Hill *et al.*, 2011). Laccase-like genes were found in the genomes of diverse bacteria, including actinobacteria, supporting the hypothesis that the capability to modify lignin and decompose lignin derivatives is more widespread among the bacteria than previously thought (Ausec *et al.*, 2011; Bugg *et al.*, 2011). Buried soil horizons that undergo water-logging after active layer thaw might periodically turn anoxic, thereby restricting fungal growth and activity, resulting in a lower fungal abundance and creating a niche for bacterial, presumably anaerobic lignin degraders such as the actinomycetes (Boer *et al.*, 2005; DeAngelis *et al.*, 2011). Though being functionally redundant, actinobacterial activity in lignin degradation and transformation apparently does not resemble fungal activities as decomposition in buried soils is strongly retarded. This discrepancy might be due to differences in bacterial and fungal biomass, lower cell-specific enzyme activities, as well as morphological restrictions such as the absence of hyphae structures to efficiently penetrate the substrate.

Interestingly, the highest correlations between potential oxidative enzyme activities (phenoloxidase, peroxidase) and taxon abundance were found for rare bacterial taxa (for example, the candidate phylum SC3, Pearson's $R>0.6$, $P<0.01$), either indicating that those might contribute to the production of key enzymes involved in lignin degradation or solely act as so-called 'cheaters' participating from the products being available from exoenzymatic breakdown of polymers (Allison 2005), as suggested in an evolutionary context in the Black Queen Hypothesis (Morris *et al.*, 2012).

In summary, our study demonstrates that microbial community structure in buried topsoil horizons is distinct from that in unburied topsoil and the surrounding mineral subsoil horizons. Opposing trends in bacterial and fungal abundances were manifested in remarkably low FB ratios in buried topsoil horizons. The decrease in abundance of ECM fungi and the extent to which bacterial decomposers are able to act as functional substitutes in SOM transformations are proposed as microbial key factors in the retarded decomposition of SOM in buried soils of Northern latitude permafrost regions. The response of the fungal community to rising temperatures and concomitant changes in environmental parameters such as soil moisture and plant cover will be of particular interest in the scope of drastic changes in the arctic climate.

Conflict of Interest

The authors declare no conflict of interest.

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