The activity and functions of subarctic soil microbial communities vary across vegetation types

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Keywords: microbial communities, transcriptomics, microbial ecology, tundra, climate change

Abstract

Global warming changes the activity of soil microbial communities in high latitudes, which might result in higher greenhouse gas emissions. However, these microbial processes involved in GHG production and consumption are not thoroughly understood. We analyzed 116 soil metatranscriptomes from 73 tundra sites and investigated how bacterial and archaeal communities and their functions vary horizontally (i.e. vegetation type) and vertically (i.e. topsoil organic and mineral layers) during the summer season, in soil types that differed in pH, moisture, soil organic matter (SOM), and carbon and nitrogen content. Active microbial communities were significantly different in the organic and mineral soil layers. Additionally, the communities differed significantly between the different vegetation types both in the organic and mineral layers. Various plant polymer degraders were particularly active in shrub-dominated ecosystems with high SOM and low pH, less known mixotrophic groups (such as Chloroflexi) were active lower SOM and higher pH. Additionally, we detected transcripts of alphaproteobacterial methanotrophs, which potentially moderate methane release from tundra soils in deeper soil layer. Our results provide new insights into the diversity and activity of microbial communities of high latitudes under climate change.

1 Introduction

The Arctic is experiencing some of the most rapid and severe climate change on Earth (IPCC 2018). Over the next 100 years, climate change is expected to accelerate, contributing to major ecological changes (Post et al. 2019). Tundra soils harbor large carbon (C) stocks that are susceptible to microbial degradation with rising global temperatures (Johnston et al. 2019, Mackelprang *et al.* 2011). Soil microorganisms are critical in C and nutrient cycling in terrestrial ecosystems (Falkowski et al. 2008). Therefore, understanding soil microbial communities and how they correspond to local environmental variation is essential to predict the impacts of environmental change on ecosystem functioning (Buckeridge et al. 2013, Mod et al. 2021, Virkkala et al. 2018).

Warming and elevated atmospheric carbon dioxide (CO₂) concentrations stimulate plant productivity and can thereby increase the net carbon sink of arctic ecosystems (Friend *et al.*, 2014; Berner *et al.*, 2020). However, warmer temperatures also increase soil decomposition (Bond-Lamberty *et al.*, 2018) and plant productivity, and this provides substrates for microbes, which subsequently stimulate decomposition and the release of CO₂ (priming effect, reviewed in Jansson & Hofmockel, 2020). Although GHG emissions from soil are measurable (Bond-Lamberty & Thomson, 2010), soil microbial communities and their functions have not been explored in detail

due to technical limitations and vast diversity. This in turn hinders a comprehensive understanding of the net contribution of microbes and their feedback to climate change.

In high-latitude soils, microbial communities are exposed to extreme environmental stressors, such as fluctuating temperatures, long periods of darkness, and intensive UV radiation and drought. In addition, vegetation is typically heterogenous in areas, where the local growing conditions (microclimate, soil moisture, soil nutrients) can vary greatly at small spatial scales (le Roux *et al.*, 2013; Kempainen *et al.*, 2021). Different vegetation types affect soil biotic and abiotic factors, through which vegetation can influence the local soil microbial community. For example, tundra soils are relatively acidic (pH 4-6; Hobbie & Gough, 2002; Männistö *et al.*, 2007) and soil acidity is generally one of the most predominant drivers of the composition and activity of microbial communities (Chu *et al.*, 2010). In addition, the quantity and quality of soil organic matter (SOM) and nitrogen (N) availability and the C/N ratio affect microbial processes and primary production in Arctic tundra soils (Koyama *et al.*, 2014, Zhang *et al.*, 2014).

Soil microbial communities are taxonomically and functionally diverse. Unlike higher organisms, microbial communities in Arctic soils can be as diverse as in other soil biomes across the globe (Neufeld & Mohn 2005; Chu *et al.* 2010). Recent studies have revealed Acidobacterial predominance in acidic soils and stability of microbial community composition over seasons in subarctic tundra (Männistö *et al.*, 2007; Männistö *et al.*, 2009), suggesting that Acidobacteria may also have a significant role in community functions. In addition, recent research has shown methanogenesis and methanotroph activity in peatlands experiencing permafrost thaw (McCalley *et al.*, 2014; Singleton *et al.*, 2018; Woodcroft *et al.*, 2018). Previous studies on high-Arctic and permafrost soil microbial communities have also shown activity of diverse organic matter decomposition, methanogenesis, and methanotrophy (eg. Hultman *et al.*, 2015; Schostag *et al.*, 2015; Tveit *et al.*, 2015). However, soil microbial studies rarely compare multiple vegetation types or investigate drier upland tundra, which is a noteworthy ecosystem that covers ca. 90% of the Arctic (Raynolds *et al.*, 2019). In general, these studies were performed with small sample sizes (Hultman *et al.*, 2015; Schostag *et al.*, 2015; Singleton *et al.*, 2018; Woodcroft *et al.*, 2018), limiting our understanding of the spatial variability in microbial communities. Further, several molecular methods have been used, including phospholipid fatty acid (PLFA), terminal restriction fragment analysis (T-RFLP) (Männistö *et al.*, 2007) and metagenomics (Pessi *et al.* 2020). In metatranscriptomics the target molecule is RNA, allowing analysis of both function and activity of organisms at the time of sampling. However, transcriptomics analyses have not yet been fully explored. Therefore, transcriptomics provides a new tool to thoroughly examine the tundra microbial community composition and functions.

Using a metatranscriptomics approach targeting total RNA, we investigated soil bacterial and archaeal diversity and activity. We analyzed 73 sites during the active growing season in a mountain tundra ecosystem. The area covers a wide range of microclimatic and soil nutrient conditions and different vegetation types. The samples represent four different tundra vegetation types (namely barren tundra, deciduous shrub, evergreen shrub and meadow; Figure 1), and both organic and mineral layers when available.

2 Materials and methods

2.1 Study setting and sampling

The study area is located in Kilpisjärvi, northwestern Finland, and extends to the Scandinavian Mountains. The 3 km² area covers parts of two mountains, Mount Saana (69°02'N, 20°51'E) and Mount Korkea-Jehkas (69°04'N, 20°79'E) and the valley between them. The elevation range in the study area is 320 m, with the highest point on Mount Saana at 903 m a.s.l. The study area is topographically heterogeneous and part of the arctic-alpine tundra biome. Consequently, the area has relatively broad environmental gradients of microclimate, soil moisture, and soil pH, for example (Kemppinen *et al.*, 2021). The vegetation type is mainly mountain heath dominated by dwarf shrubs, such as *Empetrum nigrum* and *Betula nana*, and to a lesser extent by *Juniperus communis*, *Vaccinium vitis-idaea*, *V. uliginosum*, and *V. myrtillus* (Kemppinen *et al.*, 2019). However, due to the fine-scale environmental variation and broad gradients, the landscape forms a mosaic of different vegetation types, as both vegetation cover and plant species composition can vary over very short distances (le Roux *et al.*, 2013). The soils in the area are mostly poorly developed leptosols with shallow organic layers and occasional podzolization; however, the meadows have soils with thicker organic layers. Permafrost is absent from soils but can be found in the bedrock above 800 m a.s.l. (King and Seppälä 1987). The average air temperature and precipitation sum in July measured at the Kilpisjärvi meteorological station (69°05'N 20°79'E, 480 m a.s.l.) for the period 1981-2010 is 11.2°C and 73 mm, respectively (Pirinen *et al.* 2012).

Samples were collected in July 2017 from 73 sites (Figure 1). Sampling sites consisted of four vegetation types, which were classified using a similar system as in the Circumpolar Arctic Vegetation Map, namely barren soil, deciduous shrub, evergreen shrub, and meadow (Walker *et al.* 2005). All sampling equipment was disinfected with 70% ethanol before and between samples to avoid contamination. The soil was bored with a 50-mm diameter stainless-steel soil corer with a plastic inner casing. When available, both organic and mineral layer sub-samples were collected. The samples were collected below the rhizosphere, with a 5-cm target depth for the organic layer

sample, whereas the mineral layer sample was taken from the lowest part of the core from 10-15 cm. The samples were placed in a Whirl-Pak sampling bag (Nasco, Fort Atkinson, WI, USA) with a metal spoon and immediately frozen on dry ice and kept frozen at -80°C until nucleic acid extraction. Samples were collected from 73 sites, from which 116 metatranscriptomes were sequenced.

2.2 Soil physicochemical data

For analysis of soil physicochemical properties, approximately 0.2 dm³ of soil was collected with a steel cylinder (diameter 5.5 cm, height 7.5) and stored at 4 °C. The soils were lyophilized according to the Finnish standard SFS300 and pH analysis was performed according to international standard ISO 10390. SOM content was analyzed by loss on ignition analysis according to standard SFS 3008. CNS analysis (carbon, nitrogen, and sulfur) was performed with a Vario Micro Cube (Elementar, Langenselbold, Germany). Mineral samples were sieved through a 2-mm plastic sieve and the organic samples for the CNS analysis were homogenized by hammering the material into smaller pieces.

2.3 Nucleic acid extraction

Three replicate DNA/RNA extractions were performed for each sample. Samples were kept on ice during weighing and extraction and all steps were performed promptly with nuclease-free labware to avoid RNA degradation. Solutions and water were treated with 0.1% diethylpyrocarbonate. Nucleic acids were extracted using a modified hexadecyltrimethylammonium bromide (CTAB), phenol-chloroform, and bead-beating protocol (Griffiths *et al.*, 2000; DeAngelis *et al.*, 2009). On dry ice, approximately 0.5 g of frozen soil was transferred to a 2-mL Lysing Matrix E tube (MP Biomedicals, Heidelberg, Germany) and 0.5 mL of CTAB buffer (consisting of equal amounts of 10% CTAB in 1 M sodium chloride and 0.5 M phosphate buffer in 1 M NaCl), 50 µl 0.1 M ammonium aluminum sulfate (NH₄(SO₄)₂ · 12 H₂O), and 0.5 mL phenol:chloroform:isoamyl alcohol (25:24:1) was added. After bead-beating with FastPrep (MP Biomedicals, Heidelberg, Germany) at 5.5 m s⁻¹ for 30 s, 0.5 mL chloroform was added. Nucleic acids were precipitated with polyethylene glycol 6000 (PEG6000, 30% in 1.6 M NaCl) and washed with ethanol. Nucleic acids were once again extracted from the leftover soil pellet to maximize RNA. Nucleic acids were resuspended in 25 µl of Buffer EB and 250 µl Buffer RLT with β-mercaptoethanol added. Buffers EB and RLT were from an AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). All centrifugations were performed at 4°C. Finally, RNA and DNA were purified with AllPrep

DNA/RNA Mini Kit, where RNA was treated with DNase I (Qiagen, Hilden, Germany). The amount and integrity of RNA were assessed on a Bioanalyzer RNA 2100 Nano/Pico Chip with Total RNA Assay (Agilent, Santa Clara, CA, USA). To ensure that RNA was DNA-free, a check-up PCR with universal primers and gel electrophoresis was performed. Triplicates were pooled by combining equal amounts of RNA from each triplicate.

2.4 Sequencing

Complementary DNA (cDNA) libraries were constructed from the pooled RNAs with Ultra II RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). cDNA concentrations were measured using a Qubit fluorometer with a dsDNA BR/HS kit (Invitrogen, Carlsbad, CA, USA). Before sequencing, the libraries were analyzed with a Fragment analyzer (Advanced Analytical, Ames, IA) and small cDNA fragments were removed to avoid primer binding to the flowcell and to reduce cluster density. Single-end sequencing was performed on an Illumina NextSeq 500 (Illumina, San Diego, CA, USA) with 150 cycles at the Institute of Biotechnology, Helsinki, Finland.

2.5 Data processing and analysis

As the total RNA approach was used (Urich *et al.*, 2008), ribosomal RNA was not depleted after extraction and RNA transcripts were sequenced. One sample (site 11202, organic layer) yielded 80.4 million reads and it was reduced randomly with seqtk (Li 2016) with random seed 100 to a 4-million read subset. The quality of the sequences was assessed using default settings with FastQC (v. 0.11.5, Andrews 2010) and MultiQC (v. 1.3, (Ewels *et al.*, 2016)) before and after trimming. Trimming and quality filtering was performed with Cutadapt (v. 1.10, (Martin, 2011)) with quality cutoff 25 and minimum adapter overlap 10. A mineral layer sample from site 11210 was discarded due to a low number of reads. Metaxa2 (v. 2.1.3, (Bengtsson-Palme *et al.*, 2018)) was used to identify reads mapping to the small subunit (SSU) rRNA. These were then classified using the mothur (v. 1.40.5, (Schloss *et al.*, 2009)) classify.seqs command with a confidence cutoff of 60% and SILVA release 132 (Quast *et al.*, 2013) as the reference database. The taxonomy of abundant taxa was manually updated according to the Genome Taxonomy database (Parks *et al.*, 2018; Parks *et al.*, 2020). Reads were mapped onto the Kyoto Encyclopedia of Genes and Genomes (KEGG) Prokaryote database release 86 (Kanehisa & Goto, 2000) using DIAMOND blastx (v. 2.1.3, Buchfink *et al.* 2015) with an E-value of 0.001. Blast results were parsed with KEGG-tools (v. 1.3, Pessi 2020). Briefly, the KEGG orthology (KO) identifier of the best BLAST hit was assigned to

each read and mapped to the KEGG module hierarchy, and spurious pathways were removed using MinPath (v. 1.4, (Ye & Doak, 2009). Since genes for methane and ammonia oxidation are not distinguished in KEGG classification we performed BLAST (Altschul *et al.*, 1990) for these genes against a manually curated database of eight *pmoA* and eight *amoA* genes from bacteria and archaea.

Due to differences in read numbers per sample, the metatranscriptome data were subsampled for statistical analyses of protein-encoding genes to avoid skewing caused by the low number of observations in low-read samples. After subsampling, the number of samples represented in each vegetation type was 6 (barren), 7 deciduous shrub, 18 (evergreen shrub) in the mineral layer and 5 (barren), 6 (deciduous shrub), 19 (evergreen shrub), and 8 (meadow) in the organic layer. For the mineral layer, meadow samples were discarded from statistical analyses due to a low sample number (n=4) after subsampling. Statistical analyses and visualization were performed with R (v. 3.6.2, R Core Team 2020). Differences between microbial communities and functional genes among vegetation types (Table 1) were tested with permutational multivariate analysis of variance using distance matrices (PERMANOVA, function *adonis*, R-package *vegan* v. 2.5-6, Oksanen et al. 2019) and pairwise permutation MANOVA (functions *pairwise.perm.manova* R-package *RVAideMemoire* v.0.9-78 (Hervé, 2021) and *vegdist*, R-package *vegan* v. 2.5-6). For the statistical analyses of functional genes, samples were subsampled from 116 to 73 samples to avoid skewing caused by the low number of observations, using a limit of 9654 KEGG hits (function *rrarefy*, R-package *vegan* v. 2.5-6). Differences between vegetation types for bacterial and archaeal genera and between soil types for selected genes were tested with one-way analysis of variance (ANOVA, functions *lm* and *aov*, R-core package) and pairwise t-test (function *pairwise.t.test*, R-core package). Soil physicochemical properties were tested with Kruskal-Wallis test and pairwise Wilcoxon test with Bonferroni correction (functions *kruskal.test* and *pairwise.wilcox.test*, R-core package). Differences in microbial communities in metagenomic and metatranscriptomic datasets were visualized in principal coordinates analysis plot, using Bray-Curtis dissimilarities (function *ordinate*, *Phyloseq* v. 1.30.0 (McMurdie & Holmes, 2013).

Table 1. Number of samples sequenced from each of the vegetation type in the study area.

Soil layer	Barren	Deciduous shrub	Evergreen shrub	Meadow	Total
Organic	6	14	37	12	69
Mineral	7	10	23	7	47

209   **2.6 Data availability**

210   Sequences have been deposited to the European Nucleotide Archive under accession number
211   PRJEB45463.

212   **3 Results**

213   **3.1 Soil conditions across vegetation types**

214   We observed high variability in soil conditions between organic and mineral layers and across
215   vegetation types. Among all samples collected, SOM content varied from 2% to 92%, gravimetric
216   soil water concentration from 9% to 432%, and pH from 3.9 to 5.8 (Figure 1). Soil properties
217   differed between soil layers (Kruskal-Wallis test; $P < 0.01$, Figure 1). The organic layer in the
218   meadow sites was less acidic (Pairwise Wilcoxon test; $P < 0.05$) and contained less SOM than the
219   organic layers in sites with deciduous or evergreen shrubs ($P < 0.05$). Sites with meadow vegetation
220   also had a lower C/N ratio than sites with deciduous or evergreen shrubs or barren soil ($P < 0.01$).
221   The physicochemical properties of the mineral layer were not significantly different between
222   vegetation types. In mineral layer pH was overall higher than in the organic layer whereas SOM,
223   N%, C/N and gravimetric soil water concentration were lower.

Figure 1. a) Map of sampling sites. Saana fell is located in the southern part of the study area. Typical vegetation in meadow (b), evergreen shrub (c), deciduous shrub (d), and barren (e) sites. f) Soil physicochemical properties (pH, soil organic matter (SOM), nitrogen (N), carbon-nitrogen ratio (C/N), and gravimetric water content) in each vegetation type. Categories with the same letter are not statistically different. One outlier from mineral C/N plot was removed.

3.2 Microbial community composition across sites and vegetation types

We analyzed 281.1 million sequence reads obtained with a total RNA approach (Urich *et al.*, 2008) from 116 samples (Supplementary table S1). First, we analyzed the small subunit ribosomal RNA (SSU rRNAs) from the metatranscriptomes. SSU rRNA represented $35 \pm 2\%$ (mean $\pm$ standard deviation) of the reads. On the domain level, over 80% of the assigned SSU rRNAs in the total metatranscriptomes were bacterial, 0.1 % Archaeal, and 19% were of eukaryotic origin, with fungal SSU rRNA representing 12% of the sequences. Out of the identified fungal phyla, Ascomycota represented 8.2% and Basidiomycota 2.8%. Furthermore, 0.2% of the sequences in both soils were recognized as SSU rRNA but could not be assigned to the domain level.

Figure 2. Order-level bacterial abundances in metatranscriptomes (RNA) and metagenomes (DNA) in organic (upper panel) and mineral (lower panel) layers. Samples of the same vegetation type were pooled and unclassified taxa were removed.

According to the analysis of bacterial 16S rRNA, the predominant active phyla in the microbial communities were Actinobacteria ($27 \pm 9\%$) with order Acidothermales and Solirubrobacterales; Acidobacteria ($17 \pm 3\%$) with orders Acidobacteriales and Solibacteriales; Alphaproteobacteria ($16 \pm 3\%$) with orders Rhizobiales and Acetobacterales, and Planctomycetes

(14 ± 3%) with order Gemmatales (Figure 2). Moreover, Deltaproteobacteria, Chloroflexi, Gammaproteobacteria, and Verrucomicrobia were also active in these soils. Out of the Archaeal reads Thaumarchaea (0.12 ± 0.15%) were most active, with Group 1.1c (0.05 ± 0.05%) and Nitrososphaerales (0.05 ± 0.11%) being the most active taxa. The relative abundances at the genus level in each vegetation type and soil layer are listed in Supplementary table S2.

Figure 3. a) Genus-level classified square root transformed relative abundances of the 100 most abundant bacterial genera for each vegetation type and each site, and b) Square root transformed Archaeal taxa with mean relative abundance > 0.001, lowest rank classification level shown. A column of pixels represents a site.

The most active genera were *Acidothermus* (Class Actinobacteria, 13 ± 5.7%) and *Candidatus Solibacter* (Acidobacteriia, 3.11 ± 0.83%), followed by *Bryobacter* (Acidobacteriia, 2.06 ± 0.38%) *Pajarococcus* (Deltaproteobacteria, 1.64 ± 0.55%), and *Roseiarcus* (Alphaproteobacteria, 1.48 ± 1.07%, Figure 3a). Archaea were not particularly abundant in the

metatranscriptomes of these soils, but the most active archaeal groups belonging to Thaumarchaea were more active in the mineral than organic layer (Figure 3b).

When comparing the 16S rRNA from metatranscriptomes (i.e., RNA; active microbes) to the 16S rRNA genes from metagenomes (i.e., DNA, Pessi *et al.*, 2020), the most abundant and active microbial groups were the same but notable differences appeared among the datasets (Figures 2 and 4a). Orders with greater relative abundance in metatranscriptomes included Gemmatales (Planctomycetes), Acidothermales (Actinobacteria), Solibacterales (Acidobacteria), and Myxococcales (Deltaproteobacteria), whereas Subgroup 2 (Acidobacteria), Rickettsiales (Alphaproteobacteria), Dormibacterota, Chitinophagales (Verrucomicrobia), and Gemmatimonadales (Gemmatimonadetes) showed higher relative abundance in metagenomes. In metatranscriptomes, the orders with relative abundance more than 1% covered $78 \pm 0.02\%$ of active orders, but only $64 \pm 0.02\%$ in metagenomes. Additionally, more than 1500 bacterial and archaeal genera were identified from metatranscriptomes and less than 500 bacterial and archaeal genera from metagenomes.

Figure 4. a. Principal coordinates analysis plot showing differences between 16S rRNA genes from metagenomes and 16S rRNA from metatranscriptomes among vegetation types and soil layers, based on square root-transformed Bray-Curtis dissimilarities. b. Ordination plot showing the differences in active microbial communities through 16S rRNA genes in organic and mineral layer.

Active microbial communities were different in organic and mineral layers (PERMANOVA; $R^2 = 0.06642$; $P < 0.001$, Figure 4b) and therefore further analyses were conducted separately for each layer. Interestingly, the communities also differed significantly between the four different vegetation types both in organic (PERMANOVA; $R^2 = 0.15866$; $P < 0.001$) and in mineral layer (PERMANOVA; $R^2 = 0.13057$; $P < 0.01$). The communities in the organic layer were significantly

different at the meadow site compared to the deciduous or evergreen shrub sites (pairwise PERMANOVA, $P < 0.01$) or barren sites (pairwise PERMANOVA, $P < 0.05$), whereas communities in the mineral layer were significantly different only at the meadow and evergreen shrub sites (pairwise PERMANOVA, $P < 0.01$).

Genus-level comparisons were conducted for the metatranscriptomes among vegetation types and between different soil layers. Considering genera with mean abundance twofold larger than the 'mean of all genera, in the organic layer alphaproteobacterial genera *Bradyrhizobium*, *Nitrobacter*, *Rhodoplanes* and *Rhodomicrobium* were significantly (ANOVA/pairwise T-test; $P < 0.01$) more active in meadow sites than sites with other vegetation types (Figure 5a). In addition, *Acidothrmus* (Actinobacteria) was significantly more active in deciduous and evergreen shrub sites than in meadow sites, whereas *Roseiarcus* (Rhizobiales), *Acidipila* (Acidobacteriales), *Granulicella* (Acidobacteriales), *Edaphobacter* (Acidobacteriales), and *Gemmata* (Gemmatales) were more active in deciduous and evergreen shrub and barren sites than in meadow sites. The following five genera were significantly more active in the mineral layer across all vegetation types: *Anaeromyxobacter* (Myxococcales) and uncultured lineages from Ktedonobacterales, Pedosphaerales, and Pyrinomonadales. However, these were more active in meadow site compared to others (Figure 5b) and *Bauldia* (Rhizobiales) was more active in meadow and barren sites than in evergreen shrub sites (Figure 5b).

Figure 5. Boxplot showing genera with mean abundance larger than the twofold mean of all genera that are significantly more active in certain vegetation types in a) organic layer ($P < 0.01$) and b) mineral layer ($P < 0.01$).

3.3 Active microbial community functions across sites and vegetation types

The differences between the organic and mineral layer for protein-encoding genes were small but significant (PERMANOVA, $R^2 = 0.03044$, $P < 0.001$). Protein-encoding genes were significantly different (PERMANOVA, $R^2 = 0.09852$, $P < 0.001$) between vegetation types in the organic layer, and pairwise comparisons showed that meadow sites were different from shrub and barren sites (pairwise PERMANOVA, $P < 0.01$) and evergreen shrub sites differed from barren sites (pairwise PERMANOVA, $P < 0.05$). No significant differences for functional genes were observed between vegetation types in mineral layer.

The most abundantly transcribed genes that were mapped to KEGG (Kanehisa & Goto, 2000) pathways are involved in genetic information processing, including i) folding, sorting, and degradation, ii) transcription, and iii) metabolism (Figure 6a). ABC transporter genes were widely transcribed, including transport system substrate-binding proteins for ribose (*rbsB*), D-xylose

(*xylF*), and sorbitol/mannitol (*smoE*, *mtlE*), and multiple sugar transport system ATP-binding proteins (*msmX*, *msmK*, *malK*, *sugC*, *ggtA*, *msiK*), indicating the decomposition of plant polymers composed of these sugars. Other widely transcribed ABC transporters included branched-chain amino acid transport system protein genes (*livKGFHM*) and urea transport system substrate-binding protein genes (*urtA*). In addition to transporters, chaperon genes *groEL*, *dnaK*, and cold shock protein gene *cspA*, involved in survival in cold temperatures, were among the most transcribed genes in all samples. Gene *coxL/cutL* encoding the large subunit of aerobic carbon-monoxide dehydrogenase was widely expressed. Two genes involved in nitrogen uptake, glutamine synthetase *glnA* and ammonium transporter *amt*, were among the most abundant transcripts.

Interestingly, *pmoCAB-amuCAB* genes involved in methane and ammonia oxidation were transcribed significantly more (*pmoC*: $R^2 = 0.2539$, *pmoB*: $R^2 = 0.1541$, *pmoA* $R^2 = 0.2372$; $P < 0.001$; ANOVA) in the mineral layer than in the organic layer (Figure 6b). In KEGG classification, genes for methane and ammonia oxidation are not distinguished and therefore we performed BLAST (Altschul *et al.*, 1990) for these genes against a manually curated database of eight *pmoA* and eight *amoA* genes from different organisms. Results suggested that 96 % of the *pmoA-amOA* hits were *pmoA*, indicating activity of methane oxidation by particulate methane monooxygenase (pMMO) in mineral layers. There was virtually no transcription of soluble methane monooxygenase (sMMO) genes *mmoXYZBCD* or genes *mxoF* or *xoxF*, which encode the methanol dehydrogenases for methanol oxidation to formaldehyde (Figure 6b). Genes for formaldehyde assimilation in serine pathway, including *glyA* encoding glycine hydroxymethyltransferase, were transcribed. This indicates utilization of the serine cycle instead of the ribulosemonophosphate (*RuMP*) cycle for formaldehyde assimilation in these microbial communities.

Although some individual genes showed significantly different transcription in vegetation types (data not shown), transcriptome profiles based on KEGG hits across the vegetation types were mostly homogenous. Genes with a KEGG classification represented only a small fraction ($1.39 \pm 0.27\%$) of the reads that were recognized as protein-encoding genes. While genes with no KEGG classification were not analyzed in this study, they were mostly proteins with unknown function (Supplementary Figure S3).

differences at the genus level in the active microbes. Bacterial and archaeal communities and their activities in the organic layers differed across vegetation types. In particular, meadow sites were distinct from deciduous and evergreen shrub sites. Actinobacteria (*Acidothermus*), Acidobacteria (*Acidipila*, *Granulicella*, and *Edaphobacter*), and Planctomycetes (*Gemmata*) were significantly more active in the organic layer of the deciduous and evergreen shrub sites or barren sites than in meadow sites, whereas Alphaproteobacteria (*Bradyrhizobium*, *Nitrobacter*, *Rhodoplanes*, and *Rhodomicrobium*) were significantly more active in meadow sites.

In the organic layer, soil physicochemical factors also varied according to vegetation type, but not in the mineral layer. These linkages are likely related to the vegetation being the primary source of material for the organic layer, whereas the mineral layer properties are more affected by bedrock and soil texture, for example (Jenny 1941). Soil samples from shrub sites had significantly higher SOM concentrations and lower pH, whereas meadow sites had lower SOM concentrations and higher pH. The difference between shrub and meadow sites is presumably linked to the differences in organic matter quality; evergreen shrubs, such as *Empetrum nigrum*, (the dominant plant species of the study area) produce recalcitrant, acidic, and slowly decomposing litter, whereas meadows are dominated by forbs, grasses, and sedges, which produce litter that decomposes faster than the litter of *E. nigrum* (Hobbie *et al.*, 2000) and have higher nutrient concentrations. However, the two shrubs are difficult to separate as they usually form mixed communities within the study area. In the mineral layer, the environmental conditions can be more homogeneous and plant roots may not have a direct effect on the microbial community, which leads to more uniform microbial communities between different vegetation types. Although mineral samples were collected below the true rhizosphere, the indirect impact of plant roots is considerable in the proximate soil (Haichar *et al.*, 2008).

Acidobacteria were the most active phylum in all vegetation types, consistent with a previous PLFA study on the same area (Männistö *et al.*, 2007). The genera *Acidipila*, *Granulicella*, and *Edaphobacter* from order Acidobacteriales (also known as Subgroup 1), were particularly active in the organic layer with shrub vegetation. Of these, *Granulicella* are chemoorganotrophic bacteria capable of degrading various heteropolysaccharides, and several described species have been found in acidic upland soils (Pankratov & Dedysh, 2010; Männistö *et al.*, 2013). Acidobacteriales were also predominant in Northern Russian raised bogs (Ivanova *et al.*, 2020).

In this study, *Candidatus Solibacter* Ellin6076 and *Bryobacter* from Acidobacterial order Solibacterales were the second and third most active genera, with no significant variation according to vegetation type. Both have been found in acidic boreal soils (Kulichevskaya *et al.*, 2009; Rawat *et al.*, 2012). Genome analysis of *Candidatus Solibacter* revealed the ability not only to utilize

complex plant cell-wall polysaccharides and simple sugars but might also be able to use carbon monoxide (CO), a toxic gas, as a complementary energy source in a mixotrophic lifestyle (Ward *et al.*, 2009). Moreover, the carbon monoxide dehydrogenase gene *coxL/cutL* was widely expressed here, which may indicate the usage of CO in these soils. Thus, these bacteria are likely to have a role in carbon cycling in these tundra soils. Additionally, *Candidatus Solibacter* can participate in nitrogen cycling as they harbor candidate nitrate-reduction genes. *Bryobacter* are moss-associated, acidotolerant, strictly aerobic, slow-growing chemoorganotrophic bacteria, which inhabit acidic wetlands and soils and are also capable of hydrolyzing various heteropolysaccharides (Kulichevskaya *et al.*, 2009). These acidophilic bacteria likely have a role as diverse degraders of organic matter in tundra soils, as they have been shown to survive temperature and moisture fluctuation and low-nutrient conditions (Ward *et al.*, 2009; Rawat *et al.*, 2012).

Actinobacteria were widely represented in all vegetation types, with a large number of unclassified taxa. The most abundant active genus, *Acidothermus*, is the only described genus in the family *Acidothermaceae* and has one described species (*Acidothermus cellulolyticus*, Mohagheghi *et al.*, 1986). This thermophilic, acidophilic, and cellulolytic species was first isolated from acidic hot springs and has been studied mostly because of its endoglucanase for biotechnological applications (Mohagheghi *et al.*, 1986). In tundra soils, *Acidothermus* likely has a role as a degrader of cellulose from plant litter. Another active actinobacterial group abundant in these soils, order Solirubrobacterales, is not well characterized. These are mesophilic heterotrophs and based on 16S rRNA gene analysis, Solirubrobacterales have been encountered in diverse environments, such as weathered serpentine rock (Khilyas *et al.*, 2019), Antarctic soil (Chong *et al.*, 2012), agroecosystem soils (Shange *et al.*, 2012), *Sphagnum* peat bog (Ivanova *et al.*, 2016), and shrub-associated desert soil (Saul-Tcherkas & Steinberger, 2011). This wide range of habitats suggests that this order includes mixotrophic bacteria with the ability to survive in environments with fluctuating conditions, including temperature, nutrients, and UV radiation.

In addition to Actinobacteria and Acidobacteria, the following three orders from phylum Planctomycetes were among the most active bacterial groups in these soils: Gemmatales, Isosphaerales, and Tepidisphaerales. These orders are abundant in acidic raised bogs with high organic matter but low nutrient content (Ivanova *et al.*, 2020) and they have chitinolytic potential in *Sphagnum*-dominated wetlands (Ivanova *et al.*, 2018). *Aquisphaera* and *Singulisphaera* from order Isosphaerales were among the most active genera. These acidophilic bacteria have been isolated from northern Russian peatlands (Kulichevskaya *et al.*, 2009; Kulichevskaya *et al.*, 2012) and can degrade several biopolymers under acidic, microaerobic, and low-temperature conditions. Intriguingly, metatranscriptomes revealed that Planctomycetes were more active than what could be

expected based on the metagenomes, indicating that interpretation based solely on metagenomic data would underestimate their importance. As Planctomycetes were widely active in all vegetation types and genus *Gemmata* was significantly more active in the shrub sites, the results suggest that Planctomycetes are potentially active and diverse OM degraders in tundra soils.

Diverse Alphaproteobacteria were active in all sites, and the order Rhizobiales was particularly predominant in all vegetation types. *Roseiarcus* was among the most active genera in shrub sites with low pH and high OM. The only described species from this genus, *Roseiarcus fermentas*, was isolated from a methanotrophic consortium enriched in acidic *Sphagnum* peat (Kulichevskaya *et al.*, 2014). These bacteria belong to a cluster of environmental sequences, which have been obtained from wetlands and forest soils by molecular methods and are probably adapted to live in acidic soils in alternating high UV radiation and dark periods and varying levels of oxygen (Kulichevskaya *et al.*, 2014).

Potential CH₄ oxidation was observed particularly in the mineral layer, as particulate methane monooxygenase (pMMO) was transcribed together with the activity of methanotrophic bacteria, such as *Methylocapsa* (Rhizobiales). This genus has only a few described species, including *M. acidiphila* isolated from Siberian acidic *Sphagnum* bog (Dedysh *et al.*, 2002), *M. palsarum* isolated in subarctic palsa soil (Dedysh *et al.*, 2015), and *M. gorgona* from subarctic soil (Tveit *et al.*, 2015). These bacteria possess pMMO and use the serine pathway for one-carbon compound utilization, which was also transcribed here. Additionally, *Methylocystis*, *Methylovirgula*, and *Methylocella* showed activity in these soils. Contrastingly, Singleton *et al.* (2018) found gammaproteobacterial but no alphaproteobacterial methanotrophs active in permafrost thaw fens. Based on the results shown here and by others (Belova *et al.*, 2020), *Methylocapsa* and other methanotrophs from order the Rhizobiales could have a significant role as CH₄ oxidizers in subarctic tundra soils.

Several other Rhizobiales with diverse metabolism were particularly active in meadows. Although many known members of *Bradyrhizobium* are chemoorganotrophs and symbiotic N-fixers, some grow chemolithotrophically in the presence of H₂, CO₂, and low levels of O₂. Genes involved in N fixation were not observed in these soils. Nitrite-oxidizing *Nitrobacter* and anaerobic photoheterotrophic *Rhodoplanes* with capacity for complete denitrification were active in meadow sites. Overall, Alphaproteobacteria are an active and metabolically diverse group in these tundra soils.

We observed the activity of several candidate divisions of phylum Chloroflexi, especially in the mineral layer. One of the most diverse and active divisions were Ktedonobacterales, which are abundant in extreme environments with fluctuating temperature, intense solar radiation, and low

OM content. Ktedonobacterales are also potential atmospheric CO and H₂ oxidizers (King & King, 2014; Islam *et al.*, 2019). In addition to Chloroflexi, members of the uncultivated phyla Dormibacterota and Eremiobacterota were active in the mineral layer. As Ktedonobacteria, they can exploit atmospheric H₂, CO₂, and CO for energy and carbon sources (Ji *et al.*, 2017). Transcription of carbon monoxide dehydrogenase might indicate Ktedonobacterales, Dormibacterota and Eremiobacterota and the previously mentioned *Candidatus* Solibacter oxidize CO in these soils. *Candidatus* Udaeobacter and uncultured lineage ADurb.Bin063-1 from phylum Verrucomicrobia were particularly active in meadows. *Candidatus* Udaeobacter potentially oxidizes trace gas H₂ to generate energy and benefits antibiotics release in soil (Willms *et al.*, 2020). These are poorly characterized bacteria, yet they were active and potentially have traits to survive in harsh, competitive conditions with the ability to scavenge atmospheric trace gases.

Archaeal 16S rRNA represented only 0.1% in the studied tundra samples. Thaumarchaeal genera *Nitrososphaera* and *Nitrosotalea* and candidate division Group 1.1c were the most active Archaea in the soils and were significantly more active in the mineral layer. *Nitrososphaera* and *Nitrosotalea* are capable of NH₃ oxidation but are suspected of a mixotrophic or even heterotrophic lifestyle (Tourn *et al.*, 2011; Lehtovirta-Morley *et al.*, 2014). The cultured strains were isolated from temperate soils and their temperature optima were at mesophilic scale. Group 1.1c is the dominating archaeal taxa in temperate acidic forest and forest peat soils (Kemnitz *et al.*, 2007; Stopnišek Nejc *et al.*, 2010). These archaea grow at low pH (Lehtovirta *et al.*, 2009) at an optimal temperature of 30°C (Weber *et al.*, 2015). Group 1.1c does not oxidize NH₃ or carry the gene *amoA*, in contrast to cultured relatives from groups 1.1a and 1.1b do, which is why they are suspected to be heterotrophic (Weber *et al.*, 2015). Temperature may be the growth-limiting factor of these Archaea, and their activity in carbon-rich northern soils may be higher with warming climate. Indeed, they do have a role in the tundra soil ecosystem that is yet to be discovered.

5 Conclusions

Understanding the microbial communities and their functions is key to acquiring a process-level understanding about tundra biogeochemistry and accurate models of greenhouse gas release. It is of global relevance to investigate this particularly in the Arctic, where soil organic carbon stocks are large and vulnerable to climate change and have drastic impacts on the global carbon cycle. Our results revealed the large number of active bacteria and archaea across tundra soils. The activity of these communities varied according to vegetation types; in particular, communities in the organic layer at the meadow sites were distinct from deciduous and evergreen shrub sites. Communities at

the deciduous and evergreen shrub sites had a similar microbial community composition and activity. Vegetation type-driven differences in microbial communities in the mineral layer were less evident. Potential organic matter degraders belonging to Actinobacteria, Acidobacteria, Alphaproteobacteria, and Planctomycetes were active in shrub soils high SOM content, whereas poorly known Chloroflexi and Dormibacterota were active in the deeper, low SOM mineral layer. Chloroflexi and Dormibacterota use potentially atmospheric trace gases for growth. In addition, alphaproteobacterial methanotrophs are potential moderators of methane release, particularly in the mineral layers of subarctic tundra soils. Genes involved in nitrogen fixation were not observed in these soils, however nitrite-oxidizing *Nitrobacter* sp. and *Rhodoplanes* sp. with capacity for complete denitrification were active in meadow sites. Microbes in these soils thus impact the tundra ecosystem functioning in various ways and play an important role in climate change feedbacks.

6 Acknowledgments

The study was funded by the Academy of Finland (Grant 1314114) and the University of Helsinki three-year grant to JH. SV was funded by the Doctorate Program in Microbiology and Biotechnology. PN was funded by Kone Foundation and Nessling Foundation. JK was funded by the Arctic Interactions at the University of Oulu and Academy of Finland (grant no. 318930, Profi 4). AMV acknowledges Gordon and Betty Moore Foundation (Grant 8414). Permission to perform fieldwork was granted by Metsähallitus. We acknowledge the CSC IT center for science LTD for the computational resources and Mr. Kimmo Mattila for assistance. We acknowledge the Kilpisjärvi Biological Station and staff and members of the BioGeoClimate Modelling Lab for use of the premises and assistance with fieldwork.

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