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

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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 methanothrophs, 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 (CO_2) 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 CO_2 (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; Kemppinen *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 metatransctriptomics 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 km^2 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

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

122 **2.2 Soil physicochemical data**

123 For analysis of soil physicochemical properties, approximately 0.2 dm^3 of soil was collected with a
124 steel cylinder (diameter 5.5 cm, height 7.5) and stored at 4 °C. The soils were lyophilized according
125 to the Finnish standard SFS300 and pH analysis was performed according to international standard
126 ISO 10390. SOM content was analyzed by loss on ignition analysis according to standard SFS
127 3008. CNS analysis (carbon, nitrogen, and sulfur) was performed with a Vario Micro Cube
128 (Elementar, Langenselbold, Germany). Mineral samples were sieved through a 2-mm plastic sieve
129 and the organic samples for the CNS analysis were homogenized by hammering the material into
130 smaller pieces.

131 **2.3 Nucleic acid extraction**

132 Three replicate DNA/RNA extractions were performed for each sample. Samples were kept on ice
133 during weighing and extraction and all steps were performed promptly with nuclease-free labware
134 to avoid RNA degradation. Solutions and water were treated with 0.1% diethylpyrocarbonate.
135 Nucleic acids were extracted using a modified hexadecyltrimethylammonium bromide (CTAB),
136 phenol-chloroform, and bead-beating protocol (Griffiths *et al.*, 2000; DeAngelis *et al.*, 2009). On
137 dry ice, approximately 0.5 g of frozen soil was transferred to a 2-mL Lysing Matrix E tube (MP
138 Biomedicals, Heidelberg, Germany) and 0.5 mL of CTAB buffer (consisting of equal amounts of
139 10% CTAB in 1 M sodium chloride and 0.5 M phosphate buffer in 1 M NaCl), 50 µl 0.1 M
140 ammonium aluminum sulfate ($NH_4(SO_4)_2 \cdot 12\ H_2O$), and 0.5 mL phenol:chloroform:isoamyl
141 alcohol (25:24:1) was added. After bead-beating with FastPrep (MP Biomedicals, Heidelberg,
142 Germany) at 5.5 m s^{-1} for 30 s, 0.5 mL chloroform was added. Nucleic acids were precipitated with
143 polyethylene glycol 6000 (PEG6000, 30% in 1.6 M NaCl) and washed with ethanol. Nucleic acids
144 were once again extracted from the leftover soil pellet to maximize RNA. Nucleic acids were
145 resuspended in 25 µl of Buffer EB and 250 µl Buffer RLT with β-mercaptoethanol added. Buffers
146 EB and RLT were from an AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). All
147 centrifugations were performed at 4°C. Finally, RNA and DNA were purified with AllPrep

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

153 **2.4 Sequencing**

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

162 **2.5 Data processing and analysis**

163 As the total RNA approach was used (Urich *et al.*, 2008), ribosomal RNA was not depleted after
164 extraction and RNA transcripts were sequenced. One sample (site 11202, organic layer) yielded
165 80.4 million reads and it was reduced randomly with seqtk (Li 2016) with random seed 100 to a 4-
166 million read subset. The quality of the sequences was assessed using default settings with FastQC
167 (v. 0.11.5, Andrews 2010) and MultiQC (v. 1.3, (Ewels *et al.*, 2016)) before and after trimming.
168 Trimming and quality filtering was performed with Cutadapt (v. 1.10, (Martin, 2011)) with quality
169 cutoff 25 and minimum adapter overlap 10. A mineral layer sample from site 11210 was discarded
170 due to a low number of reads. Metaxa2 (v. 2.1.3, (Bengtsson-Palme *et al.*, 2018)) was used to
171 identify reads mapping to the small subunit (SSU) rRNA. These were then classified using the
172 mothur (v. 1.40.5, (Schloss *et al.*, 2009)) classify.seqs command with a confidence cutoff of 60%
173 and SILVA release 132 (Quast *et al.*, 2013) as the reference database. The taxonomy of abundant
174 taxa was manually updated according to the Genome Taxonomy database (Parks *et al.*, 2018; Parks
175 *et al.*, 2020). Reads were mapped onto the Kyoto Encyclopedia of Genes and Genomes (KEGG)
176 Prokaryote database release 86 (Kanehisa & Goto, 2000) using DIAMOND blastx (v. 2.1.3,
177 Buchfink *et al.* 2015) with an E-value of 0.001. Blast results were parsed with KEGG-tools (v. 1.3,
178 Pessi 2020). Briefly, the KEGG orthology (KO) identifier of the best BLAST hit was assigned to

179 each read and mapped to the KEGG module hierarchy, and spurious pathways were removed using
180 MinPath (v. 1.4, (Ye & Doak, 2009). Since genes for methane and ammonia oxidation are not
181 distinguished in KEGG classification we performed BLAST (Altschul *et al.*, 1990) for these genes
182 against a manually curated database of eight *pmoA* and eight *amoA* genes from bacteria and
183 archaea.
184 Due to differences in read numbers per sample, the metatranscriptome data were
185 subsampled for statistical analyses of protein-encoding genes to avoid skewing caused by the low
186 number of observations in low-read samples. After subsampling, the number of samples represented
187 in each vegetation type was 6 (barren), 7 deciduous shrub, 18 (evergreen shrub) in the mineral layer
188 and 5 (barren), 6 (deciduous shrub), 19 (evergreen shrub), and 8 (meadow) in the organic layer. For
189 the mineral layer, meadow samples were discarded from statistical analyses due to a low sample
190 number (n=4) after subsampling. Statistical analyses and visualization were performed with R (v.
191 3.6.2, R Core Team 2020). Differences between microbial communities and functional genes
192 among vegetation types (Table 1) were tested with permutational multivariate analysis of variance
193 using distance matrices (PERMANOVA, function adonis, R-package vegan v. 2.5-6, Oksanen et al.
194 2019) and pairwise permutation MANOVA (functions pairwise.perm.manova R-package
195 RVAideMemoire v.0.9-78 (Hervé, 2021) and vegdist, R-package vegan v. 2.5-6). For the statistical
196 analyses of functional genes, samples were subsampled from 116 to 73 samples to avoid skewing
197 caused by the low number of observations, using a limit of 9654 KEGG hits (function rrarefy, R-
198 package vegan v. 2.5-6). Differences between vegetation types for bacterial and archaeal genera and
199 between soil types for selected genes were tested with one-way analysis of variance (ANOVA,
200 functions lm and aov, R-core package) and pairwise t-test (function pairwise.t.test, R-core package).
201 Soil physicochemical properties were tested with Kruskal-Wallis test and pairwise Wilcoxon test
202 with Bonferroni correction (functions kruskal.test and pairwise.wilcox.test, R-core package).
203 Differences in microbial communities in metagenomic and metatranscriptomic datasets were
204 visualized in principal coordinates analysis plot, using Bray-Curtis dissimilarities (function
205 ordinate, Phyloseq v. 1.30.0 (McMurdie & Holmes, 2013).
206
207 **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**

208

2.6 Data availability

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

3 Results

3.1 Soil conditions across vegetation types

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

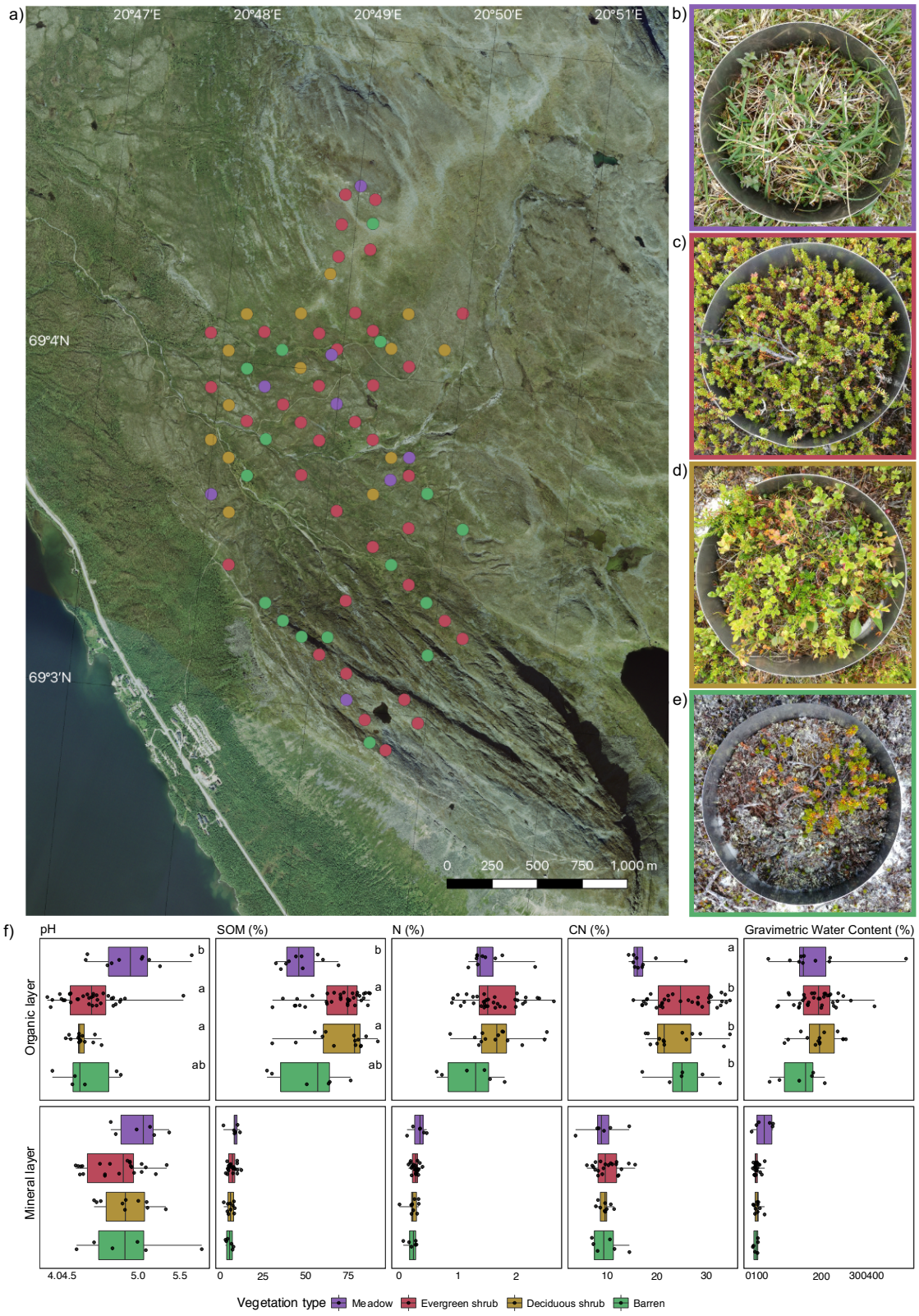
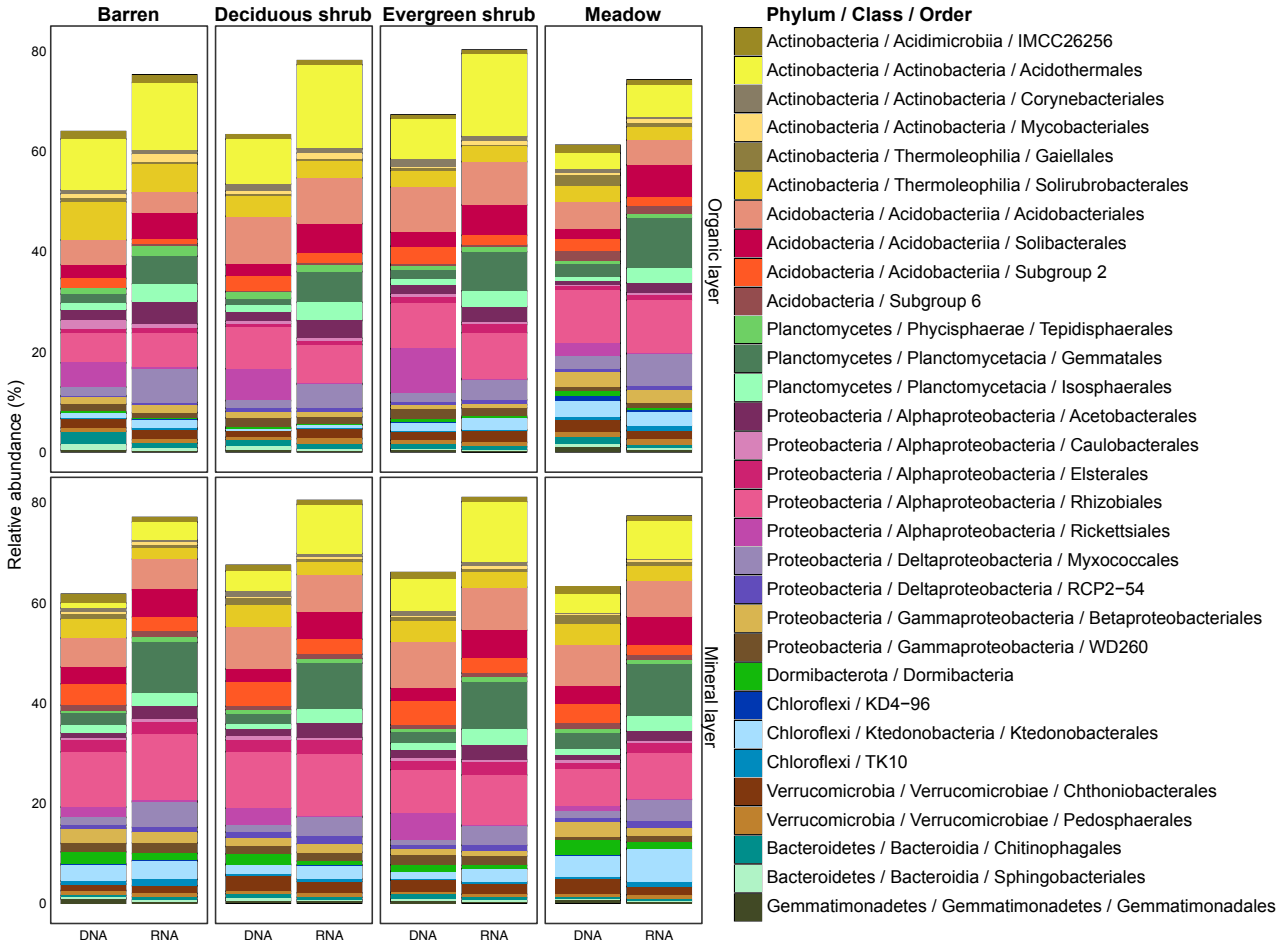


Figure 1. a) Map of sampling sites. Saana fell is located in the southern part of the study area. Typical vegetation in meadow (b), evergreen shrub (c), deciduous shrub (d), and barren (e) sites. f) Soil physicochemical properties (pH, soil organic matter (SOM), nitrogen (N), carbon-nitrogen ratio (C/N), 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.

230 **3.2 Microbial community composition across sites and vegetation types**

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

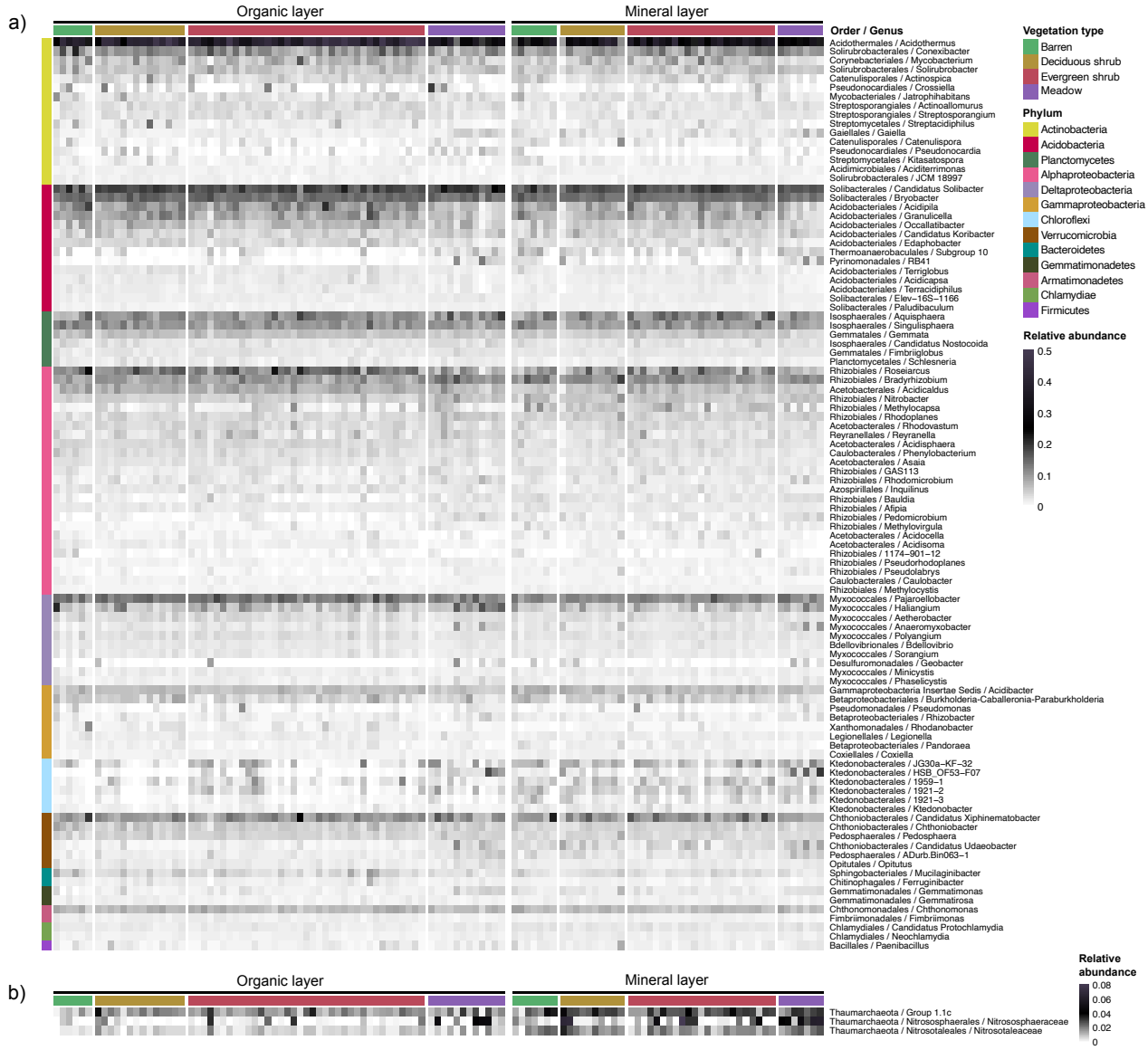


239

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

243 According to the analysis of bacterial 16S rRNA, the predominant active phyla in the
244 microbial communities were Actinobacteria (27 ± 9%) with order Acidothermales and
245 Solirubrobacterales; Acidobacteria (17 ± 3%) with orders Acidobacteriales and Solibacterales;
246 Alphaproteobacteria (16 ± 3%) with orders Rhizobiales and Acetobacterales, and Planctomycetes

247 (14 ± 3%) with order Gemmatales (Figure 2). Moreover, Deltaproteobacteria, Chloroflexi,
248 Gammaproteobacteria, and Verrucomicrobia were also active in these soils. Out of the Archaeal
249 reads Thaumarchaea (0.12 ± 0.15%) were most active, with Group 1.1c (0.05 ± 0.05%) and
250 Nitrososphaerales (0.05 ± 0.11%) being the most active taxa. The relative abundances at the genus
251 level in each vegetation type and soil layer are listed in Supplementary table S2.

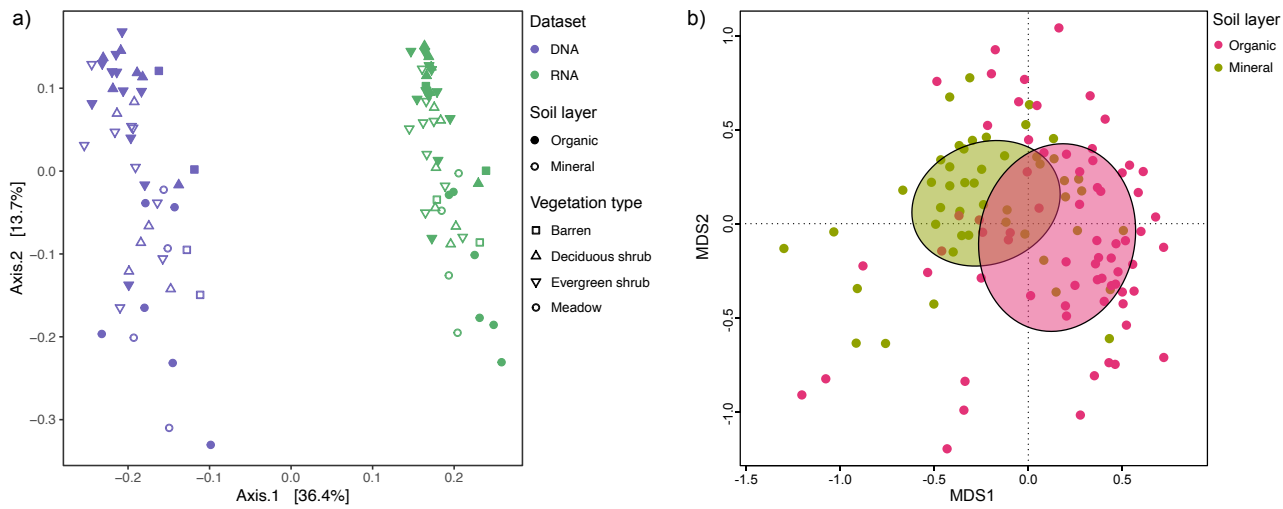


252

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

257 The most active genera were *Acidothermus* (Class Actinobacteria, 13 ± 5.7%) and
258 *Candidatus* Solibacter (Acidobacteriia, 3.11 ± 0.83%), followed by *Bryobacter* (Acidobacteriia,
259 2.06 ± 0.38%) *Pajaroellobacter* (Deltaproteobacteria, 1.64 ± 0.55%), and *Roseiarcus*
260 (Alphaproteobacteria, 1.48 ± 1.07%, Figure 3a). Archaea were not particularly abundant in the

261 metatranscriptomes of these soils, but the most active archaeal groups belonging to Thaumarchaea
262 were more active in the mineral than organic layer (Figure 3b).
263 When comparing the 16S rRNA from metatranscriptomes (i.e., RNA; active microbes) to
264 the 16S rRNA genes from metagenomes (i.e., DNA, Pessi *et al.*, 2020), the most abundant and
265 active microbial groups were the same but notable differences appeared among the datasets (Figures
266 2 and 4a). Orders with greater relative abundance in metatranscriptomes included Gemmatales
267 (Planctomycetes), Acidothermales (Actinobacteria), Solibacterales (Acidobacteria), and
268 Myxococcales (Deltaproteobacteria), whereas Subgroup 2 (Acidobacteria), Rickettsiales
269 (Alphaproteobacteria), Dormibacterota, Chitinophagales (Verrucomicrobia), and
270 Gemmatimonadales (Gemmatimonadetes) showed higher relative abundance in metagenomes. In
271 metatranscriptomes, the orders with relative abundance more than 1% covered 78 ± 0.02% of active
272 orders, but only 64 ± 0.02% in metagenomes. Additionally, more than 1500 bacterial and archaeal
273 genera were identified from metatranscriptomes and less than 500 bacterial and archaeal genera
274 from metagenomes.
275



276
277 **Figure 4.** a. Principal coordinates analysis plot showing differences between 16S rRNA genes from
278 metagenomes and 16S rRNA from metatranscriptomes among vegetation types and soil layers,
279 based on square root-transformed Bray-Curtis dissimilarities. b. Ordination plot showing the
280 differences in active microbial communities through 16S rRNA genes in organic and mineral layer.

281 Active microbial communities were different in organic and mineral layers (PERMANOVA; R^2 =
282 0.06642; $P < 0.001$, Figure 4b) and therefore further analyses were conducted separately for each
283 layer. Interestingly, the communities also differed significantly between the four different
284 vegetation types both in organic (PERMANOVA; $R^2 = 0.15866$; $P < 0.001$) and in mineral layer
285 (PERMANOVA; $R^2 = 0.13057$; $P < 0.01$). The communities in the organic layer were significantly

286 different at the meadow site compared to the deciduous or evergreen shrub sites (pairwise
287 PERMANOVA, P <0.01) or barren sites (pairwise PERMANOVA, P <0.05), whereas communities
288 in the mineral layer were significantly different only at the meadow and evergreen shrub sites
289 (pairwise PERMANOVA, P <0.01).
290 Genus-level comparisons were conducted for the metatranscriptomes among vegetation
291 types and between different soil layers. Considering genera with mean abundance twofold larger
292 than the ´mean of all genera, in the organic layer alphaproteobacterial genera *Bradyrhizobium*,
293 *Nitrobacter*, *Rhodoplanes* and *Rhodomicrobium* were significantly (ANOVA/pairwise T-test;
294 P<0.01) more active in meadow sites than sites with other vegetation types (Figure 5a). In addition,
295 *Acidothermus* (Actinobacteria) was significantly more active in deciduous and evergreen shrub sites
296 than in meadow sites, whereas *Roseiarcus* (Rhizobiales), *Acidipila* (Acidobacteriales), *Granulicella*
297 (Acidobacteriales), *Edaphobacter* (Acidobacteriales), and *Gemmata* (Gemmatales) were more
298 active in deciduous and evergreen shrub and barren sites than in meadow sites. The following five
299 genera were significantly more active in the mineral layer across all vegetation types:
300 *Anaeromyxobacter* (Myxococcales) and uncultured lineages from Ktedonobacterales,
301 Pedospherales, and Pyrinomonadales. However, these were more active in meadow site compared
302 to others (Figure 5b) and *Bauldia* (Rhizobiales) was more active in meadow and barren sites than in
303 evergreen shrub sites (Figure 5b).

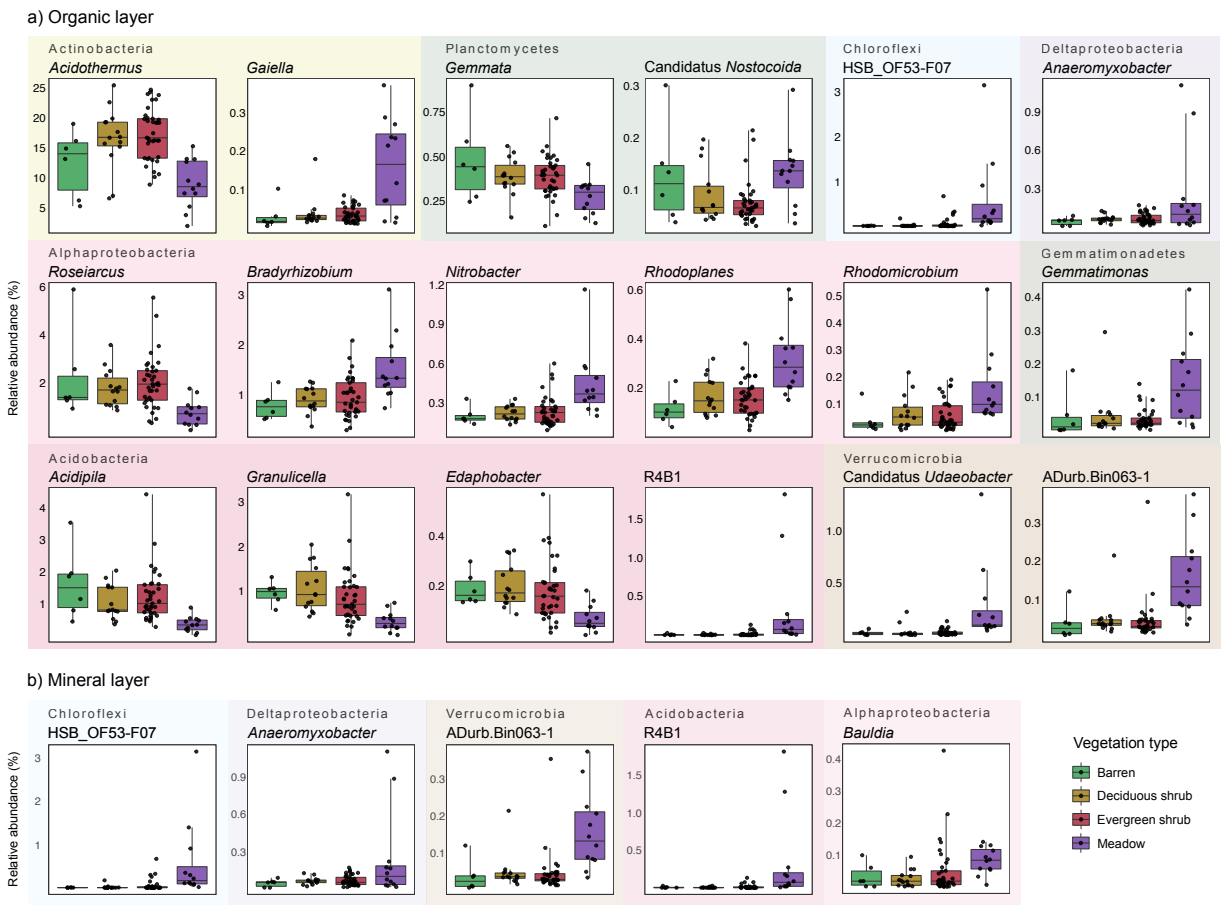


Figure 5. Boxplot showing genera with mean abundance larger than the twofold mean of all genera that are significantly more active in certain vegetation types in a) organic layer ($P < 0.01$) and b) mineral layer ($P < 0.01$).

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

320 (*xylF*), and sorbitol/mannitol (*smoE*, *mtlE*), and multiple sugar transport system ATP-binding
321 proteins (*msmX*, *msmK*, *malK*, *sugC*, *ggtA*, *msiK*), indicating the decomposition of plant polymers
322 composed of these sugars. Other widely transcribed ABC transporters included branched-chain
323 amino acid transport system protein genes (*livKGFHM*) and urea transport system substrate-binding
324 protein genes (*urtA*). In addition to transporters, chaperon genes *groeL*, *dnaK,* and cold shock
325 protein gene *cspA*, involved in survival in cold temperatures, were among the most transcribed
326 genes in all samples. Gene *coxL*/*cutL* encoding the large subunit of aerobic carbon-monoxide
327 dehydrogenase was widely expressed. Two genes involved in nitrogen uptake, glutamine synthetase
328 *glnA* and ammonium transporter *amt*, were among the most abundant transcripts.
329 Interestingly, *pmoCAB-amoCAB* genes involved in methane and ammonia oxidation were
330 transcribed significantly more (*pmoC:* R^2 = 0.2539, *pmoB:* R^2 = 0.1541, *pmoA* R^2 = 0.2372; $P <$
331 0.001; ANOVA) in the mineral layer than in the organic layer (Figure 6b). In KEGG classification,
332 genes for methane and ammonia oxidation are not distinguished and therefore we performed
333 BLAST (Altschul *et al.*, 1990) for these genes against a manually curated database of eight *pmoA*
334 and eight *amoA* genes from different organisms. Results suggested that 96 % of the *pmoA-amoA*
335 hits were *pmoA*, indicating activity of methane oxidation by particulate methane monooxygenase
336 (pMMO) in mineral layers. There was virtually no transcription of soluble methane monooxygenase
337 (sMMO) genes *mmoXYZBCD* or genes *mxaF* or *xoxF*, which encode the methanol dehydrogenases
338 for methanol oxidation to formaldehyde (Figure 6b). Genes for formaldehyde assimilation in serine
339 pathway, including *glyA* encoding glycine hydroxymethyltransferase, were transcribed. This
340 indicates utilization of the serine cycle instead of the ribulosemonophosphate (*RuMP*) cycle for
341 formaldehyde assimilation in these microbial communities.
342 Although some individual genes showed significantly different transcription in vegetation
343 types (data not shown), transcriptome profiles based on KEGG hits across the vegetation types were
344 mostly homogenous. Genes with a KEGG classification represented only a small fraction (1.39 ±
345 0.27%) of the reads that were recognized as protein-encoding genes. While genes with no KEGG
346 classification were not analyzed in this study, they were mostly proteins with unknown function
347 (Supplementary Figure S3).

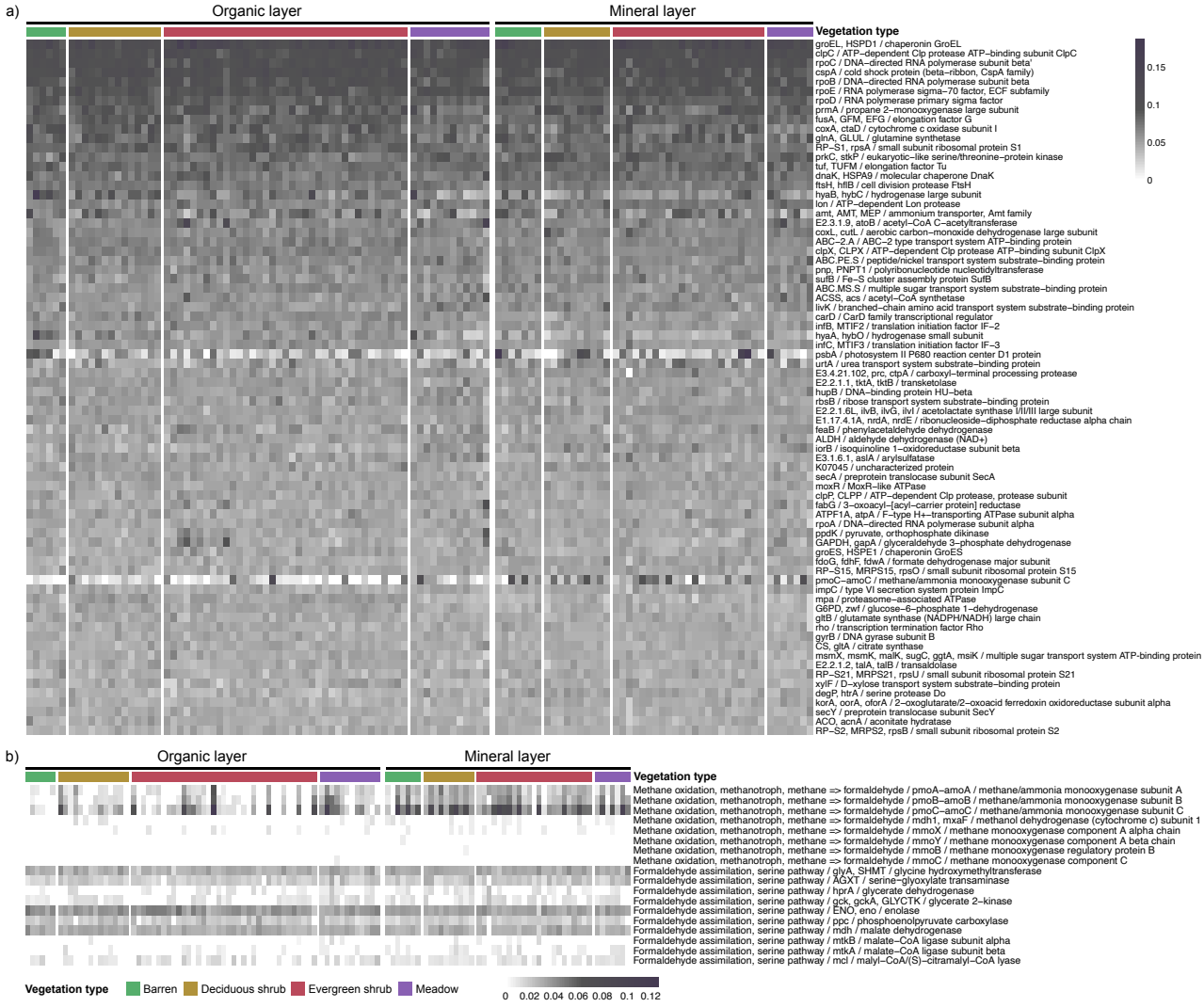


Figure 6. a) KEGG classified square-root transformed gene transcripts with mean relative abundance > 0.002. b) KEGG classified square-root transformed gene transcripts for methane oxidation and serine pathway formaldehyde assimilation. A column of pixels represents a site.

4 Discussion

We investigated over 100 soil samples across a subarctic landscape to gain new insights into the microbial community composition together with their previously uncharacterized functions in the tundra. This study suggests that microbial community composition and activity are strongly linked to vegetation types via soil moisture as well as organic matter quality and quantity.

The most active microbial phyla were Actinobacteria, Acidobacteria, Alphaproteobacteria, and Planctomycetes, consistent with previous studies from Arctic regions (Hultman *et al.*, 2015; Tas *et al.*, 2018; Tripathi *et al.*, 2019). These phyla, excluding Planctomycetes, were also among the most abundant in the metagenomics dataset from these samples (Pessi et al., 2020). Although the microbial community composition was similar across vegetation types, there were significant

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

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

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

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

396 complex plant cell-wall polysaccharides and simple sugars but might also be able to use carbon
397 monoxide (CO), a toxic gas, as a complementary energy source in a mixotrophic lifestyle (Ward *et*
398 *al.*, 2009). Moreover, the carbon monoxide dehydrogenase gene *coxL*/*cutL* was widely expressed
399 here, which may indicate the usage of CO in these soils. Thus, these bacteria are likely to have a
400 role in carbon cycling in these tundra soils. Additionally, *Candidatus* Solibacter can participate in
401 nitrogen cycling as they harbor candidate nitrate-reduction genes. *Bryobacter* are moss-associated,
402 acidotolerant, strictly aerobic, slow-growing chemoorganotrophic bacteria, which inhabit acidic
403 wetlands and soils and are also capable of hydrolyzing various heteropolysaccharides
404 (Kulichevskaya *et al.*, 2009). These acidophilic bacteria likely have a role as diverse degraders of
405 organic matter in tundra soils, as they have been shown to survive temperature and moisture
406 fluctuation and low-nutrient conditions (Ward *et al.*, 2009; Rawat *et al.*, 2012).
407 Actinobacteria were widely represented in all vegetation types, with a large number of
408 unclassified taxa. The most abundant active genus, *Acidothermus*, is the only described genus in the
409 family *Acidothermaceae* and has one described species (*Acidothermus cellulolyticus,* Mohagheghi
410 *et al.*, 1986). This thermophilic, acidophilic, and cellulolytic species was first isolated from acidic
411 hot springs and has been studied mostly because of its endoglucanase for biotechnological
412 applications (Mohagheghi *et al.*, 1986). In tundra soils, *Acidothermus* likely has a role as a degrader
413 of cellulose from plant litter. Another active actinobacterial group abundant in these soils, order
414 Solirubrobacterales, is not well characterized. These are mesophilic heterotrophs and based on 16S
415 rRNA gene analysis, Solirubrobacterales have been encountered in diverse environments, such as
416 weathered serpentine rock (Khilyas *et al.*, 2019), Antarctic soil (Chong *et al.*, 2012), agroecosystem
417 soils (Shange *et al.*, 2012), *Sphagnum* peat bog (Ivanova *et al.*, 2016), and shrub-associated desert
418 soil (Saul-Tcherkas & Steinberger, 2011). This wide range of habitats suggests that this order
419 includes mixotrophic bacteria with the ability to survive in environments with fluctuating
420 conditions, including temperature, nutrients, and UV radiation.
421 In addition to Actinobacteria and Acidobacteria, the following three orders from phylum
422 Planctomycetes were among the most active bacterial groups in these soils: Gemmatales,
423 Isosphaerales, and Tepidisphaerales. These orders are abundant in acidic raised bogs with high
424 organic matter but low nutrient content (Ivanova *et al.*, 2020) and they have chitinolytic potential in
425 *Sphagnum*-dominated wetlands (Ivanova *et al.*, 2018). *Aquisphera* and *Singulisphaera* from order
426 Isosphaerales were among the most active genera. These acidophilic bacteria have been isolated
427 from northern Russian peatlands (Kulichevskaya *et al.*, 2009; Kulichevskaya *et al.*, 2012) and can
428 degrade several biopolymers under acidic, microaerobic, and low-temperature conditions.
429 Intriguingly, metatranscriptomes revealed that Planctomycetes were more active than what could be

430 expected based on the metagenomes, indicating that interpretation based solely on metagenomic
431 data would underestimate their importance. As Planctomycetes were widely active in all vegetation
432 types and genus *Gemmata* was significantly more active in the shrub sites, the results suggest that
433 Planctomycetes are potentially active and diverse OM degraders in tundra soils.
434 Diverse Alphaproteobacteria were active in all sites, and the order Rhizobiales was
435 particularly predominant in all vegetation types. *Roseiarcus* was among the most active genera in
436 shrub sites with low pH and high OM. The only described species from this genus, *Roseiarcus*
437 *fermentas*, was isolated from a methanotrophic consortium enriched in acidic *Sphagnum* peat
438 (Kulichevskaya *et al.*, 2014). These bacteria belong to a cluster of environmental sequences, which
439 have been obtained from wetlands and forest soils by molecular methods and are probably adapted
440 to live in acidic soils in alternating high UV radiation and dark periods and varying levels of oxygen
441 (Kulichevskaya *et al.*, 2014).
442 Potential CH_4 oxidation was observed particularly in the mineral layer, as particulate
443 methane monooxygenase (pMMO) was transcribed together with the activity of methanotrophic
444 bacteria, such as *Methylocapsa* (Rhizobiales). This genus has only a few described species,
445 including *M. acidiphila* isolated from Siberian acidic *Sphagnum* bog (Dedysh *et al.*, 2002), *M.*
446 *palsarum* isolated in subarctic palsa soil (Dedysh *et al.*, 2015), and *M. gorgona* from subarctic soil
447 (Tveit *et al.*, 2015). These bacteria possess pMMO and use the serine pathway for one-carbon
448 compound utilization, which was also transcribed here. Additionally, *Methylocystis*,
449 *Methylovirgula,* and *Methylocella* showed activity in these soils. Contrastingly, Singleton et al.
450 (2018) found gammaproteobacterial but no alphaproteobacterial methanotrophs active in permafrost
451 thaw fens. Based on the results shown here and by others (Belova *et al.*, 2020), *Methylocapsa* and
452 other methanotrophs from order the Rhizobiales could have a significant role as CH_4 oxidizers in
453 subarctic tundra soils.
454 Several other Rhizobiales with diverse metabolism were particularly active in meadows.
455 Although many known members of *Bradyrhizobium* are chemoorganotrophs and symbiotic N-
456 fixers, some grow chemolithotrophically in the presence of H_2, CO_2, and low levels of O_2. Genes
457 involved in N fixation were not observed in these soils. Nitrite-oxidizing *Nitrobacter* and anaerobic
458 photoheterotrophic *Rhodoplanes* with capacity for complete denitrification were active in meadow
459 sites. Overall, Alphaproteobacteria are an active and metabolically diverse group in these tundra
460 soils.
461 We observed the activity of several candidate divisions of phylum Chloroflexi, especially in
462 the mineral layer. One of the most diverse and active divisions were Ktedonobacterales, which are
463 abundant in extreme environments with fluctuating temperature, intense solar radiation, and low

OM content. Ktedonobacterales are also potential atmospheric CO and H_2 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 H_2, CO_2, 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 H_2 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 *Nitrosophaera* 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. *Nitrosophaera* and *Nitrosotalea* are capable of NH_3 oxidation but are suspected of a mixotrophic or even heterotrophic lifestyle (Tourna *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 NH_3 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.

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