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Depth-specific distribution of bacterial MAGs in permafrost active layer in Ny Ålesund, Svalbard (79°N)

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ABSTRACT

Arctic soil microbial communities may shift with increasing temperatures and water availability from climate change. We examined temperature and volumetric liquid water content (VWC) in the upper 80 cm of permafrost-affected soil over 2 years (2018–2019) at the Bayelva monitoring station, Ny Ålesund, Svalbard. We show VWC increases with depth, whereas *in situ* temperature is more stable vertically, ranging from −5°C to 5 °C seasonally. Prokaryotic metagenome-assembled genomes (MAGs) were obtained at 2–4 cm vertical resolution collected while frozen in April 2018 and at 10 cm vertical resolution collected while thawed in September 2019. The most abundant MAGs were *Acidobacteriota*, *Actinomycetota*, and *Chloroflexota*. *Actinomycetota* and *Chloroflexota* increase with depth, while *Acidobacteriota* classes *Thermoanaerobaculia* Gp7-AA8, *Blastocatellia* UBA7656, and *Vicinamibacteria Vicinamibacterales* are found above 6 cm, below 6 cm, and below 20 cm, respectively. All MAGs have diverse carbon-degrading genes, and *Actinomycetota* and *Chloroflexota* have autotrophic genes. Genes encoding β -glucosidase, N-acetyl-β-D-glucosaminidase, and xylosidase increase with depth, indicating a greater potential for organic matter degradation with higher VWC. *Acidobacteriota* dominate the top 6 cm with their classes segregating by depth, whereas *Actinomycetota* and *Chloroflexota* dominate below ~6 cm. This suggests that *Acidobacteriota* classes adapt to lower VWC at the surface, while *Actinomycetota* and *Chloroflexota* persist below 6 cm with higher VWC. This indicates that VWC may be as important as temperature in microbial climate change responses in Arctic mineral soils. Here we describe MAG-based Seqcode type species in the *Acidobacteriota*, *Onstottus arcticum, Onstottus frigus*, and *Gilichinskyi gelida* and in the *Actinobacteriota*, *Mayfieldus profundus*.

Introduction

The high Arctic archipelago, Svalbard, experiences amplified effects of climate change because it receives warm water from the Atlantic Ocean and warm air from greenhouse gas amplification at the pole (Lawrence & Slater et al., 2005). Temperatures on Svalbard are often close to the freezing point, especially in the vicinity of the coasts in the southern archipelago, hence soil has been observed to be warming since observations began in 1998 (Boike et al., 2018; Cohen et al., 2014; Pörtner et al., 2019; Grünberg et al., 2024). Air temperature warming on Svalbard has been even stronger than the Arctic average, with warming rates of about 1.5 °C per decade from 1991 to 2020 (Isaksen et al., 2022;

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Nordli et al., 2020) linked to strong sea-ice decline in that region. However, to fully assess the thermal status of the soil, volumetric liquid water content (VWC) measurements are required in addition to temperatures. Geological models investigating the impact of soil destabilization on the greenhouse gas budget commonly incorporate the microbial degradation of soil organic matter (Bardgett et al., 2008; Johnston et al., 2019; Tarnocai et al., 2009). Permafrost temperatures on Svalbard are highest in the vicinity of the coasts and often are close to the freezing point (Keating et al., 2018), so most of the energy from temperature changes goes into phase changes of water (Boike et al., 2018). Therefore, microbial communities will also experience climate change as having access to liquid water more frequently, over longer time intervals, and in greater volumes. VWC is also related to osmolarity in the frozen soils, since water excludes solutes when it freezes, leaving behind higher osmolarity liquid water, which can have an effect on the microbial community (Schostag et al., 2015). Given that seasonal freezing and thawing are transmitted vertically, microbial reactions to the increased access to liquid water are likely to be depth-dependent over the scale of centimeters. These soils contain diverse microbial populations capable of degrading a wide range of carbon substrates, shown through enzyme activity measurements, metagenomics, and microbial isolates (Sipes et al., 2022a; Sipes et al., 2022b). In this study, we investigated the changes in microbial communities responsible for soil organic matter degradation in fine-scale intervals of Svalbard active layer soils using metagenomes, temperature, and VWC.

Previous bacterial taxonomy studies in Arctic permafrost-affected soils, including those from Svalbard, have focused on the upper surface (1–5 cm (Ivanova et al., 2020), and deeper horizons of the active layer (e.g., <20 cm (Schostag et al., 2015; Wilhelm et al., 2011), 30 cm (Mackelprang et al., 2011; Woodcroft et al., 2018), 40 cm (Morgalev et al., 2017), and 60 cm (Hultman et al., 2015; Woodcroft et al., 2018). All these studies have found microbial communities dominated by *Actinomycetota, Acidobacteriota* and *Chloroflexota* which contain key carbon degrading genes (Tveit et al., 2013), establishing them as important players in the microbial response to global climate change in mineral Arctic soils. However, little is known about how microbial communities vary over the small spatial scales that are relevant to these vertically layered and horizontally heterogeneous soils. Since physicochemical characteristics change with depth within the active layer (Boike et al., 2018), different microbial communities may be adapted to unique conditions at different depth layers. To fill this knowledge gap, the microbial community and its metabolic properties need to be investigated at a fine depth scale (Fierer et al., 2010). Understanding these depth-dependent variations in microbial degradation of soil organic carbon in the active layer is important for determining the response of these communities to climate change (Yi et al., 2018). The redistribution of thawed carbon-rich soil into a cold unfrozen layer near the permafrost could either slow down (Bockheim, 2007) or speed up (Turetsky et al., 2020) microbial activity and organic matter degradation.

We conducted a study in the upper layer of permafrost affected soils at five sites in the Bayelva site, near Ny Ålesund, Svalbard, located on the Leirhaugen hill, 2 km north of the rapidly retreating tounge of Austre Brøggerbreen glacier (Fig. 1). The Leirhaugen hill is covered with permafrost patterned ground and sparse vegetation. The annual physical characteristics of soil and snow at the Bayelva site have been continuously monitored since 1998 (Boike et al., 2018). To improve understanding of the microbial communities from this poorly characterized environment, frozen winter cores collected in April 2018 were subsampled at high stratigraphic resolution (1 cm to 2 cm depth intervals). To test the hypothesis that the microbial community changes according to depth variations throughout the year, thawed summer pit samples were obtained in early September 2019 and the same measurements were made.

Studying microorganisms within permafrost-affected soils is difficult for many reasons; first, low microbial biomass results in negligible DNA concentrations, which is troublesome for getting objective sequencing data; second, constrained cultivation approaches lead to poor species diversity; and third, rapidly changing environmental characteristics make it difficult to make broadly representative measurements of microbial activity (Jansson and Taş, 2014). Amplicon studies of 16S rRNA genes are heavily biased toward known microorganisms (Lloyd et al., 2018) because the relative abundance of microorganisms is often skewed due to primer bias (Suzuki and Giovannoni, 1996). Therefore, we used metagenomic approaches to analyze microbial DNA (Tyson et al., 2004). The bulk genomic DNA and the metagenomic-assembled genomes (MAGs) were analyzed to assess the relative abundance of different taxonomic groups and their genetic material for metabolic potential. MAGs are a reliable method to assign functions at lower taxonomic groups across environmental microbial communities, however, they are often difficult to assemble and bin from soils samples due to high microbial diversity (Mackelprang et al., 2011). In our approach, we 1) binned MAGs separately in 56 similar soil metagenomic libraries, increasing the chances of binning microorganisms endemic to each

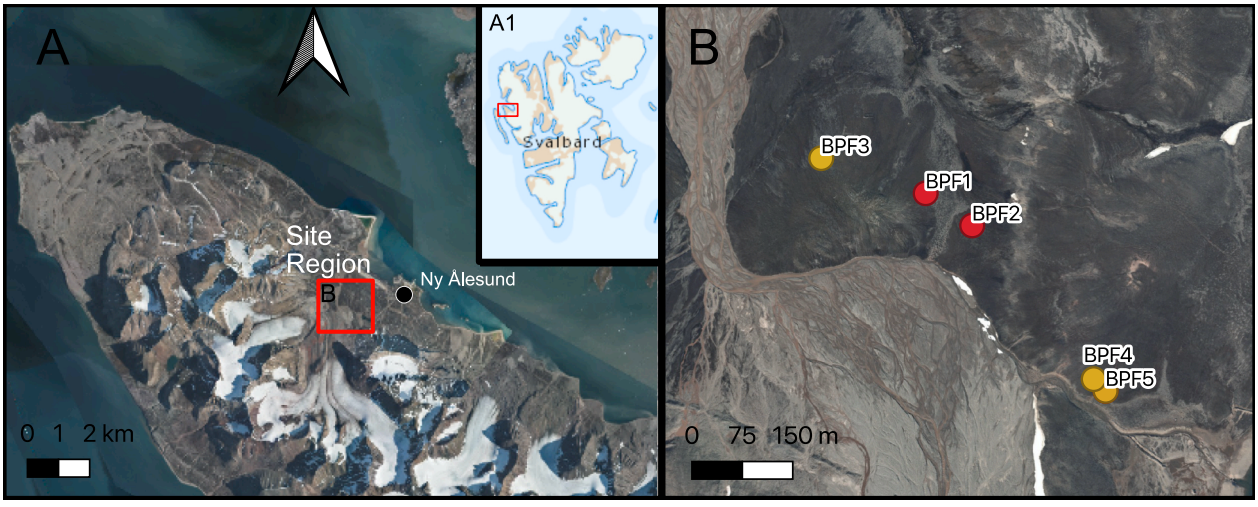

Fig. 1. Sampling location near the research station, Ny Ålesund (A) on the Svalbard archipelago (A1), close to the Bayelva long term permafrost observatory (B – fence can be seen above 'BPF3 marker'). Site region is expanded in panel B. Samples collected in April 2018 are in red and are investigated in Sipes et al., 2022a, samples collected in September 2019 are in gold (this study). Satellite photos are from Norwegian Polar Institute Map Data and Services and map was rendered in QGIS v3.18. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

horizon, 2) recruited metagenomic reads from each of the 56 soil samples to the full set of MAGs that were produced from all the samples so that a MAG's distribution across samples could be determined even if it was not directly binned in all soil samples, and 3) determined taxonomic distributions across the total metagenomic assemblies to determine if MAG diversity is representative of the microbial community.

Results

Soil physical processes and properties

The quality-controlled dataset collected in the late winter 2018 and summer 2019 from the Bayelva high Arctic permafrost long-term observation site revealed that the onset of freezing, defined as temperatures dropping below zero, started from the top down and was delayed toward the deeper layers (Fig. 2 and Table 1). The water in the upper layer soils froze first, before the layers below experienced a decrease in temperature. Consequently, the lower layers of soil reached subzero temperatures only after the upper layers froze permanently for the winter season. Due to the proximity of temperatures of the permafrost affected soils to the freezing point of water (within the soil freezing characteristic curve (Groenke et al., 2023), the vertical temperature transmission is buffered by phase changes between ice and liquid water.

The duration of the zero-curtain period (the time when the soils is below 0 °C and remain there until spring) varied between 18 to 35 days among the soil layers, indicating that differences in soil texture, properties and volumetric liquid water content (VWC) influenced the soil's ability to freeze uniformly. During the winter when the frozen cores were drilled, the VWC in the frozen soil varied between layers, ranging from ~ 4 % to 10 % with similar mean temperatures between −8 to −9°C. This variation could be attributed to different soil properties, such as the amount of organic matter and mineral content.

Description of metagenome-assembled genomes

A total of 56 samples collected across five sites in the Bayelva plain in the Laugerhain glacier moraine were sequenced on Illumina NovaSeq, totaling 1.5 TB of metagenomic reads (Table S1) and 169 MAGs of medium to high quality, based on the MIMAGs specification, with ≥50 % completeness and ≤10 % contamination (Figure S1, Table S2). Of the 56 metagenomes assembled, 52 yielded MAGs; exceptions were BPF2 (10–12 cm, 18–20 cm), BPF4 (1–2 cm), and BPF5 (43–45 cm), likely due to their smaller assembly sizes. Individual MAGs' assembly size, number of MAGs, or MAG completeness do not strongly correlate with percent of contigs binned, soil depth, or site ($R^2 < 0.2$, Table S2), suggesting that the ability to retrieve MAGs was similar in all samples. MAG assembly size increases slightly with completeness ($R^2 = 0.61$, $p < 10^{-16}$, Figure S2, Table S3). The percentage of each metagenome assembly that cumulatively maps to any of the 169 MAGs varies from 8.3 % (BPF1 56–58 cm) to 87.89 % (BPF4 0–2 cm) (Figure S3, Tables S2, S3).

The 169 MAGs belong to 12 phyla. *Acidobacteriota* account for the most MAGs (n = 60) from 5 of this phylum's 13 classes (*Blastocatellia* 37 MAGs, *Vicinamibacteria* 13, *Thermoanaerobaculia* 8, *Acidobacteriae*, and *Mor1* 1). *Actinomycetota* have the second highest number of MAGs (n = 52) spanning four of this phylum's nine classes (*Thermoleophilia* 45 MAGs, *Acidimicrobiia* 3, *Actinomycetia* 2, and *UBA4738* 2). *Chloroflexota* have 24 MAGs from three classes (*Ellin6529* had 21 MAGs, *Anaerolineae* 2, and *Dehalococcoidia* 1). *Proteobacteria* have 16 MAGs (5 from *Alphaproteobacteria* and 11 from *Gammaproteobacteria* classes). *Gemmatimonadota* have 3 MAGs all from class *Gemmatimonadetes*. *Planctomycetota* have 5 MAGs, 3 in *Phycisphaerae* and 2 in *Planctomycetes* classes. Three MAGs are assigned to *Verrucomicrobiota*, all within the class *Verrucomicrobiae*. Four phyla are represented by one MAG each: *Bacteroidota, Cyanobacteria, Eisenbacteria, and Methlomirabilota.* The only archaeal MAGs are 2 *Thermoproteota,* both in the class *Nitrososphaeria*. These MAGs represent the most abundant phyla as shown in the (unbinned) metagenomic assemblies (Table S4).

MAG read mapping to all metagenomic samples

The three most abundant phyla by metagenomic read-mapping across all samples are *Acidobacteriota, Actinomycetota*, and *Chloroflexota* (Figs. 3, S4, and S5). Thirty of the most abundant MAGs from the major clusters discussed below are shown in Fig. 3 because the complete heatmap with 169 MAGs was too large to be easily visualized in the main manuscript (Fig. S5). The *Acidobacteriota* classes *Thermoanaerobaculia* and *Blastocatellia* vary greatly in abundance with depth in the upper 20 cm. *Actinomycetota* (mostly the class *Thermoleophilia*) and *Chloroflexota* (mostly the class *Ellin6529*) are consistently abundant regardless of depth below the upper few centimeters (Fig. 4 and S3), suggesting that

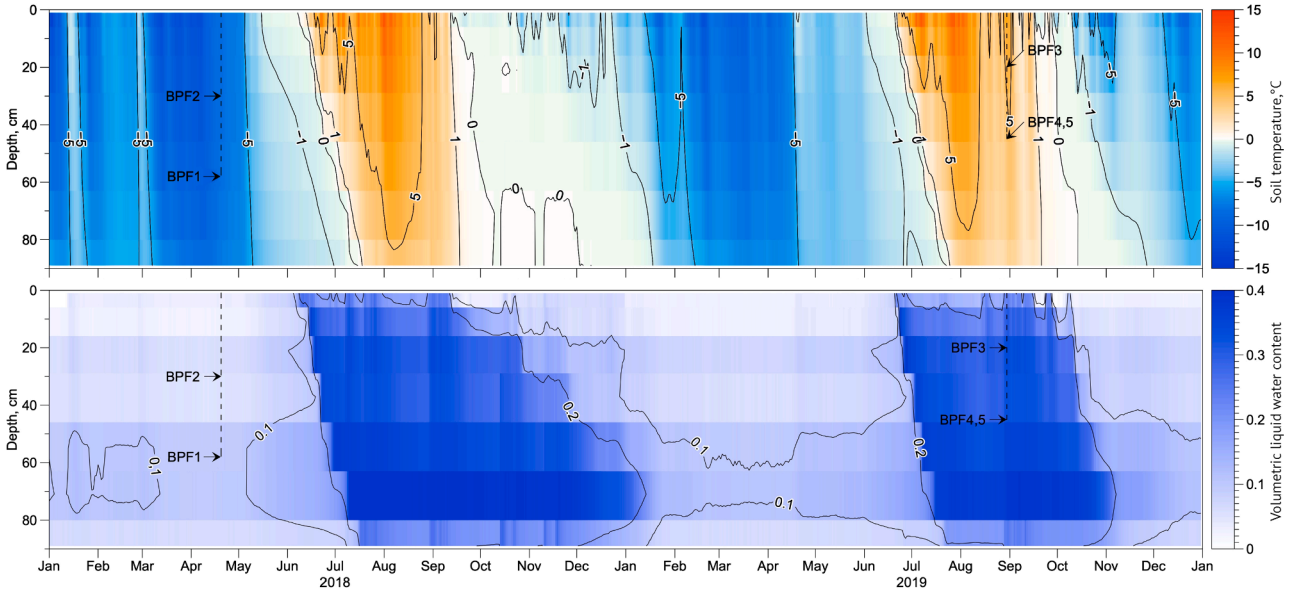

Fig. 2. Contour plots displaying the (top) soil temperature and (bottom) volumetric liquid water content profiles for the soil at the Bayelva site between January 2018 to December 2019, mean daily values are based on hourly data collected at depths of 1, 11, 21, 37, 55, 71, and 89 cm below the surface. The dates of sampling of soil material in frozen (April 2018) and unfrozen (September 2019) are added to the graph.

Table 1
Summary of freeze back characteristics (temperature-T, volumetric water content- VWC) analyzed from the Bayelva site. The start of freeze back was defined at drop of temperatures below −0.1 °C and continuously remaining below −0.1 °C. The duration of the period of zero curtain was defined as period constantly around 0 °C until the hourly soil temperature dropped and remained below −0.5C°. The mean remaining VWC was calculated for 24 h for the last day of the period of zero curtain.

Depth of sensor (cm)	Drop below −0.1 °C (start of freeze back)	Duration of freeze back (zero curtain, soil temp. <−0.5 °C)	Duration of freeze back (days)	Mean Remaining VWC at end of freeze back (%)	Mean soil temp. April 15. 2018 (°C)	Mean VWC April 15. 2018 (%)	Mean soil temp. September 15. 2019 (°C)	Mean VWC September 15. 2019 (%)
1	4.10.2017	4. – 24.10.2017	20	2.4	−9	4.3	3.6	20
11	7.10.2017	7. – 25.10.2017	18	10.8	−8.9	4.5	3.8	29.1
21	8.10.2017	8. – 30.10.2017	22	11.2	−8.9	6.9	3.7	32.4
37	11.10.2017	11.10. – 3.11.2017	23	12.4	−9	5.9	3.4	32.4
55	14.10.2017	14.10. – 9.11.2017	26	18.7	−8.8	9.5	3.1	35.4
71	22.10.2017	22.10. – 21.11.2017	30	22.6	−8.6	10.5	2.6	38.9
89	22.10.2017	22.10. – 26.11.2017	35	10.5	−8.1	6.6	2	21.2

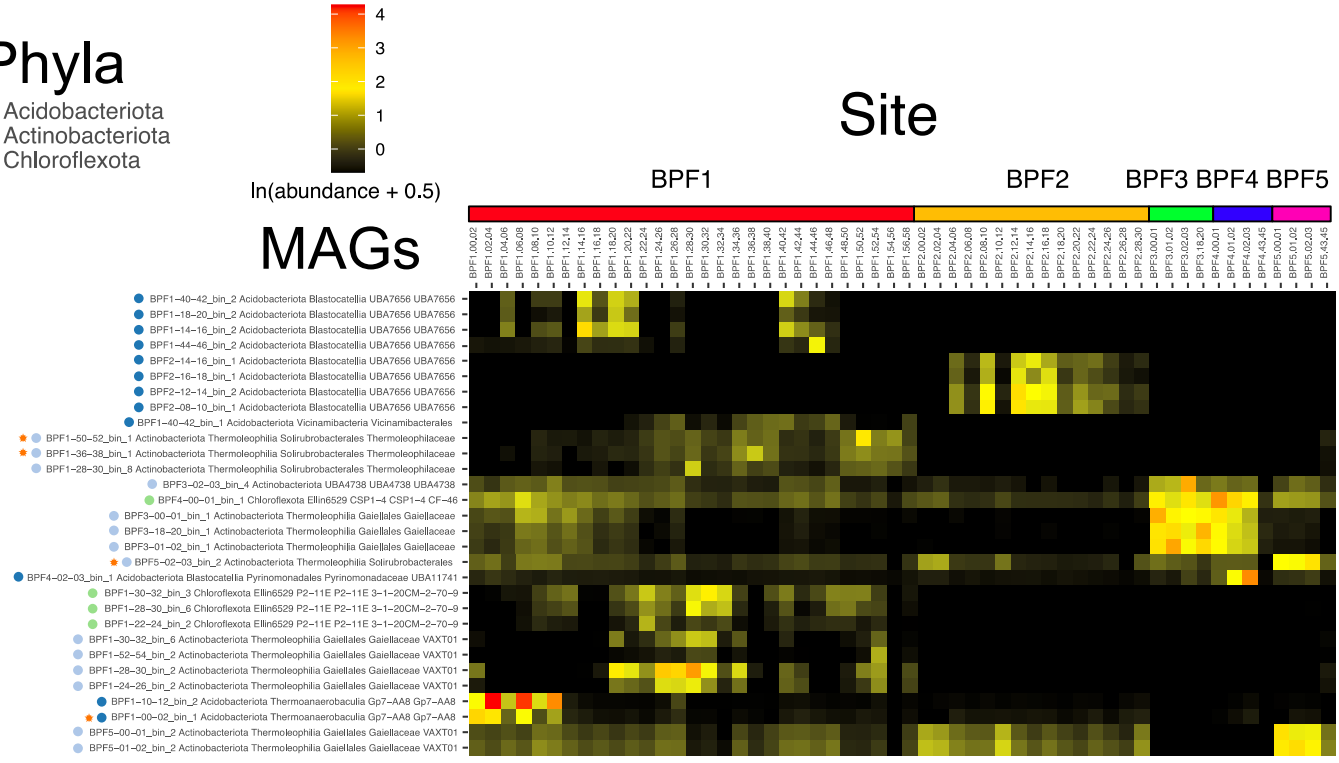

Fig. 3. Heatmap of organismal abudnace displayed through genome copies per million for 30 representatives of the total MAGs (n = 169) against metagenome read libraries (n = 56). Shown are the 30 MAGs from the full heatmap (Figure S5) that represent the most abundant MAGs from each of the three major clusters on the hierarchical clustering shown in Figure S5. Abundance plus 0.5 has been natural log transformed for visual representation on a positive scale. Sites are ordered left to right from surface to bottom depth. Colored dot at beginning of the MAG name depicts phylum. Orange stars next to the MAG name denote the present of autotrophic metabolic pathways. All autotrophic MAG abundance shown in Figure S2. Heatmap depiction inspired from others (Rogers et al., 2022). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

any possible depth-related abundance trends for these groups may occur at the lower family or genus levels.

The highest read abundance for each MAG is from the assembly from which they originated, followed by assemblies from the same site (Fig. 3 and S5). Ninety-two MAGs recruited reads from other site assemblies while 57 MAGs are constrained to a subset of locations or depths. Only 20 MAGs (9 *Actinomycetota*, 3 *Chloroflexota*, 1 *Proteobacteria*, 1 *Gemmatimonadota*, 2 *Planctomycetota*, 1 *Eisenbacteria* and 3 *Acidobacteriota*) recruited reads from all 56 samples (Fig. 3, S3, S5). A Spearman correlation-based matrix between all the MAG relative abundances at the sites was used to determine relationships between MAGs, based on their read abundances across the 56 metagenome samples. Stronger correlation coefficients (higher than 0.5) between MAGs result in closer dendrogram clustering (Figure S5). Dendrogram nodes do not always cluster by phylum or MAG's origin sample assembly, but rather by abundance similarities; for example, *Actinomycetota* do not all cluster in the same dendrogram branch nor do all BPF1 MAGs cluster together. This suggests that the approach of recruiting reads from all metagenomes to all MAGs assembled from the different soil samples is an effective way to explore the ecological significance of the MAGs. Reads from most MAGs are present in at least two sites, with a few MAGs from the same phylum found only in a single borehole site. For instance 11 *Actinomycetota Blastocatellia* MAGs are only in BPF2; 12 *Acidobacteriota Vicinamibacteria* MAGs are only below 22 cm in BPF1; 8 *Actinomycetota Blastocatellia* MAGs are only in the upper 32 cm of BPF1, 5 *Proteobacteriota Gammaproteobacteria* MAGs are found at 24–40 cm and 50–58 cm in BPF1; 3 *Planctomycetota phycisphaerae* MAGs are in low abundance and only present above 50 cm in BPF1 (Fig. 3).

Dendrogram clustering based on Spearman rank correlations identified three major patterns in groupings of MAGs (Figure S5): A) those that increase abundance with depth but were predominantly found in BPF1; B) those that increase abundance with depth in both winter and

summer cores but are more abundant in winter core BPF2; and C) those that decrease abundance with depth in all cores. The most abundant MAGs from each of these three clusters are shown in Fig. 3. The microbial communities vary with depth, and microbial abundances persist between the frozen cores (BPF1 and BPF2) vs the thawed soils (BPF3, BPF4, and BPF5). The phylum level membership is similar in these three clusters (Fig. 3 and S6). At the class level, however, *Acidobacteriota* differentiate within the three clusters. In cluster C, *Thermoanaerobaculia* Gp7-AA8 is in both frozen cores (5 MAGs are present in all 6 samples from the upper 12 cm of BPF1) and thawed cores (2 MAGs are abundant in all 3 thawed cores). Farther (clusters A and B), *Blastocatellia* UBA7656 MAGs are dominantly found in the frozen cores 6–28 cm (12 MAGs) and 40–48 cm (4 MAGs) in BPF1 and 0–30 cm in BPF2 (15 MAGs), with the 12 BPF1 MAGs and 13 BPF2 MAGs recruiting reads only from their own core. 92 % of *Blastocatellia* UBA7656 MAGs are present only in frozen cores. *Acidobacteriota* order *Vicinamibacterales* increases in abundance in frozen core BPF1 below 20 cm (19 MAGs in layer 20–58 cm) and is absent in all thawed cores. *Pyrinomonadales* is present in similar abundance in all samples, though different MAGs recruited reads from the frozen (8 MAGs) and thawed (3 MAGs) samples.

Actinomycetota are more broadly distributed along the depth gradient and among sites than other phyla, with each class recruiting reads from all depths of winter cores and summer samples. However, some family- and genus-level taxonomic groups of *Actinomycetota* are present only at a subset of depths. Thus, the family *Thermoleophilaceae* within the class *Solirubrobacterales* are abundant below 24 cm in all cores. In contrast, the members of the family *Gaiellaceae* that could not be classified at the genus level are more abundant in the upper 30 cm in all cores. The *Actinomycetota* lineages such as order *Solirubrobacterales* family 70–9, family *Gaiellaceae* genus VAXT01 and family *Gaiellaceae* unclassified contain some MAGs that are mostly found at deeper layers and others that are more abundant at upper depths.

Almost all *Chloroflexota* MAGs increase in abundance with depth (17 MAGs in winter cores and 3 MAGs in both winter and summer samples); only 3 MAGs of *Chloroflexota* Ellin6529 originating from the summer samples are abundant in the surface. *Alphaproteobacteria* MAGs are more often found at the surface, while *Gammaproteobacteria* MAGs are present at all depths in both winter and summer samples except only below 22 cm in winter core BPF1. *Planctomycetota* MAGs are usually more often found at the surface. *Gemmatimonadota* MAGs are present at all depths in both winter and summer samples. *Verrucomicrobiota* and *Nitrosphaeria* MAGs are either present in both summer and surficial winter cores or found in deeper layers in winter cores and are not found in the summer samples. The *Methylomirabilota* MAG is abundant at deeper layers in frozen cores and is not found in summer samples. *Eisenbacteria* MAGs are in deeper samples in both winter and summer samples. The single *Cyanobacteria* MAG is present in the surface sample of summer core BPF5. The *Bacteroidota* MAG is present in the upper 4 cm of winter core BPF1.

The fraction of the cumulative total of reads mapped from each assembly to any of the 169 MAGs decrease with increasing sample depth (Figure S2). The surface samples show the highest cumulative fraction of reads mapped to the residing MAGs for those samples, while the low fraction of reads mapped from the deeper samples suggests that the community members inhabiting these layers are not a complete assessment of microorganisms that are present in the permafrost active layer. To obtain substantial diversity coverage, a higher metagenomic sequencing depth with more source material is required. Additionally, the higher fraction of reads mapped in surface assemblies suggest that those MAGs are a better representation of the microbes present in the surface samples.

Correlation networks

Co-existence networks evaluated using monotonic Spearman's correlation with coefficient of $\geq$0.7 reveal that distributions of the 169 MAGs correlate to those of other MAGs from the same location or depth but not from different sites (Fig. 4A). At some depths, MAGs strongly correlate with each other (orange to red color, Fig. 4A), but MAGs from the same depths do not always have strong correlations. Layers between 16 and 48 cm in BPF1 contain 58 MAGs, which form a large network where each MAG correlates with a minimum of two others. Thus, two *Acidobacteriota* MAGs in this cluster each correlate with 10 other MAGs. BPF2 show similar results, with a tighter network in the middle of the core with 14 MAGs that correlate strongly, while the clusters above and below it do not correlate with MAGs from other depths. All 10 MAGs in BPF3 correlate to each other at a maximum of four connections. Limited to selected layers, BPF4 shows three groups of correlating MAGs at different depths with the highest Spearman's correlation coefficients at 43–45 cm (Fig. 4A). In BPF5, no MAGs were available from deeper layers, while all 6 MAGs from upper layers correlated through the three *Actinomycetota* present, one at each of the sample depths.

At the phylum level, MAGs form two complete correlated groups, suggesting that similar environmental factors may drive abundances of these phyla. Multilateral correlation occurs between *Actinomycetota, Chloroflexota,* and *Proteobacteria* (group 1) and *Actinomycetota, Chloroflexota*, *Methylomirabilota* and *Proteobacteria* (group 2) (Fig. 4B). Two groups with incomplete correlations were also observed. Thus, *Acidobacteriota* correlate with *Thermoproteota* and *Gemmatimonadota*, but the latter two phyla do not correlate with each other. *Plantomycetota* correlate positively with both *Verrucomicrobiota* and *Bacteroidota*, but the latter two phyla do not correlate with each other.

Autotrophic and heterotrophic genes

The only carbon fixation pathway identified in the MAGs is the Calvin-Benson Cycle. It is present in 10 MAGs of *Actinomycetota* (classes *Thermoleophilia* and *UBA4738*), 2 *Chloroflexota* (class *Ellin6529*), and 1 *Cyanobacteria* (Figs. 3, S5, S6). Each of 169 MAGs contain at least one gene encoding one of the seven extracellular carbon-degrading enzymes (Fig. 5, Tables S5, S6), which were previously assayed in these cores and in the isolates derived from these cores (Sipes et al., 2022a). Leucine aminopeptidase and phosphatase have the highest gene counts per MAG (Fig. 5; Table S6), while α-glucosidase and β-D-cellubiosidase have the lowest gene counts per MAG. In BPF1, the only core spanning 60 cm, β-glucosidase, N-Acetyl-β-D-glucosaminidase, and β-xylosidase gene counts per MAG increase below ~ 30 cm.

Metaproteomics of BPF2

The mapped peptides from the single metaproteomics library (BPF2 15–30 cm) classified into five phyla, with at least one representative from each of 26 COG categories, yielding a total of 793 proteins (Figure S7A). The Acidobacteriota are associated with 84 % of the metaproteome (n = 669). Most of these (n = 286) are from class *Blastocatellia*, with smaller contributions from *Mor1* and *Thermoanaerobaculia* (Figure S7B). The COG category 'Post-translational modifications, protein turnover and chaperons' has 88 identified proteins, mainly from *Acidobacteriota* (79/88) (Figure S7B).

MAGs for SeqCode

Only 43 MAGs met the requirements of $\geq$ 90 % completeness and $\leq$ 5 % contamination, and they are from *Acidobacteriota* (17 MAGs), *Actinomycetota* (15 MAGs), *Chloroflexota* (5 MAGs), *Gammaproteobacteria* (5 MAGs), and *Planctomycetota* (1 MAG). These MAGs were taxonomically classified using GTDB-Tk v2.3.2 (Fig. S4). Then MAGs were compared based on the whole-genome Average Nucleotide Identity (ANI) using FastANI v.0.1.3 and MAGs were considered to be the same species if they shared $\geq$ 95 % ANI. MAGs that were candidates to be named were selected from the same species group based on: lowest number of contigs (from 15 to 323), length of 16S rRNA from 906 bp to

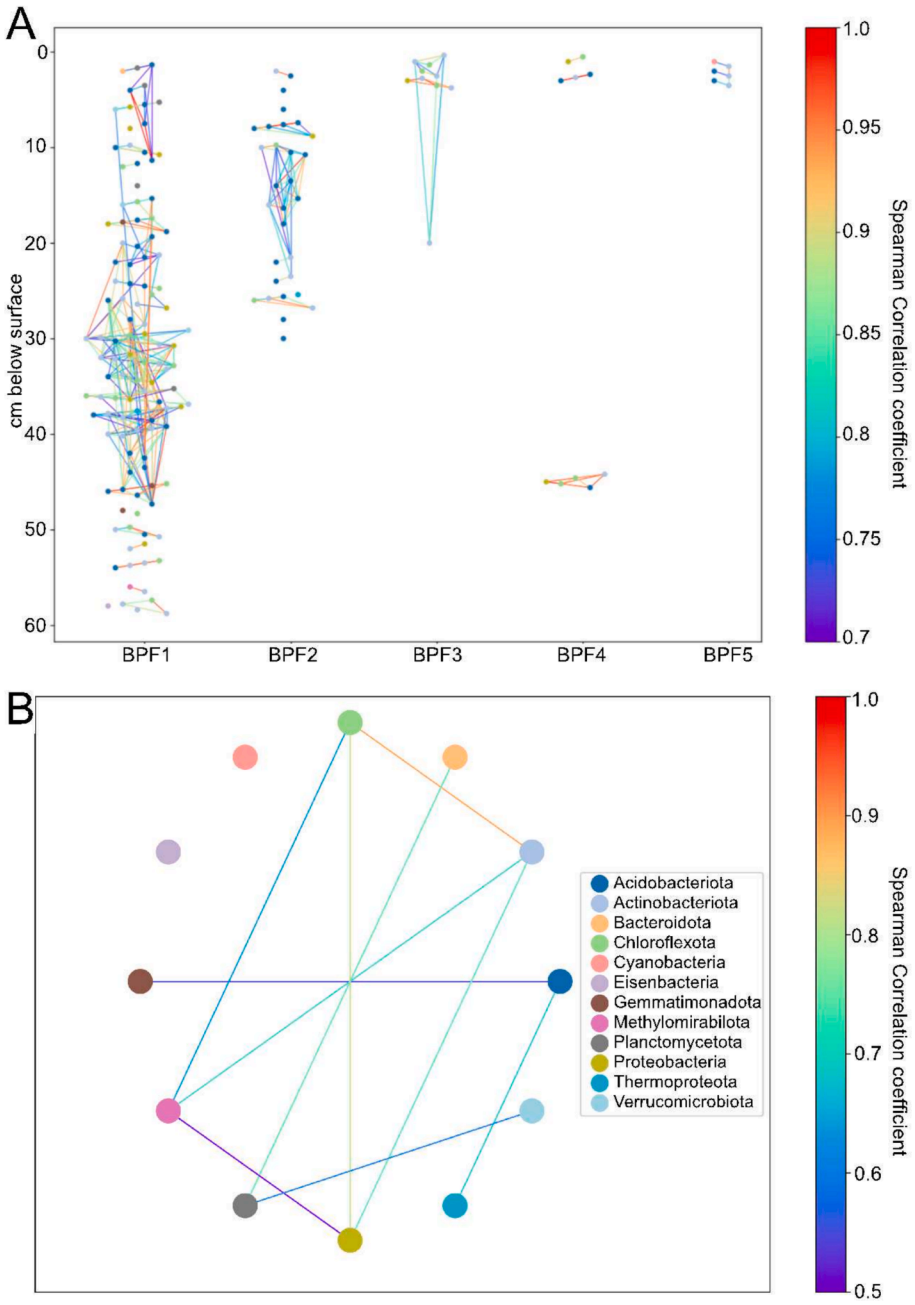

Fig. 4. Network analysis A) Each MAG correlation based on the unique genome copies per million (CPM) across all samples. Spearman correlation coefficient is represented with the line between MAGs and corresponds to the colors in the legend. MAGs originating from the same location are jittered along the 2 cm origin source in order to visualize correlations between MAGs from the same location B) Phylum level co-correlation relationships based on read abundances across all samples.

1413 bp, total number of tRNA from 78 to 157, and originating from the different soil locations. A total of 19 high quality MAGs, namely *Acidobacteriota* (7 MAGs), *Actinomycetota* (6 MAGs), *Chloroflexota* (3 MAGs), *Gammaproteobacteria* (2 MAGs), and *Planctomycetota* (1 MAG) were uploaded to the NCBI under BioProject ID number PRJNA1040778. Based on MAGs' annotation with PROKKA v1.14.6 (Seemann, 2014); 3 *Acidobacteriota* and 1 *Actinomycetota* MAGs passed the requirements of the SeqCode such as > 75 % 16S rRNA gene completeness and number of contigs less than 100, so these were given names, following the nomenclature rules of SeqCode.

Two MAGs, BPF1-18-20_bin_3 and BPF2-00-02_bin_2, were taxonomically classified to be members of the class Blastocatellia, order UBA7656, family UBA7656, and genus JADGNW01 but the fact that the MAGs have 75.6 % ANI between each other suggests that the MAGs belong to different species. Further analysis showed that these MAGs have <70 % ANI to 2 available Blastocatellia genomes (*Pyrinomonas methylaliphatogenes* and *Chloracidobacterium thermophilum*). MAG BPF1-18-20_bin_3 had 74.8 % ANI to the species *Luteitalea pratensis* (class Vicinamibacteria), and MAG BPF2-00-02_bin_2 had 77.4 % to the *Terriglobus saanensis* (class Terriglobia). There are metabolic similarities between these three classes. Acidobacteria of the classes Blastocatellia, Vicinamibacteria and Terriglobia are aerobic, psychrotolerant, grow on different simple sugars and dominate soils with a neutral or slightly basic pH in case of Blastocatellia and Vicinamibacteria (Männistö et al., 2011), or at a neutral to slightly acidic pH in case of Terriglobia (Männistö et al., 2011). The genome phylogenetic tree (Fig. S8) shows

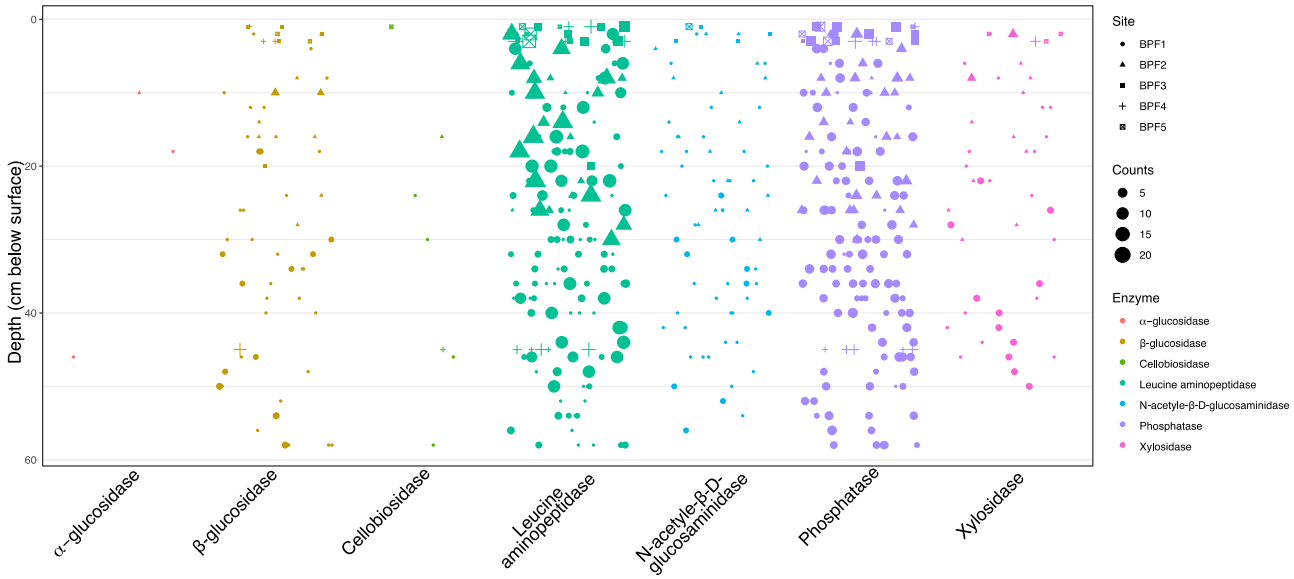

Fig. 5. Gene counts for carbohydrate- and peptide-degrading enzyme per MAG by depth and site. Number of single genes that encode for an enzyme is represented by the size of the symbol, color represents the enzyme type, and shape represents the sample site. Number of individual enzyme-coding genes present in each MAG can be found in Table S4.

low identity between these MAGs and available closest genomes. The information on the origin and etymology of novel name proposed to each MAGs is given in protologue Table 2.

Discussion

The active layer soils of the Bayelva site near Ny Ålesund, Svalbard, experience large variations in temperature and VWC between depths and seasons/locations (Fig. 2). Thawing and freezing are transmitted from the top of the soil downward seasonally (Table 1). Since the soils remain close to 0 °C much of the year, the vertical temperature transmission is buffered by phase changes between ice and VWC. Microorganisms in deeper layers have longer exposures to VWC in the fall and upper layers have earlier access to liquid water in the spring. The presence of VWC and its association with osmolarity can significantly impact the microbial community (Schostag et al., 2015; Wu et al., 2023). The entire seasonal temperature fluctuations at 40 cm depth span 20 °C, notably from −10 °C to 10 °C between April and September (Boike et al., 2018). Other geochemical parameters, such as total inorganic and organic carbon, total nitrogen, and the stable isotopic ratios of carbon and nitrogen, vary gradually with depth as shown previously for BPF1 and BPF2 (Sipes et al., 2022a).

Previous 16S rRNA gene amplicon surveys showed systematic changes at the phylum level with depth of the active layer soils in Svalbard (Müller et al., 2018) and Alaska (Tripathi et al., 2019), as well as seasonal variations in the active layer bacterial community due to dramatic annual changes in temperature and soil chemistry (Schostag et al., 2015), suggesting that VWC and osmolarity might serve as key factors driving the microbial community. Our approach focused on MAG read abundance, since this avoids the bias of primer amplification (16S rRNA gene amplicon surveys omit organisms whose 16S rRNA genes do not match the primers) and allows a more precise taxonomic resolution. However, MAG assembly and binning has the caveat that rare microorganisms from phyla with high intraphylum diversity may be missed (Ghurye et al., 2016). The metaproteomics and MAGs' analysis corroborate that *Acidobacteriota*; *Actinomycetota*, and *Chloroflexota* are the most abundant phyla found in permafrost active layer samples (Dziurzynski et al., 2022; Mackelprang et al., 2017; Müller et al., 2012; Tripathi et al., 2019; Woodcroft et al., 2018). The fact that most of the MAGs are from uncultured genera, or even families, classes, or orders, emphasizes the vast unknown diversity within this environment.

Phylum Acidobacteriota

60 MAGs are from Acidobacteriota, more than from any other phylum in this data set. Acidobacteriota is one of the most abundant and diverse phyla on Earth (Giguere et al., 2021) that comprises up to 52 % of the soil microbial community commonly found in surficial soils (Sui et al., 2022; Xue et al., 2020). Acidobacteriota were found to dominate a thaw gradient in northern Sweden from carbon-rich palsa through a bog (thawing permafrost) to a fen (no underlying permafrost) (Mondav et al., 2017) and during increases in soil respiration in a range of Antarctic soils (Yergeau et al., 2012), suggesting they are particularly important as soils warm.

The *Acidobacteriota Thermoanaerobaculia* Gp7-AA8 MAGs are dominant in the surface layer, suggesting a high tolerance to freeze–thaw and desiccation in surficial soils. Other studies have indicated metabolic versatility of members of the class *Thermoanaerobaculia* that were found to have pathways for sulfate reduction in low-temperature marine sediments of Svalbard (Flieder et al., 2021), and genes for reductive carboxylation that were identified in an isolate from a hot spring with the ability to grow as strict anaerobic chemoorganotroph on proteinaceous compounds (Stamps et al., 2014). Acidobacterial class *Blastocatellia* is represented by two families, UBA7656 and *Pyrinomonadales*. The family UBA7656 is found only below 6 cm, while dominating both the metagenome and metaproteome described in this study. MAGs from the family *Pyrinomonadales* are ubiquitous. Based on OTU analyses, the class *Blastocatellia* was shown to be one of the most common in tundra aeolian sands and was associated with unvegetated habitats (Venkatachalam et al., 2021). The presence of MAGs from the acidobacterial class *Vicinamibacteria* increase below 20 cm. A recent metabolic reconstruction study of *Acidobacterial* MAGs from activated sludge wastewater treatment plants showed that *Acidobacteriota* including *Vicinamibacteria* are involved in nitrogen and phosphorus removal and iron reduction and utilization of organic compounds like xylose, acetate and fatty acids (Kristensen et al., 2021).

Collectively, the phylum *Acidobacteriota* shows a great deal of metabolic flexibility. The vertical depth variations in abundance of different acidobacterial classes is supported by properties and characteristics that are inherent to *Acidobacteriota*, such as natural resistance to

Table 2
Protologue table for MAGs proposed as a new genus and species.

Guiding Code for Nomenclature	SeqCode	SeqCode	SeqCode	SeqCode
Nature of the type material	**Genome sequence**	**Genome sequence**	**Genome sequence**	**Genome sequence**
Phylum name	Acidobacteriota	Acidobacteriota	Acidobacteriota	Actinomycetota
Class name	Blastocatellia	Blastocatellia	Thermoanaerobaculia	Thermoleophilia
Order name	UBA7656	UBA7656	Gp7-AA8	Solirubrobacterales
Family name	UBA7656	UBA7656	Gp7-AA8	70–9
Genus name	*Onstottus*	*Onstottus*	*Gilichinskyi*	*Mayfieldus*
Species name	*Onstottus arcticum*	*Onstottus arcticum*	*Gilichinskyi gelida*	*Mayfieldus profundus*
Genus status	**gen. nov., nom. rev.**	**gen. nov., nom. rev.**	**gen. nov., nom. rev.**	**gen. nov., nom. rev.**
Genus etymology	On.s'to.ttus. N.L. masc. n. *Onstottus* derived from Tullis C. Onstott, an American geoscientist recognized for his contribution to research o endolithic life deep under the Earth's surface.	On.s'to.ttus. N.L. masc. n. *Onstottus* derived from Tullis C. Onstott, an American geoscientist recognized for his contribution to research o endolithic life deep under the Earth's surface.	Gili.'chin.skyi N.L. masc. n *Gilichinskyi* derived from David A. Gilichinsky, an international leader and cryologist recognized for his research of ancient permafrost microorganisms and astrobiology.	May.'fieldus N.L. masc. n *Mayfieldus* derived from John E. Mayfield, an American microbiologist recognized or his contribution to education in field of microbiology.
Type species of the genus	*Onstottus arcticum*	*Onstottus frigus*	*Gilichinskyi gelida*	*Mayfieldus profundus*
Specific epithet	*arcticum*	*frigus*	*gelida*	*profundus*
Species status	**sp. nov., nom. rev.**	**sp. nov., nom. rev.**	**sp. nov., nom. rev.**	**sp. nov., nom. rev.**
Species etymology	ar.cti.'cum, N.L. masc. adj. *arcticum* living in the Arctic.	'fri.gus, L. masc. n. *frigus* cold, living in the cold	gelida, N.L. fem. sing. adj. *gelida* glacial, very cold, living in the cold	pro'fun.dus, L. adj. *profundus* deep, extreme, living at the depth
Designated Genome, MAG or SAG	BPF1-18-20_bin_3[TS]	BPF2-00-02_bin_2[TS]	BPF1-10-12_bin_2[TS]	BPF1-56-58_bin_1[TS]
Type Genome, MAG or SAG accession Nr. [INSDC databases]	GenBank = GCA_038040485.1[TS]	GenBank = GCA_038040445.1[TS]	GenBank = GCA_038040385.1[TS]	GenBank = GCA_038040325.1[TS]
Access to raw data (e.g., SRA accession)	BioProject: PRJNA971579 BioSample: SAMN41246659 SRA: SRS21240154	BioProject: PRJNA971579 BioSample: SAMN41246679 SRA: SRS21240121	BioProject: PRJNA971579 BioSample: SAMN41246655 SRA: SRS21240144	BioProject: PRJNA971579 BioSample: SAMN41246678 SRA: SRS21240120
Registry number	We cannot get a SeqCode number until we get a DOI.			
Genome status	Incomplete	Incomplete	Incomplete	Incomplete
Genome size, kbp	6,907	6,750	5,458	2,308
GC mol%	57.25	56.88	65.90	69.56
Description of the new taxon and diagnostic traits	*Onstottus arcticum* likely has an aerobic heterotrophic lifestyle, with genes encoding glycolysis and tricarboxylic acid cycle, as well as a low affinity cytochrome *c* oxidase, F-type ATPase, and succinate dehydrogenase. It may produce nitrous oxide due to the presence of a nitric oxide reductase. It encodes 1 glycoside hydrolases and 26 glycosyl transferases. It was retrieved from 18-20 cm soil depth, sampled from site BPF1, Bayelva Permafrost site 1, while frozen in April 2018. Total carbon was 2.5 %, dissolved organic carbon was 0.93 mg/g soil, total nitrogen was 0.17 %, nitrate was 0.007 μg/g soil, and the soil C/N ratio was 14.3.	*Onstottus frigus* likely has an aerobic heterotrophic lifestyle, with genes encoding glycolysis and tricarboxylic acid cycle, as well as a low affinity cytochrome *c* oxidase, F-type ATPase, and succinate dehydrogenase. It may produce nitrous oxide due to the presence of a nitric oxide reductase, and may reduce nitrate and nitrite. It encodes 2 glycoside hydrolases and 31 glycosyl transferases. It was retrieved from 0-2 cm soil depth, sampled from site BPF2, Bayelva Permafrost site 2, while frozen in April 2018. Total carbon was 1.9 %, dissolved organic carbon was 2.55 mg/g soil, total nitrogen was 0.02 %, nitrate was 0.02 μg/g soil, and the soil C/N ratio was 45.1.	*Gilichinskyi gelida likely has an aerobic heterotrophic lifestyle, with genes encoding glycolysis and tricarboxylic acid cycle, as well as a low affinity cytochrome c oxidase, F-type ATPase, and succinate dehydrogenase. It encodes seven glycoside hydrolases and 23 glycosyl transferases. It was retrieved from 10-12 cm soil depth, sampled from site BPF1, Bayelva Permafrost site 1, while frozen in April 2018. Total carbon was 2.0 %, dissolved organic carbon was 1.17 mg/g soil, total nitrogen was 0.14 %, nitrate was* 0.04 μg/g soil, *and the soil C/N ratio was 14.5.*	*Mayfieldus profundus likely has an aerobic heterotrophic psychrotolerant lifestyle, with genes encoding glycolysis and tricarboxylic acid cycle, as well as a low affinity cytochrome c oxidase, F-type ATPase, and succinate dehydrogenase. It may reduce nitrite. It encodes 2 glycoside hydrolases and 24 glycosyl transferases. It was retrieved from 0-2 cm soil depth, sampled from site BPF2, Bayelva Permafrost site 2, while frozen in April 2018. Total carbon was 1.9 %, dissolved organic carbon was 2.55 mg/g soil, total nitrogen was 0.02 %, nitrate was* 0.02 μg/g soil, *and the soil C/N ratio was 45.1.*
Country of origin	Norway	Norway	Norway	Norway
Region of origin	Svalbard	Svalbard	Svalbard	Svalbard
Date of isolation (dd/mm/yyyy)	21/03/2021	21/03/2021	21/03/2021	21/03/2021
Source of isolation	Permafrost active layer	Permafrost active layer	Permafrost active layer	Permafrost active layer
Sampling date (dd/mm/yyyy)	20/04/2018	20/04/2018	20/04/2018	20/04/2018
Latitude (xx°xx′ xx″N/S)	78°55′26″N	78°55′26″N	78°55′26″N	78°55′26″N
Longitude (xx°xx′ xx″E/W)	11°50′49″N	11°50′29″E	11°50′49″N	11°50′49″N
Altitude (meters above sea level)	21	20	21	21
Depth (meters below land surface)	0.18–0.20	0.00–0.02	0.10–0.12	0.56–0.58

(*continued on next page*)

Table 2 (*continued*)

Guiding Code for Nomenclature	SeqCode	SeqCode	SeqCode	SeqCode
Number of MAGs in study	31	8	31	31
Source of isolation of non-type MAGs [opt]	Permafrost active layer	Permafrost active layer	Permafrost active layer	Permafrost active layer
Information related to the Nagoya Protocol	USDA permit # P330-21–00059	USDA permit # P330-21–00059	USDA permit # P330-21–00059	USDA permit # P330-21–00059

multiple cycles of freezing and thawing (Männistö et al., 2009), tolerance to drought conditions (Bu et al., 2018; Zhang et al., 2015); and ability to withstand variable soil conditions including nutrient fluctuations and water content variations (Huber et al., 2022) Therefore, our study suggests that *Acidobacteriota* are better equipped to respond quickly to climate change than other clades and their higher relative abundance may occur due to recent rapid changes caused by ongoing warming.

Two lineages of *Acidobacteriota*, the *Vicinamibacteria Vicinamibacterales* and *Blastocatellia* UBA7656, recruit almost no reads from the summer samples. Either we missed the depths in the thawed soil that contained these clades, or these microorganisms develop better at low-temperature conditions during winter season (November-April) and become less dominant when the season shifts and the active layer thaws. Another possibility is that this is accounted for by the high degree of spatial variability in microbial communities previously noted at this site (Sipes et al., 2022a). The possibility that we missed the samples containing these clades is supported by results from a recent study showing that *Acidobacterial* families, e.g., *Vicinamibacteraceae* and *Blastocatellaceae*, are equally abundant in 16S rRNA gene amplicon libraries in surficial soils collected during summer and winter near Ny Ålesund (Loganathachetti et al., 2022). However, we cannot exclude the possibility that some members of the microbial population could be adapted to negative temperature and high osmolarity conditions, as has been observed previously in active microbial populations (Ernakovich and Wallenstein, 2015) that survive due to presence of thin films of unfrozen brine (Gilichinsky et al., 2003) in frozen soils.

Phyla Actinomycetota and Chloroflexota

Actinomycetota and *Chloroflexota* tend to increase in relative abundance with depth and do not form subgroups that occupy particular depths, like the *Acidobacteriota* do. These phyla are found in a variety of Arctic environments, including ancient Siberian permafrost (Sipes et al., 2021), old Canadian permafrost (Goordial et al., 2017), Alaskan cave permafrost (Burkert et al., 2019), active layer and upper permafrost of the Canadian High Arctic (Wu et al., 2021), and thermokarst bog (Woodcroft et al., 2018). Characteristically for these phyla, the same MAGs often recruited reads from all cores, which were distributed spatially and seasonally, with similar depth trends in all of them. This suggests that similar populations of *Actinomycetota* and *Chloroflexota* are widespread spatially and persist year-round, with depth trends driven by their tolerance to the depth-related changes in exposure to VWC. Similar to the current study, previous analyses of 16S rRNA gene distributions showed that many types of *Actinobacteria* and *Chloroflexota* were present in both thawed and frozen samples from surficial soils around Ny Ålesund, Svalbard (Müller et al., 2018) and another study reported an increase in relative abundance of *Actinomycetota* and *Chloroflexota* with depth of permafrost soil (Loganathachetti et al., 2022). Our results suggest that this pattern is true for all classes within the *Actinobacteria* and *Chloroflexota*. The fact that *Actinomycetota* are more evenly distributed with depth and more abundant in deeper layers than *Acidobacteriota* is consistent with other active layer and permafrost research (Li et al., 2022; Xue et al., 2020).

Actinomycetota have the second most individual MAGs, the second most abundant proteins, and the most cumulative reads in every core. The most abundant class of *Actinomycetota*, *Thermoleophilia*, was shown to be abundant in soil and hot springs and contain genes for carbon–nitrogen hydrolase (42). The *Actinomycetota* class UBA4738 is a relatively new classification and MAGs of this class derived from the Antarctic Mackay Glacier soils were shown to be able to use atmospheric H_2 and CO to meet energy, carbon, and hydration needs (Ortiz et al., 2020). MAGs from the *Thermoleophilia* and UBA4738 classes contain the Calvin-Benson-Bassham cycle (CBB pathway) (Figure S6). While *Actinomycetota* have not been shown to be widely autotrophic, some MAGs previously characterized contain autotrophic pathways (Selensky et al., 2021), including other carbon fixation pathways, like Wood-Ljungdahl (Merino et al., 2020). Two *Chloroflexota* MAGs also contain the CBB pathway. MAGs containing autotrophic pathways were found in all samples, demonstrating the potential for carbon fixation at all depths. The only MAG of *Actinomycetota* class *Acidimicrobiia* is highly abundant in all soil samples studied here, possibly due to its putative ability to contribute to iron cycling as suggested for a *Acidimicrobiia* MAG discovered in thawed discontinuous permafrost in Sweden (32). Cultured isolates of *Actinomycetota* class *Actinomycetia* retrieved from sulfide ore deposits in Russia were shown to consume O_2 and emit CO_2 (41). Given the abundance of *Actinomycetota* in the Bayelva active layer, they might play a central role in carbon cycling there.

The correlation networks suggest that phyla *Actinomycetota* and *Acidobacteriota* inhabit different niches (Fig. 4B). The phylum *Chloroflexota* co-correlated with three other phyla (*Actinomycetota, Proteobacteria,* and *Methylomirabilota* with Spearman coefficient of 0.9, 0.7, and 0.7; respectively, Fig. 4B). These phyla were previously found in cold Arctic habitats. Thus, *Chloroflexota*, *Actinomycetota,* and *Proteobacteria* were repeatedly found in active layer soils in the high Arctic of Canada and Sweden (Mackelprang et al., 2011; Wilhelm et al., 2011), whereas *Methylomirabilota* was discovered in the bottom water of permafrost thaw pond in the Northern Canada (39). Positive correlation between *Actinomycetota* and *Chloroflexota* was shown in permafrost of the Greater Khingan Mountain region where they increased in abundance with depth (Li et al., 2022). These two phyla may correlate with each other due to their ability to adapt to low nutrient environments and use recalcitrant organic compounds (Kou et al., 2020).

Other studies of the active layer/permafrost cores showed a shift from *Acidobacteriota* and *Proteobacteria*-dominated microbial community in the active layer to *Actinomycetota, Bacteroidetes, Chloroflexota* and *Proteobacteria*-dominated community in the permafrost up to 2 m down (16). *Actinomycetota* are able to survive in more oligotrophic conditions than *Acidobacteriota* which prefer copiotrophic environments (Li et al., 2022). The episodic distribution of *Acidobacteriota* in our study suggests that they evolve quickly in response to availability of organic matter substrates due to changes from seasons or spatial heterogeneity, whereas *Actinomycetota* and *Chloroflexota* are less responsive to small-scale changes. Likewise, *Acidobacteriota* classes' vertical distribution may follow patterns according to their desiccation tolerance, with *Thermoanaerobaculia* Gp7-AA8 being the most tolerant, followed by *Blastocatellia* UBA7656 and then *Vicinamibacteria Vicinamibacterales*. Distribution and abundance of *Actinomycetota* and *Chloroflexota* correlated positively

with the increase of water content with depth.

Carbon-degrading potential, as shown by organic carbon hydrolyzing enzymes.

Five of the seven organic carbon-degrading enzymes assayed previously (Sipes et al., 2022a) were abundant at all depths of the cores (Fig. 5). The putative ability of bacteria to excrete these enzymes was demonstrated through metaproteomic discovery of proteins from COG categories for export/secretion in five phyla (Figure S7A). Genes encoding α-glucosidase and cellobiosidase were in low abundance (Fig. 5), commensurate with the low rates of potential activity for these enzymes measured previously (Sipes et al., 2022a). In general, predicted enzymatic activity based on the MAGs' count of enzyme-encoding genes parallel the results of enzymatic activity measured from the bulk soil samples and cultured isolates. Thus, gene counts for leucine aminopeptidase are the highest, followed by phosphatase; and both of these decrease with depth, matching the results from enzyme assays (Sipes et al., 2022a). Gene counts for three enzymes (β-glucosidase, N-acetyl-β-D-glucosaminidase, and xylosidase) increase in abundance with depth, suggesting that primary degradation of soil organic matter occurs below the surface at depths where environmental conditions are more stable. The latter statement is corroborated by the results that these three enzymes likely are psychrophilic with higher catalytic activity at 5 °C and 15 °C than at 25 °C (Sipes et al., 2022a). Two of these (β-glucosidase and N-acetyl-β-D-glucosaminidase) were shown to have similar potential activities in surface and deeper samples (Sipes et al., 2022a). Two-year-long monitoring effort during this study demonstrated that soil temperature is more vertically homogenous than the VWC. The VWC and the duration water stays liquid increases with depth in the upper 80 cm of active layer soils near Ny Ålesund, Svalbard (Fig. 2). Therefore, these results demonstrate that more liquid water and less frequent freeze–thaw events deeper in the soil profile may support development of microbial communities with higher capacity for organic matter degradation as opposite to the surface layer (Figs. 2, 6).

Conclusion

We discovered a highly heterogenous microbial community that occupies different depth-dependent soil niches showing higher organic matter degrading capabilities in deeper horizons of permafrost-affected active layer. Microbes in permafrost-affected soils are likely to experience a dramatic environmental shift as the climate continues to warm leading to permafrost degradation, ground ice thawing, and changes in soil hydrology. Our results suggest that different classes of *Acidobacteriota* may react differently to these vertically varying environmental conditions, with some classes specialized for the longer periods with low VWC in surficial layers. *Actinomycetota* and *Chloroflexota*, on the other hand, are more broadly distributed and active, perhaps driving organic matter degradation in deeper layers at the forefront of permafrost thaw, which may have greater exposure to liquid water due to insulation from upper layers.

Methods

Sample collection

Two active layer soil cores, BPF1 (58 cm) and BPF2 (30 cm), were collected in April 2018 from the Bayelva site, Ny Ålesund, Svalbard, (78°55.237′N, 011°50.495′E) 84 m apart from each other, as described previously (Sipes et al., 2022a). We will call these the winter core samples, since they were taken at the end of winter when the ground was still frozen. Additionally, small soil pits were dug from three other sites in early September 2019 at three additional sites in the same area: BPF3 (1–3 cm and 18–20 cm maximum depth), BPF4 (1–3 cm and 43–45 cm) and BPF5 (1–3 cm and 43–45 cm; Fig. 1). We will call these the summer samples since they were taken at the end of summer before the soils refroze for the winter. These are on Bayelva hill and are not directly influenced by seasonal glacial melt water flow which runs in the large (flood)plain channel. BPF1 and BPF2 cores were sliced in 2 cm increments with a sterile knife using aseptic techniques in the Kings Bay Marine Laboratory in Ny Ålesund, Svalbard. For BPF3, BPF4, and BPF5, four depths were cut from the wall of soil pits using a sterile scraper, put directly into sterile Whirl-Pak® sample bags, and frozen within hours. Samples were transported frozen to the laboratory at the University of Tennessee in Knoxville where they were stored at −80 °C until analysis.

Permafrost thermal and hydrologic properties

We investigated the thermal and hydrologic properties of the Bayelva long-term permafrost monitoring site using a quality-controlled dataset of soil temperature and VWC (Boike et al., 2018). We analyzed the thermal and hydrologic properties during four seasons of 2018 and 2019 (Fig. 2), which overlaps with sampling events for winter cores BPF1 and BPF2 as well as for summer pit samples BPF3, BPF4, and BPF5. The start of fall re-freeze was defined as when soil temperatures dropped and remained below −0.1 °C. The period of zero curtain was defined as a constant temperature around 0 °C until the hourly soil temperature dropped and remained below −0.5 °C. The mean VWC for the last day of the zero-curtain period was calculated over a 24-hour period. Methods are more extensively described in (Boike et al., 2018), and all data are available online (https://doi.pangaea.de/10.1594/PANGAEA.882060).

DNA extraction, sequencing, and bioinformatic analysis

DNA was extracted from ~ 0.5 g taken from the inside middle portion of soil sample and extracted with a QIAGEN PowerSoil DNA Kit (Hilden, Germany) within 24 h of collection (BFP1 and BFP2) or after being transported frozen to the University of Tennessee Knoxville (BPF3, BPF4, and BPF5). DNA libraries were prepared using the SMARTer ThruPlex DNA-seq libraries (350 bp average fragment size) and sequenced on Illumina NovaSeq 6000 S4 (2 x 150 bp) at Science for Life Laboratory in Stockholm, Sweden.

All bioinformatic operations were performed using the Infrastructure for Science Applications and Advanced Computing (ISAAC) high-performance computing resource at Oak Ridge National Laboratory, Knoxville, Tennessee USA in conjuction with the University of Tennessee, Knoxville, Tennessee, USA. Each of 56 libraries were assembled and binned separately. MetaWrap v1.3.2 (Uritskiy et al., 2018) was used for read quality control, assembly was done with metaSPAdes v3.11.1 (Nurk et al., 2017), and binning was performed using MaxBin2 v2.2.5 (Wu et al., 2016) and MetaBAT2 v2.12.1 (Kang et al., 2019). Bins were refined on a medium, or higher, quality category (Bowers et al., 2017) of ≥50 % completeness and <10 % contamination scores via CheckM v1.4.0 (Parks et al., 2015) and taxonomically identified with GTDB-Tk v1.5.1 (Parks et al., 2018). Taxonomic identities of unbinned metagenomic assemblies was determined with Kaiju (Menzel et al., 2016). MAG distribution was computed with metaWRAP's quant_bins module which assesses the average length-weighted TPM average per MAG in each sample to be used as a proxy for genomic copies per million (CPM) via read-mapping with salmon v1.10.1 (Patro et al., 2017).

R Statistical Software v4.1.0 (Wilson and Norden, 2015) was used to compute a Spearman's rank correlation coefficient matrix of the relative abundance of MAGs in the 56 samples. The correlation coefficients were used as connections in snetwork analyses across cores, sample depths, and phyla. MAG genes were annotated with PROKKA v1.14.6 (Seemann, 2014) and assessed for genes encoding enzymes whose activities were assayed in two cores previously (Sipes et al., 2022a): α-glucosidase (AG), β-glucosidase (BG), cellobiosidase (CB), leucine aminopeptidase (LAP), N-acetyl-b-D-glucosaminidase (NAG), phosphatase (PHOS), and xylosidase (XYL). Genes for autotrophic pathways were identified as described previously (Rogers et al., 2022) and a carbon fixation pathway was

considered present if it was > 60 % complete and contained the pathway's key genes (Berg, 2011; Probst et al., 2017). FastANI v.0.1.3 was used to compare MAGs to each other.

Metaproteomics

Metaproteomics analyses were done using 15 g of material from BPF2 only. Briefly, the soil was resuspended in a 4 % SDS/0.1 M NaOH lysis buffer prior to vortexing and centrifugation. The resulting supernatant was subjected to sonication to lyse bacterial cells. Protein clean-up and digestion were conducted via a protein aggregation capture (PAC) method (Batth et al., 2019) with slight modifications. An acidified aqueous (1 % Formic acid) wash was included to remove soluble fulvic acids from the sample prior to proteolytic digestion. Tryptic peptides were measured by 1D RP LC-MS/MS on a Q Exactive Plus as previously described (Peters et al., 2022). De novo-assisted database searches were conducted using PEAKS StudioX (Tran et al., 2019) against custom protein databases constructed using core and depth-matched metagenomes. Peptides with a false discovery rate of <1 % and that uniquely mapped to a single phylum were used for protein inference and quantification. Functional annotations and orthology assignments for all identified proteins were found using eggNOG-mapper v2 (Cantalapiedra et al., 2021).

Importance

The high Arctic is warming rapidly. Understanding the fate of organic matter is crucial for the permafrost-thawing based greenhouse effect. Soil microbes convert organic matter to carbon dioxide (CO_2) or methane (CH_4). We examined these microbes in Svalbard's permafrost-affected soils with high depth resolution and considered liquid water availability. Our results show organic matter-degrading enzymes in deeper soils, suggesting they can break down complex carbon compounds. These microbial populations respond quickly to seasonal changes in soil temperature and water content, indicating that future fluctuations driven by Arctic warming could significantly impact microbial community shifts.

CRediT authorship contribution statement

Katie Sipes: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Joy Buongiorno:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Andrew D. Steen:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Andrey A. Abramov:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Chukwufumnanya Abuah:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Samantha L. Peters:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Richard J. Giannonne:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Robert L. Hettich:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julia Boike:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sarahi L. Garcia:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation. **Tatiana A. Vishnivetskaya:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Karen G. Lloyd:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The 56 metagenomic libraries can be found under NCBI Metagenomic BioProject PRJNA971479. 19 MAGs were submitted to the NCBI under BioProject PRJNA1040778 and 4 out from these 19 MAGs were submitted to the SeqCode.

All raw mass spectra for the metaproteome measurement from this study have been deposited into the ProteomeXchange repository with accession numbers: ProteomeXchange-PXD041734; MassIVE-MSV000091780. The deposited data also includes the metagenome derived protein sequences used for the database search.

PANGAEA data citation: Boike, Julia; Juszak, Inge; Lange, Stephan; Chadburn, Sarah; Burke, Eleanor J; Overduin, Pier Paul; Roth, Kurt; Ippisch, Olaf; Bornemann, Niko; Stern, Lielle; Gouttevin, Isabelle; Hauber, Ernst; Westermann, Sebastian (2017): Soil data at station Bayelva (1998-2017, level 1, version 1), link to archive. PANGAEA, https://doi.org/10.1594/PANGAEA.882060.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2024.126544.

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