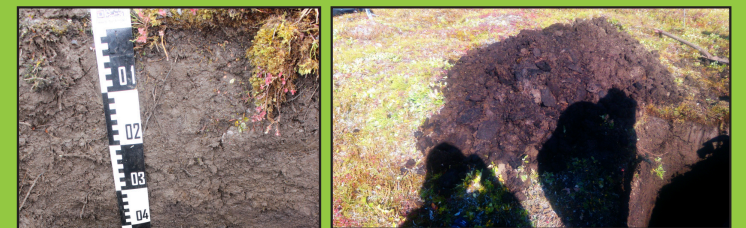


Milan Varsadiya

Effect of distinct soil horizon and vegetation on soil microbiome abundance, composition, and activity of Arctic permafrost



School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice • Faculty of Science
Ph.D. Thesis Series, 2022, No. 9

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School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz

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**Effect of distinct soil horizon and vegetation
on soil microbiome abundance, composition,
and activity of Arctic permafrost**

Ph.D. Thesis

MSc. Milan Varsadiya

Supervisor: Assoc. Prof. Ing. Jiří Bárta, Ph.D.

Department of Ecosystem Biology, Faculty of
Science
University of South Bohemia in České Budějovice

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❖ Annotation

In the present work, I studied the abundance, composition, and activity of soil microbial communities involved in the decomposition of soil organic matter (OM) in different horizons and under distinct tundra vegetation of Arctic permafrost soils. Special emphasis was given to buried topsoil caused by cryoturbation processes. The enzyme activity and their stoichiometry were also analyzed to determine the limitation of microbial carbon, nitrogen, and phosphorus. I also applied the metatranscriptomics approach to study the active microbial community in a complex view of all three domains of life (bacteria, archaea, Eukaryota). With this approach, we could better understand the potential role of microbial interactions (e.g., bacterial predation) in the carbon and nitrogen cycles of permafrost soils.

❖ Declaration

I hereby declare that I am the author of this dissertation and that I have used only those sources and literature detailed in the list of references.

Place: České Budějovice

Date: 30/04/2022

Milankumar Varsadiya

This thesis originated from of Faculty of Science, University of South Bohemia, supporting doctoral studies in the Ecosystem Biology study program



Přírodovědecká
fakulta
Faculty
of Science

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
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“Research is what I’m doing when I don’t know what I’m doing.” – Wernher von Braun. That is how this long journey started. Alex Wassabi once said, “We know what will happen if we give up, but we don’t know what will happen if we don’t”. I am very glad I chose the latter one on a long route to accomplish what may have seemed difficult at the beginning. All credit goes to my mentor my supervision Jiri Barta. I can’t thank him enough for his immense support, the scientific input and expertise that he shared with me, the fruitful discussions over the years, and his sizable patience. His support was not restricted to academics only but socially and personally too.

This thesis would not have been possible without all co-author’s contributions respective to the articles in this thesis. I am also grateful to all technician at our department Eva Koutecká, Monika Strejčková, Hana Petrásková, Katka Kučerová, Dan Vaněk, Ondra Žampach, and Lenka Čapková for their co-operation, molecular and chemical analysis.

Big heartfelt thanks to all office mates (old and new) and the laugh we had together (not many sciences laugh though!)

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Last, I would like to thank my wife Komal without her I might not have been able to accomplish this mammoth task.

“It always seems impossible until It’s done” - Nelson Mandela. Chapter 1 is done, and the rest is to be seen.

❖ List of papers and author's contribution

The thesis is based on the following papers:

- I.** Varsadiya, M., Urich, T., Hugelius, G., Bárta, J., 2021a. Microbiome structure and functional potential in permafrost soils of the Western Canadian Arctic. *FEMS Microbiology Ecology* **97**. doi:10.1093/femsec/fiab008 (IF = 4.1)

Milan Varsadiya took part in determining soil physicochemical parameters (90%), evaluated molecular data (90%), and wrote the manuscript (70%).

- II.** Varsadiya, M., Urich, T., Hugelius, G., Bárta, J., 2021b. Fungi in Permafrost-Affected Soils of the Canadian Arctic: Horizon- and Site-Specific Keystone Taxa Revealed by Co-Occurrence Network. *Microorganisms* **9**, 1943. doi:10.3390/microorganisms9091943 (IF = 4.1)

Milan Varsadiya took part in determining soil physicochemical parameters (90%), evaluated molecular data (90%), and wrote the manuscript (70%).

- III.** Varsadiya, M., Liebmann, P., Petters S., Hugelius, G., Urich, T., Guggenberger, G., Bárta, J., 2022. Extracellular enzyme ratios reveal locality and horizon-specific carbon, nitrogen, and phosphorus limitations in Arctic permafrost soils (Manuscript)

Milan Varsadiya took part in soil samples collection (100%), determining soil physicochemical parameters (90%), evaluated molecular data (90%), and wrote the manuscript (60%).

- IV.** Petters, S., Varsadiya, M., Liebmann, P., Schnecker, J., Guggenberger, G., Bárta, J., Urich, T., 2022. Patterns of belowground active biota associated with subducted carbon pockets in permafrost soils of Greenland (Manuscript)

Milan Varsadiya took part in soil samples collection (100%), determining soil physicochemical parameters (70%), evaluated molecular data (40%), and wrote manuscripts with Petters, S (40%).

❖ Co-authors agreement

Jiří Bárta, the supervisor of the present thesis and co-author of all attached papers fully acknowledged the contribution of Milan Varsadiya as a first author and his contribution as stated above.

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Assoc. Prof. Ing. Jiří Bárta, Ph.D.

Tim Urich, a co-author of all attached papers fully acknowledged the contribution of Milan Varsadiya as a first author and his contribution as stated above.

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Prof. Dr. Tim Urich

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❖ Abbreviations

C	Carbon
C/N ratio	Carbon to nitrogen ratio
CH ₄	Methane
CO ₂	Carbon dioxide
CryoOM	Cryoturbated soil organic matter
DN	Dissolved nitrogen
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
GHGs	Greenhouse gases
HT	Hummock tussock tundra
HTNC	Hummocky tussock tundra dominated by nonsorted circles
N	Nitrogen
N ₂	Nitrogen gas
N ₂ O	Nitrous oxide
OC	Organic carbon
OM	Organic matter
RNA	Ribonucleic acid
SOC	Soil organic carbon
SOM	Soil organic matter
UT	Slightly disturbed upland tundra dominated by non-sorted circles
WT	Wet polygonal tundra

1 GENERAL INTRODUCTION

1.1 Background

Extensive studies have been conducted on the carbon (C) and nitrogen (N) pool, due to the fact that thawing permafrost could increase atmospheric concentrations of carbon dioxide (CO₂) and methane (CH₄) (Zimov 2006; Schuur et al. 2008; Kuhry et al. 2010). A recent study estimated that the soil organic C (SOC) stock in 0-100 cm and 0-300 cm of Northern circumpolar permafrost is approximately 380 Pg and 813 Pg, respectively (Figure 1) (Palmtag et al. 2022). However, it is not yet clear how much of this C is vulnerable to microbial decomposition and how important the function of soil microbial communities is.

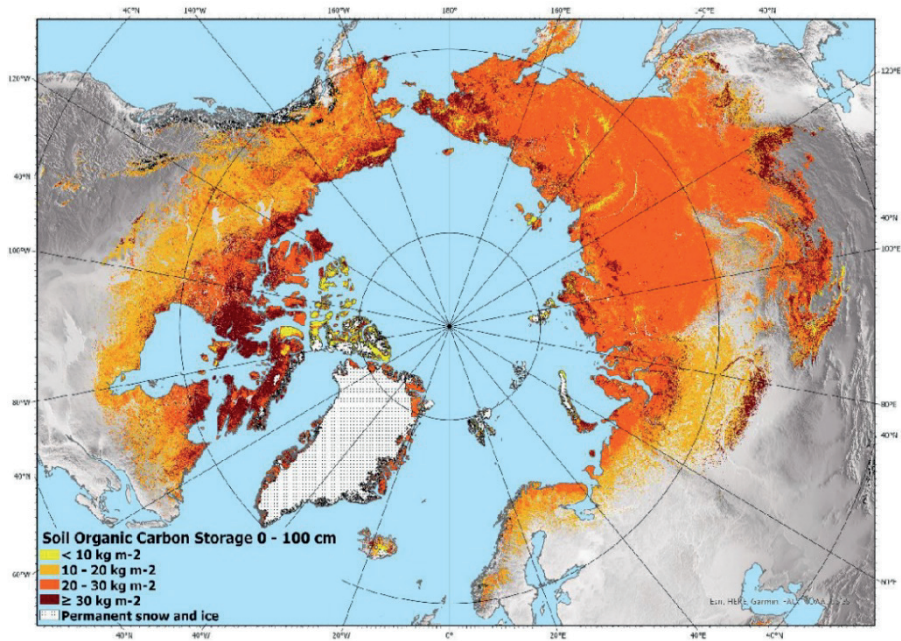


Figure 1: Estimated total storage of SOC (kg C m^{-2}) to a depth of 0-100 cm in the northern circumpolar (Palmtag et al. 2022).

The decomposition of SOC was found to be highly retarded in specific cryoturbated C-rich pockets (Kaiser et al. 2007) of permafrost soils (cryosols). Cryoturbation (Washburn 1980; Tarnocai et al. 2009), the burial of SOM deeper into the mineral soil layers (Van Vliet-Lanoë 1991) creates an uneven distribution of SOM in the active soil layer (soil layer which freezes during winter and thaws during summer, Dobiński 2020) of permafrost (i.e., pockets or layers of OM in mineral horizons, Figure 2). This translocation of SOM probably leads to the long-term stabilization of C in the permafrost (Tarnocai et al. 2009).

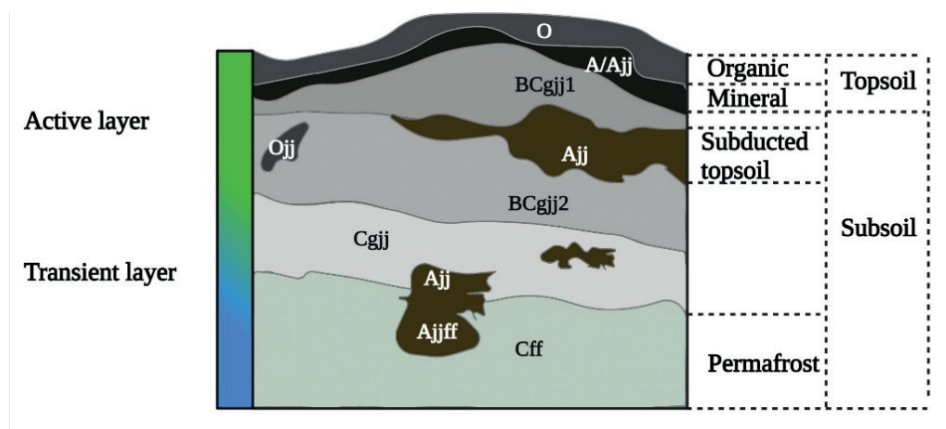


Figure 2: Schematic diagram of typical cryosols (modified from Gentsch et al. 2015a). O, organic layer; A, mineral topsoil; Ojj/Ajj, buried topsoil; B/C, mineral B/C horizons; Cff, permafrost.

Arctic permafrost is warming rapidly, and current scenarios predict a temperature rise up to 4.8 ° C until 2100 (RCP 8.5 scenario, Climate Change 2014 Synthesis Report, Intergovernmental Panel on Climate Change 2014). Higher temperatures will prolong frost-free vegetation periods and increase the thickness of the active soil layer, which may destabilize SOC pools in permafrost soils (Schuur and Abbott 2011; Dungait et al. 2012). Higher temperatures lead to the higher activity of soil microorganisms, which through their metabolism produce CO₂, CH₄, and nitrous oxide (N₂O), potent greenhouse gases (GHGs). Their higher concentration in the atmosphere increases the temperature, leading to

higher permafrost thawing and higher activity of microorganisms. This creates positive feedback on the C cycle in cryosols (Figure 3).

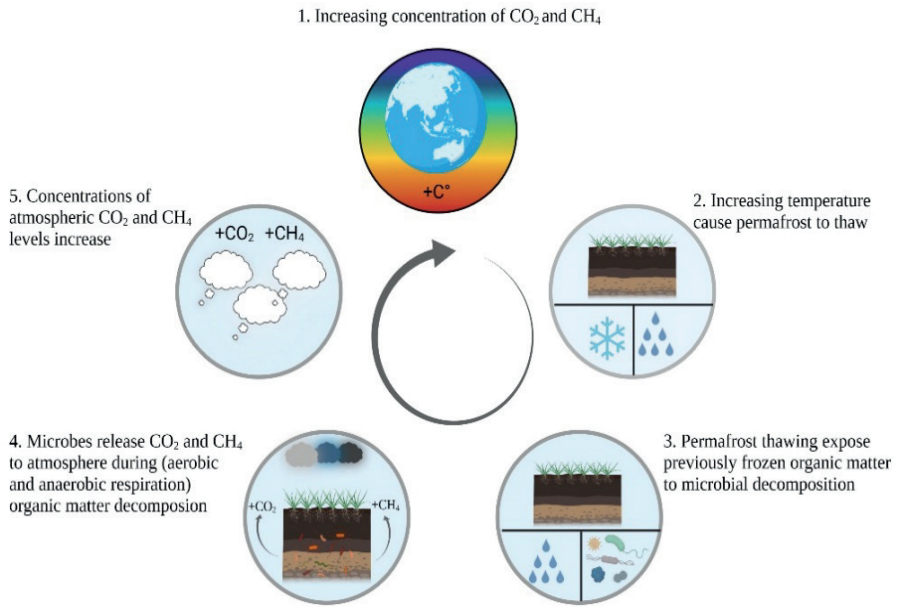


Figure 3: Positive feedback between global warming and permafrost Thaw (Original Varsadiya M).

It is well-known that microbial activity (e.g., enzyme activity, cellular respiration) responds positively to an increase in temperature. Many studies show that the extent of this response depends on both abiotic and biotic factors. Among the most important abiotic factors are the availability of oxygen for microorganisms, moisture, soil dissolved organic matter (DOM), and SOM quality and availability. On the other hand, the taxonomic composition, metabolic potential, and interactions of soil microbial communities are some of the most important biotic factors that influence the decomposition of SOM (Conant et al. 2011; Blagodatskaya et al. 2016; Zhang et al. 2022).

1.2 The microbiome of cryosols

In regular undisturbed soil, the upper organic layer tends to have more fast-growing microbes (copiotrophs or r-strategists) due to the higher availability of nutrients and energy sources (e.g., plant root exudates, and fresh litter)(Fierer et al. 2007; Thomson et al. 2010; Nemergut et al. 2010).

With increasing depth, the amount of easily degradable SOM decreases, and recalcitrant or less available SOM becomes more prevalent (Rumpel and Kögel-Knabner 2011; Eilers et al. 2012; Gentsch et al. 2015a; Palmtag et al. 2016). As a consequence, the slower-growing microbes that are more focused on the decomposition of the recalcitrant SOM (oligotrophs or k-strategists) dominate the subsoil, represented by members of Actinobacteria and Acidobacteria (Fierer et al. 2007; Goldfarb et al. 2011). Therefore, the diversity and abundance of microbial communities in regular undisturbed soils follow the gradient of SOM availability and quantity.

On the contrary, cryosols are specific in the irregular distribution of SOM in the soil profile. Gentsch et al. 2015a reported a similar amount of C and N content in buried OM (cryoOM) and topsoil, which is not observed in regular undisturbed soil. These spatial differences in SOM could lead to significantly different microbial processes in cryoOM, making predictions of cryosols SOM decomposition more difficult (Schnecker et al. 2014; Gittel et al. 2014a, b).

1.2.1 Methane and C1 compounds utilization in the Arctic

Most of the SOC stored in the permafrost can be potentially released not only in the form of CO₂ (Stackhouse et al. 2015; Drake et al. 2015) but a significant fraction (0 - 1 Tg CH₄ yr⁻¹) can be emitted as CH₄ (Saunio et al. 2020). The main producers of CH₄ are methanogenic archaea in anoxic environments that either reduce CO₂ by H₂ (hydrogenotrophic) or disproportionate acetate (acetoclastic). They are ubiquitous and have been isolated from different habitats with moderate climate conditions such as rice paddies (Breidenbach and Conrad 2015; Breidenbach et al. 2016; Masuda et al. 2018), lakes (Jurgens et al. 2000, p. 200; Keough et al. 2003; Reitschuler et al. 2016; Masuda et al. 2018; Chaya et al. 2019), freshwater sediments (Chan et al. 2005; Kadnikov et al. 2019; Wu et al. 2021a), animal rumen fluid (Paul et al. 2017; Söllinger et al. 2018), hydrothermal vents (Ver Eecke et al. 2013; Teske et al. 2014), hypersaline habitats (Li et al. 2019), and permafrost (Kobabe et al. 2004; Ganzert et al. 2007; Tveit et al. 2014; Gittel et al. 2014b; Lau et al. 2015a; Crevecoeur et al. 2016).

Permafrost soil is characterized by extreme environmental conditions (subzero temperature and anoxia), even under such harsh conditions, a high number of methanogens were detected from the permafrost active layer (3×10^8 cell g^{-1}) soil (Kobabe et al. 2004), compared to marshland (9.5×10^4 cell g^{-1}) (Wagner 2017). Most methanogenic species identified in active layers belong to hydrogenotrophic (i.e., *Methanobacteriaceae*, *Methanomicrobiaceae*) and acetoclastic families (i.e., *Methanosarcinaceae*, *Methanosaetaceae*) (Høj et al. 2005; Ganzert et al. 2007; Metje and Frenzel 2007). Several studies reported the dominance of hydrogenotrophic methanogens (*Methanobacteria*) in the top layer which were replaced by acetoclastic methanogens in the mineral subsoil horizon (Frank-Fahle et al. 2014; Gittel et al. 2014b). Recently, hydrogenotrophic methanogens were found to be more temperature-dependent (de Jong et al. 2018; Lavergne et al. 2021) which may have important consequences on the C flux in topsoils of cryosols (Tripathi et al. 2019). On the other hand, the higher relative proportions of facultative acetoclastic order Methanosarcinales in the lower horizons could be due to the fact that this group can use a broad range of substrates, for example, acetate, methanol, and hydrogen (Conrad 2005) and outcompetes hydrogenotrophic methanogens in the deeper soil horizon in cryosols (Barbier et al. 2012). Additionally, acetoclastic methanogenesis might be favored over hydrogenotrophic in deeper soils due to the strong competition of hydrogenotrophic methanogens for H_2 and CO_2 with acetogenic bacteria (i.e., Bacteroidetes) producing acetate, which can be readily available for acetoclastic methanogenesis in deeper layers of cryosols (Kotsyurbenko 2005).

Soil CH_4 fluxes are the result of the production and consumption of microbial CH_4 . Up to 90% of the CH_4 produced by methanogens in deeper soils is consumed in the upper, more aerated layers of soil by aerobic CH_4 oxidizing bacteria, methanotrophs (Le Mer and Roger 2001). Methanotrophs are of particular interest in cryosols due to their key role in the CH_4 cycle in the Arctic (Crevecoeur et al. 2017; Singleton et al. 2018) and for prediction of the further consequences in the global CH_4 budget. Methanotrophs use CH_4 as a C and energy source. The biological oxidation of CH_4 by methanotrophs is the only sink of CH_4 from permafrost soil

(Trotsenko and Khmelenina 2005). Methanotrophs can be found in almost all types of environments, where they survive unfavorable conditions by forming exospores or cysts. Using the fluorescence in situ hybridization method, Liebner and Wagner 2007 detected a high number of the soil of methanotrophs (1×10^8 cell g^{-1}) in the upper layer of the polygon rim than in the lower layer (3×10^6 cell g^{-1}) of the active layer of the permafrost soil, which corresponds with higher numbers of methanogens in the topsoil of cryosols.

Aerobic methanotrophs, in general, belong to three major groups according to the phylogeny and C assimilation pathways, which include Gammaproteobacteria (Type I), Alphaproteobacteria (type II) (Trotsenko and Murrell 2008; Semrau et al. 2010), and Verrucomicrobia (Dunfield et al. 2007; Op den Camp et al. 2009; Urbanová and Bárta 2014). Type I methanotrophs from Gammaproteobacteria are organized into 18 characterized genera within the *Methylococcaceae* and *Methylothermaceae* families (Heyer et al. 2005; Knief 2015; Skennerton et al. 2015), while type II methanotrophs from Alphaproteobacteria are less diverse and comprise only 5 genera within the *Methylocystaceae* and *Beijerinckiaceae* families (Tamas et al. 2014; Knief 2015). The distribution of methanotrophs in the soil profile does not appear to be consistent between different studies and may reflect the differences in the rates of CH₄ production by methanogens. For example, Liebner and Wagner 2007 found the dominance of type I methanotrophs in the surface layer rather than in the deeper layer, whereas another study found that type II methanotrophs outnumbered type I in surface topsoil and cryoOM (Gittel et al. 2014b). The high proportion of type II methanotrophs in the surface layer can be explained by the fact that they thrive in warmer conditions (Knoblauch et al. 2008) and have a high tolerance to the availability of oxygen in contrast to type I methanotrophs. In addition, type II methanotrophs have a low affinity for CH₄, therefore, they dominate mostly in places where CH₄ concentration is high (Bender and Conrad 1995). The warmer climate in the Arctic has already evidenced the shift of microbial community dominated from type I methanotrophs to type II (Knoblauch et al. 2008) which goes together with higher CH₄ production rates; therefore, the diversity and ratios between type I vs. type II

methanotrophs will have an effect on the mitigation of CH₄ flux from the Arctic cryosols.

In contrast to aerobic CH₄ consumption, anaerobic consumption of CH₄ was also observed in a different environment (Boetius et al. 2000; Knittel and Boetius 2009; Blazewicz et al. 2012; Martinez-Cruz et al. 2017a; Miller et al. 2019) which suggested that CH₄ consumption can also occur in anoxic condition using alternative electron acceptor (e.g. sulfates, nitrates, iron, manganese, and humic substances (Achtnich et al. 1995; Caldwell et al. 2008; Smemo and Yavitt 2011; Scheller et al. 2016)). The mechanism behind the anaerobic oxidation in CH₄ consists of altering the last step of methanogenesis that produces CH₄. Alteration of the electron acceptor has been reported to inhibit methanogenesis (Yao and Conrad 1999; Reiche et al. 2008) either by microbial competition (Bodegom et al. 2004) or indirectly through toxic intermediate products like nitrate (Klüber and Conrad 1998). Iron minerals have also been reported to stimulate methanotrophy in methanogens (Bar-Or et al. 2017; Miller et al. 2019) and are the most dominant electron acceptor for anaerobic CH₄ oxidation (Miller et al. 2019). Regardless, an alternative electron acceptor that could be released from permafrost thaw (Patzner et al. 2020) could theoretically dampen the flux of CH₄ through anaerobic CH₄ oxidation, as was already found in a previous Arctic study (Miller et al. 2019).

Another important group responsible for the utilization of C₁/C₂ compounds (e.g., methanol, methylamines) as C and energy sources are methylotrophs (Lidstrom 2006). Most of the known methylotrophs are facultative methylotrophic and strictly aerobic; however, some strict anaerobes have already been described to utilize methanol, for instance, *Morella mulderi* (Balk et al. 2003) and *Thermotoga lettingae* (Balk et al. 2002). So far, 154 species (58 genera) have been described from Alpha-, Beta-, Gamma Proteobacteria, Verrucomicrobia, Bacteroidetes, Firmicutes, and Actinobacteria (Lidstrom 2006).

The methylotrophic genera *Methylobacterium* and *Methylophilus* were found to be actively utilizing C from CH₄ in deep sediment of Alaska (He et al. 2012; Martinez-Cruz et al. 2017b; Kadnikov et al. 2019). With the deepening of the active layer in the Arctic, the lignin decomposition is

expected to increase (Dao et al. 2022), and lignin decomposition is one of the important sources of methanol (Warneke et al. 1999), therefore the importance of methylotrophs in SOM decomposition will be crucial.

1.2.2 N cycle in the Arctic

Primary production and SOM decomposition in the Arctic soil is believed to be N limited (Giblin et al. 1991; Atkin 1996; Schimel and Bennett 2004), and previous studies found a strong positive correlation between the higher C/N ratio of SOM and C mineralization in Arctic soil (Schädel et al. 2014). Furthermore, studies that focused specifically on cryosols also demonstrated strong limitations in N (Wild et al. 2013, 2014). Soil microbes and plants strongly compete to acquire different forms of N, since microbial growth also depends on the energy source (C), organic N-like amino acids appear to be a better substrate for microbial N than the inorganic N. Soil microbes are considered to be more efficient in acquiring organic N-forms (i.e., amino acids) than inorganic N-forms (nitrate, ammonium) due to high substrate affinity, rapid growth rates, and high surface-to-volume ratio when it comes to direct competition with plants (Rosswall 1982). In contrast to this, several studies have found that plants can successfully compete with soil microbes for amino acids (Schimel and Chapin 1996; Lipson and Monson 1998; Näsholm et al. 1998; Nordin et al. 2001, 2004; McKane et al. 2002). These contradictory results are likely due to the different effectiveness of different plants and the different composition of soil microbial communities. The surface soil in the Arctic is considered to have a low amount of N (Sistla et al. 2012; Wild et al. 2013). Immobilized N by plants and microbes can be stored in their biomass and therefore creates a low rate of mineralization of N and low availability of mineral N forms in the topsoil (Schmidt et al. 2002; Schimel and Bennett 2004; Kaiser et al. 2007; Wild et al. 2014). However, the C/N ratio generally decreases with depth inferring that N deficiency in topsoil turns to the limitation of SOC for microbes in subsoil (Fierer et al. 2007; Wild et al. 2014). Therefore, the decomposition of microbial SOM is not only limited by the availability of N but is also restricted by SOC in the Arctic soil. Since N could be bound in complex SOM in the subsoil, microbes need to release extracellular enzymes for N mining. During

cryoturbation, topsoil with a high C/N ratio is buried in mineral subsoil with a low C/N ratio where microbial activity is limited (Schnecker et al. 2014; Gittel et al. 2014a, b), and SOM is relatively not processed over time (Schirrmeyer et al. 2011). Although cryoturbated soil gets mixed with mineral subsoil, a low C/N ratio of the subsoil can partially mitigate the N deficiency of cryoOM by transporting different forms of N from subsoil to cryoOM either via microbial interaction or N leaching, microbes still need to release extracellular enzymes to acquire N from complexed SOM as well as OC. The low availability of N for the microbial community, along with lower enzymatic activity, is believed to be a limiting factor for SOM decomposition in cryoturbated soil (Schnecker et al. 2014; Wild et al. 2014; Čapek et al. 2015). Hence the factors influencing the N availability in this soil are crucial to infer.

Nitrogen input into the Arctic soil is scarce, but its availability in the Arctic is predicted to increase with global warming. Nitrogen, in general, can enter the soil environment by depolymerization of organic compounds, atmospheric deposition, and biological fixation of atmospheric nitrogen gas (N₂). Among them, N₂-fixation by biological processes is an important source of N input to the Arctic terrestrial systems (Barsdate and Alexander 1975; Chapin 1996; Hobara et al. 2006) because natural atmospheric N deposition alone cannot account for net organic N uptake rates (Harms and Jones 2012). Nitrogen fixation is an energy-demanding process constrained by low temperature, limited energy inputs, low pH, or limited by other nutrients (i.e., phosphors). However, N₂-fixation by heterotrophic bacteria (Nosko et al. 1994; Larmola et al. 2014; Knorr et al. 2015) can represent an important input of N in the northern ecosystem, but its importance and implications for cryosols still scare.

The diversity and abundance of N₂-fixing microbes (diazotrophs) are determined by sequencing and quantification of the *nifH* gene, which encodes a subunit of the nitrogenase complex (Hsu and Buckley 2009; Reed et al. 2010). Five primary groups of homologous genes to *nifH* have been identified, including Groups I to V (Raymond et al. 2004). Group I consists of aerobic diazotrophs belonging to Alpha-, Beta-, Gamma Proteobacteria; Group II is functionally similar to Group I and contains

mainly obligate anaerobes such as methanogens, sulfate-reducers, and clostridia; Group III comprises anaerobic diazotrophs from bacteria and archaea and primarily Deltaproteobacteria; IV and V contain *nifH* paralog and are not involved in N₂ fixation. Previous studies found varying diazotroph diversity and abundance across soil depth. Quantitative PCR (qPCR) of the *nifH* gene revealed that several diazotrophs in cryosols were more abundant in the topsoil and decreased with increasing depth (Frank-Fahle et al. 2014). The sequencing-based study reported a high relative proportion of the *nifH* gene in the mineral subsoil than in the topsoil (Tripathi et al. 2019). The metatranscriptomics study also confirmed the presence of *nifH* in the active layer and permafrost samples of 2 m depth (Yergeau et al. 2010).

The diversity and abundance of diazotrophs are influenced by many abiotic factors (i.e., temperature, soil texture, pH), among which soil moisture plays a major role (Chapin 1996; Stewart et al. 2011). The warmer climate in the Arctic leads to an increase in permafrost thaw (Schuur et al. 2015), which frequently results in soil profile compaction and higher soil moisture, and therefore supports the growth of diazotrophs (Liengen and Olsen 1997; Zielke et al. 2005; Stewart et al. 2013). High soil moisture also has an indirect effect on N₂-fixation by enhancing net plant primary production, thus increasing plant-derived root exudate input and transporting dissolved C and nutrients to deeper layers (Hartley and Schlesinger 2002). Increased supply of dissolved nutrients and C by root exudates from deep-rooting plants (Mekonnen et al. 2021) or leaching with water can liberate the N pool (Keuper et al. 2012) from deeper soil and could inhibit diazotroph activity of diazotrophs if N availability exceeds a threshold concentration (Rousk et al. 2013). However, until now, comprehensive studies of diazotrophs' activity and abundance have not been clarified in cryosols.

1.2.3 Eukaryotes involved in the C and N cycle in cryosols

Soil eukaryotes play an important role as a decomposer, symbiotrophs, plant parasites, and microbial predators in the soil. One of the most studied eukaryotic groups in the soil is fungi which represent a large portion of the biodiversity on Earth that comprises approximately 12 million fungal

species (Wu et al. 2019). Fungi are widely distributed in all terrestrial ecosystems, from the tropics to the polar regions. Fungi are ubiquitous in the cold soils of the Arctic (Newsham et al. 2009; Timling and Taylor 2012). They are found in Arctic sediments, glaciers, and permafrost and comprise a significant amount of living microbial biomass of the Arctic soil. Arctic permafrost soils exhibit considerable taxonomic diversity, which includes all major fungal phyla.

Studies of the Arctic microbiome revealed that Ascomycota and Basidiomycota are the most abundant phyla, while Glomeromycota, Rozellomycota, and Zygomycota were less abundant (Wallenstein et al. 2007; Gittel et al. 2014a; Zhang et al. 2016). The diversity and composition of fungal communities were also found to differ with the depth of the soil in the Arctic soil. The culture-independent approach showed significantly higher richness and diversity of the fungal community between the organic horizon and the mineral subsoil horizon (Deslippe et al. 2012; Gittel et al. 2014a). The C/N ratio is a significant factor in determining the structure of the fungal community (Dennis et al. 2012; Fujimura and Egger 2012). Organic horizons have a relatively higher C/N ratio, which may harbor a more diverse and highly rich fungal community, as has been observed in the Arctic and other regions (Dennis et al. 2012; Fujimura and Egger 2012; Timling and Taylor 2012; Schnecker et al. 2014; Gittel et al. 2014a). Not only C and N but also phosphorus (P) was also found to have a significant effect on the distribution of fungal communities in Arctic soil (Fujimura et al. 2008). Fungi play an essential role in solubilizing inorganic phosphate to simple forms, thus making it available to plants (Saxena et al. 2014). Permafrost thaw has been reported to increase the availability of P in soil, which could potentially be solubilized by fungi and can make it readily available for plant growth (Yang et al. 2021). Higher availability of P after permafrost thaw was also associated with changes in plant diversity and increased gross primary production in the Arctic (Yang et al. 2021). Ultimately, P availability can lead to changes in the composition and abundance of fungi, but its role in SOM decomposition in cryosols is still unknown.

One of the most important fungal lifestyles in the Arctic is mycorrhiza, which uptakes nutrients and water for plants and gets C in return from the plant host. Their role is particularly essential in the Arctic ecosystem, where low water and nutrient availability constrain plant growth (Timling and Taylor 2012). It is estimated that almost 86% of N uptake by Arctic plants is mainly through ectomycorrhizal fungi (Hobbie and Hobbie 2006). Molecular analyses of root tips and soil clones show that the most frequent ectomycorrhizal fungal genera were *Cenococcum*, *Cortinarius*, *Inocybe*, and *Thelephora* (Bjorbækmo et al. 2010; Timling and Taylor 2012). High temperature in the Arctic is associated with a decrease in ectomycorrhizal richness (Mundra et al. 2016), but it is not yet known if this response is short- or long-term. Nitrogen availability is predicted to increase with changing climate, and this may have a negative effect on the richness and abundance of the ectomycorrhizal community. If more available N is present in the soil, the host plant may have a lower dependency on ectomycorrhizal symbionts and consequently decrease the allocation of C to fungal partners (Deslippe and Simard 2011) that starve. Plants may instead use retained C to build plant biomass. This process may exceed the SOM and can thereby result in the greater richness and abundance of saprotrophic fungi, which are known to produce more efficient extracellular enzymes to decompose complex SOM. Overall, a higher proportion of saprotrophs can change the overall ecosystem functioning (Setälä and McLean 2004; van der Wal et al. 2013). Although changes in plant diversity in the Arctic (described in Chapter 1.4) are likely to influence associated fungal communities, there is still a lack of information on the occurrence and ecological importance of fungi in Arctic habitats across different horizons and tundra vegetation.

Microbes in the soil create a complex ecological network by interacting with each other (Faust et al. 2012). This interaction includes, for example, predation, competition, parasitism, or mutualism (Deng et al. 2012; Barberán et al. 2012; Lupatini et al. 2014; Shen et al. 2014; Coyte et al. 2015; Mondav et al. 2017; Qian et al. 2018; Wagg et al. 2019). However, currently, we are not able to cultivate the majority of fungal species and observe these complex interactions in vivo. The current tool partially overcomes these constraints by using molecular markers, constructing the

operational taxonomic unit tables (OTU tables), and studying these interactions in-silico through the correlation co-occurrence matrixes. Predicting ecological microbial networks is especially important to understand the microbial assembly and the potential interactions between keystone taxa resulting in ecological functions (Barberán et al. 2012; Faust et al. 2012; Ma et al. 2016; Banerjee et al. 2018). The co-occurrence network allows the identification of the positive/negative relationships between microbial taxa and identifies potential keystone taxa which are crucial for overall community stability and functioning (Rafrafi et al. 2013; Sun et al. 2017; Banerjee et al. 2018). The keystone species are most important since their absence could lead to network fragmentation (Martín González et al. 2010a). According to Olesen et al. 2007, the taxa which have more connections with other taxa but within their own separate modules (i.e., highly connected subnetwork) are called “*module hubs*”, while the taxa which have more connections with several modules but less in their own modules are called “*module connectors*” which can be viewed as the messengers between the various modules. Module hubs and connectors are considered *generalists*, while the third class, the “*peripherals*” have only a few connections to the taxa within their modules as a *specialist*. Translated to a natural ecosystem, generalists take up nutrients from a broad range of sources and grow well in many habitats, whereas specialists have narrow nutrient requirements and therefore their growth is restricted to certain habitats (Dupont and Olesen 2009; Martín González et al. 2010b; Deng et al. 2012; Tao et al. 2018). Permafrost thaw is inevitable, which can change the availability of different chemical compounds in soil (Patzner et al. 2020), and this changed soil chemistry potentially changes the role of certain taxa from being specialist to generalist or vice versa.

If it were not for protozoa and nematodes in the soil, the nutrients available to the plant would be locked up in the bacterial biomass. This interaction between prey bacteria and predatory protozoa in the soil has both a "top-down" effect on the regulation of bacterial numbers and a "bottom-up" effect on the microfauna (nematodes, small arthropods, and protozoa) that acquire nutrients in the terrestrial environment (Wardle and Yeates 1993; Bonkowski et al. 2000).

Grazing-induced changes in the composition and functioning of the microbial community can affect fundamental ecosystem properties because soil bacteria (and archaea) essentially control N cycling, such as N₂-fixation, nitrification, and denitrification (Mengel 1996). Nitrifying bacteria are stimulated by the release of ammonium (NH₄⁺) from protozoan grazers, presumably by preying on fast-growing bacterial competitors (Griffiths 1989; Alpehi et al. 1996). In addition to traditionally considered bacterial eukaryotic grazers (protozoa and nematodes), several recent studies showed that in some soils bacterial predators are also very active, for example, myxobacteria of the order Myxococcales (Petters et al. 2021).

According to the microbial loop hypothesis, some organisms (earthworms, plants, Van Breemen and Finzi 1998) can stimulate the release of mineral N from SOM by providing a low molecular weight C source to C-limited microbes (Figure 4). To initiate the microbial loop, the first step is the release of plant root exudate, which can increase the growth of bacteria and a higher bacterial growth leads to an increase in SOM degradation and mineral N demands for microbes. Stimulated bacterial growth enhances the predation rates, and high predation liberates mineral N and makes it readily available for other microbes and plants (Clarholm 1985). In the Arctic, the roots of tundra grasses are not growing deep and the N cycle and the availability of N are severely limited in cryoOM compared to topsoil (Wild et al. 2013, 2014), which could probably indicate a slow 'microbial loop', possibly due to the lack of root exudates and a low abundance of bacteria grazers (i.e., protozoa and nematodes). Future changes in the Arctic can cause vegetation shifts from shallow rooting grasses toward deeper rooting shrub vegetation (Mekonnen et al. 2021), which can exude easily available C and N and prime the decomposition of

SOM in the cryoturbated soil, as was found in previous laboratory incubation studies (Wild et al. 2013, 2014).

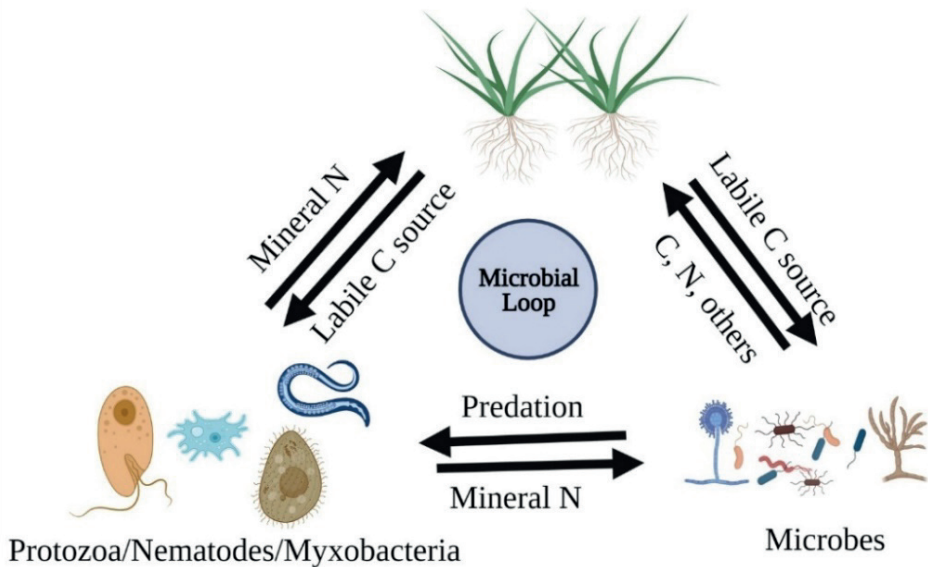


Figure 4: Schematic overview of the simplified illustration of microbial loop concept as applied to soil community (created by Varsadiya M according to Clarholm 1985).

1.3 Arctic vegetation and associated microbial communities

The growing season in the Arctic is very short, while sub-zero temperatures and snow form the harsh winter climate for most of the year. Typical vegetation that can persist under specific climatic conditions is herbaceous plants, small shrubs, mosses, and lichens (Walker et al. 2005). The increase in annual temperature in the Arctic (Intergovernmental Panel on Climate Change 2014), has led to permafrost thawing and active layer deepening, which liberated nutrients for the growth of plants and soil microbes (Anisimov et al. 1997; Zhao and Wu 2019; Desyatkin et al. 2020).

Several recent studies have predicted higher plant productivity and significant changes in the composition and diversity of Arctic vegetation (Tape et al. 2006; Elmendorf et al. 2012; Macias-Fauria et al. 2012; Pearson et al. 2013; Mekonnen et al. 2021). Higher plant productivity leads

to higher root exudates and fresh root litter fluxes which can stimulate soil microbial activity and enhance the decomposition of more recalcitrant SOM, a process known as the positive priming effect (Fontaine et al. 2003, 2007; Pegoraro et al. 2019; Parker et al. 2021) that induces losses of SOM to the atmosphere, mainly in the form of CO₂ and CH₄. In contrast to positive priming, negative priming decreases the microbial mineralization of native SOM when lower substrate addition would cause only a switch toward new substrate utilization (Kuzyakov et al. 2000). Field and laboratory studies have confirmed the stimulation of SOM decomposition when amended with easily available plant materials (Rinnan et al. 2007; Wild et al. 2014; Walker et al. 2016), but the response rate varied in different horizons. For instance, SOM decomposition was stimulated when amended with N sources in topsoil, whereas C sources in mineral subsoil (Nowinski et al. 2008; Lavoie et al. 2011; Sistla et al. 2012; Wild et al. 2014), which suggested N and C limited microbial community in topsoil and subsoil, respectively (Fontaine et al. 2007; Wild et al. 2014). Cryoturbated soil, in contrast to regular soils, exhibited enhanced SOM decomposition when amended with both C and N sources (Wild et al. 2014), likely due to mixed N and C limitations. Together, the shift in vegetation in the Arctic leads to changes in the associated microbial community through the allocation of different quality and quantities of root exudates and plant litter, which consequently either increase (positive priming) or decrease (negative priming) SOM decomposition in cryosols. One of the most crucial biotic factors controlling these processes is a microbial community, and therefore, research focused more specifically on concrete functional guilds (methanogens, diazotrophs), their diversity and abundance together with the actual soil microbial activity (reflected by an extracellular enzyme) associated with vegetation shift is much needed (Eilers et al. 2010; Ridl et al. 2016).

Various plant communities support a different structure of the soil microbial community. Since Arctic tundra vegetation varies greatly across relatively short distances due to highly heterogeneous soil conditions of cryosols across the landscape, vegetation type can be an indicator of spatial patterns in the soil microbial community (Walker 2000; Björk et al. 2007). Vegetation composition influences soil biogeochemistry (Shi et al. 2015), including soil nutrient content, OM quality (Biasi et al. 2005), and microbial diversity (Gittel et al. 2014a, b) by a specific allocation of root

exudates and root litter. For example, tussock tundra can promote the growth of slow-growing oligotrophs (i.e., Acidobacteria, Actinobacteria) due to more recalcitrant plant litter, whereas shrub tundra can enhance the growth of copiotrophs (i.e., Proteobacteria) due to higher availability of labile source (Wallenstein et al. 2007). In addition, tussock tundra, which includes nonmycorrhizal sedges and mosses, had more dominance of Ascomycota, whereas Basidiomycota and Zygomycota were more frequent in an ectomycorrhizal deciduous dwarf shrub (Wallenstein et al. 2007). Collectively, these findings emphasize that the plant community and the soil microbial community are highly dependent on each other. Under changing climate, vegetation shift in the Arctic ecosystem may have a significant effect on microbial composition and diversity compared to other temperate terrestrial ecosystems. These changes could lead to a shift in the subsurface microbial community, which consequently reflects the overall C fluxes from the Arctic ecosystem.

1.4 Microbial activity in cryosols

Soil extracellular enzymes produced by microorganisms are the most important biological machinery responsible for the transformation of SOM (Schnecker et al. 2014). Extracellular enzymes are N-rich polymers, and their production can be constitutive (constantly produced) or inducible. Their secretion and activity are sensitive to environmental changes and due to their functional characteristics (i.e., substrate specificity), they are powerful indicators of microbial metabolic activity and soil quality (if limited or enriched by certain nutrients or energy) in different ecosystems (Bell et al. 2010; Henry 2013; Luo et al. 2017). The most evaluated enzymes are those which are involved in the decomposition of cellulose (β -1,4-glucosidase and 1, 4- β -cellobiohydrolase), chitin (β -1,4-N-acetylglucosaminidase and leucine aminopeptidase), and phosphate (phosphatase). These enzymes represent the metabolic activities of C, N, and P, respectively (Allison et al. 2007). The decomposition of more complex recalcitrant SOM occurs by oxidation of aliphatic and aromatic hydrocarbons by peroxidase and oxidation of phenolic compounds by phenoloxidase (Sinsabaugh 2010). Phenoloxidase and peroxidase use molecular oxygen and peroxides, respectively, as terminal electron

acceptors to catalyze, e.g., oxidative cleavage of the complex lignin structure.

According to resource allocation theory, microbes could invest an abundant amount of nutrients to produce energetically costly extracellular enzymes to acquire the most limiting nutrient for their growth and metabolism (Allison et al. 2010; Mooshammer et al. 2012). However, the activity of a single enzyme alone cannot reflect the actual limitations of microbial resources. Resource limitation is given by the difference between the ratio of nutrients demanded by microbes and the relative availability of all major nutrients in the environment (Sinsabaugh et al. 2009). The actual limitation of the available resources for microbial metabolism is better reflected in the ratios of C, N, and P enzymes than their absolute activities. The approach of studying enzyme ratios provides insight into the limitation of energy (C) and/or nutrients (N and P) in the soil by assessing the shift in microbial metabolism from being energy limited (more C enzyme production) to nutrient-limited (more N, P enzyme production) or vice versa (Sinsabaugh et al. 2009). In general, this so-called enzyme stoichiometry is calculated from the ratio of different enzymes acquiring C, N, and P (Sinsabaugh et al. 2008).

The enzyme pool in the tundra ecosystem is smaller than in the other ecosystem (Wallenstein et al. 2009), which can be explained by two hypotheses. According to the first hypothesis, microbes produce enzymes to degrade SOM into soluble monomers that can be taken up by microbes or plants; therefore, the abundance of enzymes can reflect the availability of the substrate they degrade. Hence, the microbial allocation of resources to produce enzymes depends on the availability of N in the environment (since enzymes are N-rich proteins) and the physiological status of the microbe (Sinsabaugh and Moorhead 1994; Schimel 2003). As maintenance costs increase or N becomes limited, the resource is likely diverted from enzyme production to cell maintenance. Arctic soil has low available N (Wild et al. 2013, 2014). As a consequence, microbes possess fewer resources than they could allocate to enzyme production, and the enzyme pool in the Arctic is smaller than in other ecosystems. The second hypothesis is based on *in situ* conditions, namely, temperature because the

rate of every enzymatic reaction is positively related to temperature. The obvious temperature sensitivity of enzyme activity is the result of at least two components, i) the intrinsic response of the enzymatic reaction to temperature and ii) the effect of temperature on physiochemical protection of substrate and enzymes and the diffusion of both through the soil environment. This is especially important in the sensitivity of the cold region, as the enzyme increases with a decrease in temperature (Koch et al. 2007). Overall, the decomposition of SOM in the tundra is likely to slow due to a combination of two phenomena, the limited production of microbial enzymes (small pool of enzymes) and the harsh *in situ* conditions (i.e., low temperature) that decrease the enzyme activity.

Schnecker et al. 2014 studied the effect of the properties of SOM and the composition of the microbial community on the enzyme activity of cryoOM from the Siberian Arctic. The authors found that cryoOM had a similar content of C and N (total) as mineral topsoil and the C enzyme (β -1,4-glucosidase) and the phenoloxidase activities were comparable in both, while the activity of the N enzyme (leucine aminopeptidase) was significantly lower in the cryoOM than in mineral topsoil. The authors further found that the microbial enzyme activity in the regular genetic soil horizons (organic topsoil mineral topsoil, and mineral subsoil) mainly corresponded to substrate properties (total C and N). On the contrary, the enzyme activity in cryoOM was driven solely by the composition of the microbial community, and no significant effect on substrate quality (SOM). This study suggested that the microbial communities in the cryoOM are not equilibrated with the available substrate, resulting in slow/delayed decomposition of SOM in cryoOM (Kaiser et al. 2007; Čapek et al. 2015).

2 AIMS AND OBJECTIVES

The main objective of this work was to identify and quantify the dominant taxonomic groups and functional guilds of the soil microbiome involved in the transformation of SOM in Arctic cryosols.

Objective 1: To describe the composition and interaction of prokaryotic and eukaryotic microbial communities associated with different soil horizons and tundra vegetation.

Objective 2: To specifically characterize and quantify the microbial community associated with CH₄ production and N₂ fixation at distinct soil horizons and under different tundra vegetation.

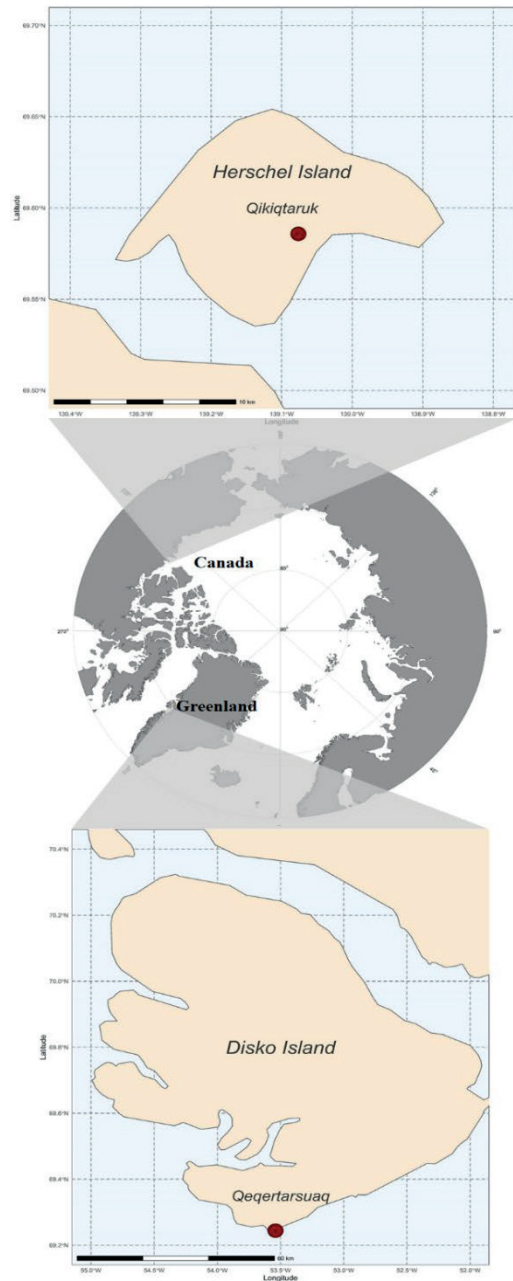
Objective 3: To identify differences in C, N, and P resource limitation in distinct soil horizons through the activity of soil C, N, and P extracellular enzymes.

Objective 4: To characterize the active microbial community include the prey-predator relationship affecting the C and N cycles.

3 SAMPLING LOCALITIES

The soil samples were collected from two Arctic localities - Herschel Island (HI), Canada (**paper I, II, and III**), and Disko Island (DI), Greenland (**paper III and IV**) (Figure 5). In total, 136 and 140 soil samples were collected across three and four sites from HI and DI, respectively. The samples were acquired from topsoil (O and A horizons), cryoOM (Ojj and Ajj soil horizons), subsoil (B, C, BCg soil horizon), and permafrost layer (PF). A detailed description of environmental conditions, sampling strategy, and sampling process is given in the attached papers.

Figure 5: Sampling locations.



4 METHODS

The following methods were applied to study the composition of the microbial community (Figure 6). The composition of the prokaryotic (archaeal and bacterial) and eukaryotic (fungal and protozoa) community and microbes with the potential for CH₄ emission (by methanogens- *mcrA* gene) and N₂ fixation (diazotrophs- *nifH* gene) were studied. Each identified bacterial and fungal genera was annotated into functional groups (**paper I**) using the FAPROTAX pipeline (Louca et al. 2016) and lifestyle (**paper II**) using the FungalTraits pipeline (Pöhlme et al. 2020), respectively. The co-occurrence network (**paper II**) was constructed to identify relationship between different OTUs, describe the properties of fungal networks in different horizons, and identify the keystone fungal taxa. Several model approaches were used to determine the resource (C, N, and P) limitations based on soil extracellular enzyme analysis (**paper III**). The holistic metatranscriptomics community profiling approach (Urich et al. 2008) was used to obtain a broad view into the three domains of life (archaea, bacteria, and eukaryote, **paper IV**) and the relationship between different trophic levels (predator/prey). The abundance of SSU rRNA was quantified per gram of dry soil by integrating total RNA amounts per gram of soil and relative transcript abundance (Söllinger et al. 2018).

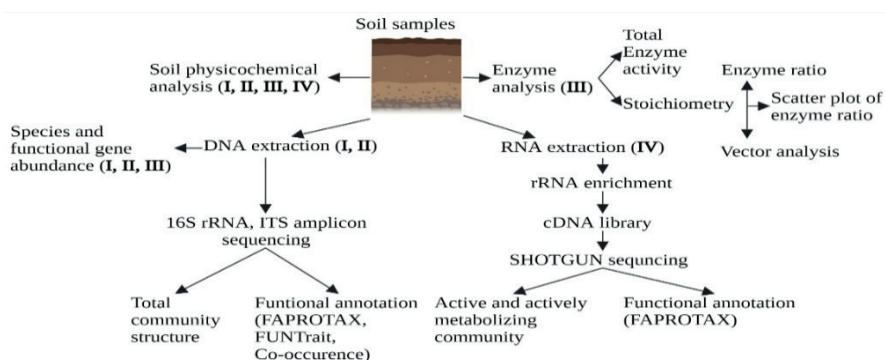


Figure 6: Analysis carried out in the present study.

5 RESULTS AND DISCUSSION

5.1 Microbial community composition in topsoil and subsoil in the active layer of Arctic cryosols

The composition of the microbial community and the functional potential are the two most important biological factors that influence the rates of SOM and the potential release of GHGs into the atmosphere from Arctic cryosols. Arctic microbiomes are unique because they have to deal with very harsh and dynamic environmental conditions, which affect their composition and functionality. Here we discuss the main prokaryotic and eukaryotic communities, their unique taxa, and functional guilds which we found to be the most important in relation to the chemical composition of SOM and its transformation. Additionally, we will try to reveal important interactions and activity within and between different trophic levels of the complex soil microbiome in Arctic cryosols.

Bacterial diversity, species richness, and evenness are the main indices that are determined in many studies. It was generally accepted that these indexes decrease with soil depth (Frank-Fahle et al. 2014; Gittel et al. 2014a, b; Tripathi et al. 2019) because they probably reflect the different SOM content and availability in distinct soil horizons (Schnecker et al. 2014; Gentsch et al. 2015a, b).

Our data confirmed the decreasing trend of these indices with soil depth and showed that the topsoil (0 to approx. 30cm) of the active layer of cryosols exhibited the highest bacterial richness and evenness compared to subsoil horizons. The topsoil microbiome was significantly enriched with copiotrophic bacteria of Alphaproteobacteria (i.e., free-living aerobic N₂-fixing *Beijerinckiaceae*) and Gammaproteobacteria (i.e., saprophytic *Pseudomonadaceae*), which can be related to higher availability of DOM from root exudates and decomposing litter (Fierer et al. 2007; Thomson et al. 2010; Nemergut et al. 2010; Frank-Fahle et al. 2014; Gittel et al. 2014a, b; Dukunde et al. 2019; Wang et al. 2020). The proportion of Alphaproteobacteria decreased from topsoil to subsoil, but no such difference was observed for Gammaproteobacteria in our study (**paper I**). Contrary to our data, the proportion of Gammaproteobacteria was higher

in the subsoil compared to the topsoil of the Arctic (Gittel et al. 2014a; Wu et al. 2021b) and the forest ecosystem (Wang et al. 2020). One potential reason for this discrepancy could be a negative correlation of this class with OC (Wu et al. 2018; Ricketts et al. 2020). Gittel et al. 2014a reported significantly lower OC in the subsoil compared to the topsoil and no significant differences in OC were evidenced from our work, which could potentially suggest the difference in the relative proportion of Gammaproteobacteria. In addition, the effect of the physical and chemical properties of the soil (that is, aggregation, mineral association, SOC, pH) on the composition of the microbial community composition became more dominant in the subsoil, contrary to topsoil. Edaphic factors such as SOC and change in soil nutrients strongly drive the composition of the subsoil microbial community (Jobbágy and Jackson 2000, 2001; Rumpel and Kögel-Knabner 2011), instead of the temperature and soil moisture which are the most common determinants factors in topsoil microbial community (Dukunde et al. 2019). Soil OM is less available for microbes in the subsoil due to mineral association or aggregate occlusion, which enforces colonization of microbes adapted to low OC availability, such as oligotrophs and lithotrophs. Our data also reported such microbes in the subsoil (i.e., Acidobacteria, Gemmatimonadetes), which were consistent with previous studies from the Arctic (Frank-Fahle et al. 2014; Gittel et al. 2014a, b; Tripathi et al. 2019), but in contrast to the forest (Dukunde et al. 2019; Guo et al. 2021) where the authors reported similarly higher proportions of these phyla in the topsoil and subsoil. Deeper taxonomy analyzes further revealed significant enrichment of the Nitrosomonadaceae bacterial family in subsoil compared to topsoil. This family plays major role in controlling the N cycle in terrestrial, freshwater, and marine environments (Prosser et al. 2014). Hence, their higher proportion in the subsoil could suggest their importance in N cycle in the deeper soil of cryosols too; however, warrant more detailed study. Collectively, the bacterial community in the topsoil can be similar between different ecosystems but markedly varied when comes to the subsoil.

The other important prokaryotic group that we analyzed was archaea. However, here we must point out that the number of archaea sequences in the total microbiome was a maximum of 4%, which in absolute numbers

corresponded to hundreds to thousands of sequences per sample. Data and trends may be influenced by this fact. Also, their abundances were several orders lower than those of bacteria as quantified by qPCR, and they did not differ significantly between topsoil and subsoil. This was in contrast to previous studies from Greenland (Gittel et al. 2014b), Canada (Frank-Fahle et al. 2014), and Alaska (Lipson et al. 2013). We think that this contrasting results could be explained by differences in composition of archaeal community in distinct horizons in the studied localities. In our study sites the methanogenic Euryarchaeota decreased dramatically from topsoil to subsoil, whereas autotrophic ammonia oxidizing Thaumarchaeota steeply increased (**paper I, IV**, Gittel et al. 2014b), which was in contrast to previous studies (Bai et al. 2017; Tripathi et al. 2019; Frey et al. 2021). Moreover, the proportion of phylum Crenarchaeota was also considerably lower in our sites (Jurgens G et al. 1997; Buckley Daniel H. et al. 1998; Mikkonen et al. 2014; Bai et al. 2017). Hence, we assume that the difference in the composition of the archaeal community could lead to change in archaeal genes at our study sites.

Similar to bacteria, the number of fungi was significantly higher in the topsoil than in the subsoil (**paper I, III**) (Schnecker et al. 2014; Gittel et al. 2014a, b). We found a higher relative proportion of phylum Basidiomycota in topsoil, whereas Ascomycota in subsoil, similar to previous study (Gittel et al. 2014a). The topsoil had a high relative proportion of soil saprotrophs and root endophyte, whereas in subsoil ectomycorrhizal, litter and wood saprotrophs dominated (**paper II**). Among endophytic fungi in the topsoil the genus *Phialocephala* was the most represented (Zhang et al. 2016; Schütte et al. 2019). Members of this genus can form a symbiotic relationship with the plant host, mineralize organic N (e.g., proteins) as the sole source of N (Caldwell et al. 2000) in the rhizosphere (Upton et al. 2009), and also decompose the complex SOM (Surono and Narisawa 2017). Their role in releasing complexed P to a more labile form was also evidenced (Della Monica et al. 2015). Collectively, all these studies indicate the essential role of this genus in nutrient uptake and SOM transformation. The presence of *Phialocephala* was not restricted only to topsoil, but we found it in subsoil too. Obviously, fungal genera that have a lifestyle of endophytes in the topsoil can switch

to a different lifestyle in the subsoil due to more competition or co-metabolism to acquire limited resources (Schlegel et al. 2016; Lennon 2020). Some species of endophytes actually exhibit morphologically and phylogenetically similar characteristics to saprotrophs such as *Colletotrichum* sp., *Fusarium* sp., *Leptosphaeria* sp. (Promputtha et al. 2010) and *Phialocephala* sp. (Schlegel et al. 2016).

To better understand the shift in fungal lifestyle, we constructed a co-occurrence network which could identify generalist (keystone taxa) and specialist taxa. The co-occurrence network analysis revealed that the saprotrophic *Penicillium* (Pölme et al. 2020) was generalist (plays crucial role in network maintenance) in the subsoil, whereas it was identified as specialist in the topsoil. As defined previously, generalist taxa have more interactions and mostly with other modules (i.e., different lifestyle), whereas specialist have selected interaction and only within its own module (i.e., similar lifestyles). We found that *Penicillium* was positively interacted (in the co-occurrence network) with the soil saprotrophic *Oidiodendron*, ectomycorrhizal *Tricholoma*, and lichenized *Bagliettoa* in the subsoil as a generalist taxon, whereas the litter saprotrophic *Tetracladium* and *Leptodontidium*, and the soil saprotrophic *Mucor* and *Mortierella* in the topsoil as a specialist taxon. These interactions can influence the presence/absence of co-occurring taxa, for example, a previous study found that metabolites produced by *Penicillium* can support the growth of ectomycorrhizal *Tricholoma* (Oh et al. 2019). The absence of certain taxa can, therefore, demolish these interactions and probably enforce certain taxa to shift their lifestyle. Another example of a shift of the fungal lifestyle between the horizons could be members ectomycorrhizal *Russula* which was identified as generalist in the topsoil and had positive interactions with *Amanita* (ectomycorrhizal), *Tetracladium* (litter saprotroph), *Meliniomyces* (root endophyte), and *Luellia* (wood saprotroph). Whereas as a specialist in subsoil, this genus had only two positive interactions with an unidentified fungal genus. These results could support the idea that generalists have a great number of interactions with different lifestyle comprised modules than a specialist. The Arctic vegetation and associated below-ground microbial community are expected to change (Mekonnen et al. 2021) that may change the

occurrence of individual taxa and as consequences, potentially change the lifestyle of co-occurring taxa.

Bacteria and fungi represent the most studied soil microbial groups, and the shift in their composition and functioning is often related to changes in the decomposition of SOM in soil through mutualistic or competitive interaction between them. However, these microbial domains are embedded in complex soil food webs in which predation can dramatically change the structure and functioning of these communities (Lal 2004; Gao et al. 2019). The bacterial and fungal populations in the soil are preferentially grazed by a large group of protozoa, mainly amoebae, flagellates (Ekelund and Rønn 1994), and nematodes (Gao et al. 2019; Thakur and Geisen 2019). The topsoil is the zone of extensive root exudation that supports quick growth and faster turnover of microbial biomass compared to the subsoil, which is usually nutrient limited (**paper I, II**). Therefore, the biomass of most microorganisms can be controlled not only by the number of available nutrients, but also by the actual abundance of individual predators. For example, active eukaryotic predators (protozoa and nematodes) decreased from topsoil to subsoil along with the prokaryotic predator Bdellovibrionales (**paper IV**). Bacteria and other protists feeding Amoebozoa abundance, in particular, decreased from the topsoil to the subsoil. Their high abundance in topsoil can be explained by their ability to consume not only bacteria and other protists prey, but also particulate OM (Stockem and Klein 1979). The number of predators declines with depth due to reduced prey biomass density (mainly due to limited availability of SOC) and, in addition, the soil pore size also decreases in the mineral subsoil, which restricts eukaryotic predators from accessing bacterial prey. The different size of soil pores has already been found to be the main driver of compartmentalization of predators and prey organisms in the soil (Griffiths 1990; Rønn et al. 2001; Erktan et al. 2020). This constraining factor, however, does not restrict small sized prokaryotic predators to prey bacteria. We found that the total count of active prokaryotic predator myxobacteria per g of soil, as well as their proportional abundance in the microbial community, was remarkably similar between horizons, which was already evidenced recently (Petters et al. 2021). Thus, microbes living

in non-continuous capillary pores (i.e., unprotected macro-pore in topsoil) are protected from bigger cell sized eukaryotic predators in subsoil but not from similar sized prokaryotic predators such as myxobacteria. Taken together, a high abundance of active eukaryotic and prokaryotic predators was found in all analyzed horizons; however, the predatory control over the microbial population in the soil profile varies according to individual horizon in cryosols.

5.2 Specific microbiome of cryoturbated organic matter (cryoOM)

The composition of the microbial community undergoes a significant change when topsoil OM material is buried in deeper soil layers by cryoturbation (cryoOM) and trapped within the mineral subsoil and is mainly influenced by i) mixing of the topsoil with the subsoil microbes and/or ii) migration of the subsoil community into the cryoOM. Our data confirmed that the composition of the cryoOM microbiome was more similar to the surrounding subsoil than to the topsoil from which it originated (**paper I, II, IV**) supporting the proposed theory of mixing and migrating microbes from subsoil to cryoOM. What was exceptional compared to the subsoil was the number of the bacteria (per gram of soil), which was as high as in the topsoil. Our findings were consistent with previous studies on permafrost-affected soils (Schnecker et al. 2014; Gittel et al. 2014a, b).

The cryoturbated organic horizon has in general a significantly larger amount of SOM than the surrounding subsoil (Kaiser et al. 2007; Gentsch et al. 2015a), however the majority of this SOM is bound to mineral particles (Gentsch et al. 2015b, a) and therefore not readily available for the microbial community. The specific conditions in cryoOM probably support the growth of slower growing oligotrophic bacterial taxa. Indeed, we found in the cryoOM microbiome a significantly higher proportion of oligotrophic families of Actinobacteria (*Intrasporangiaceae*, *Cellulomonadaceae*). The families *Intrasporangiaceae*, *Cellulomonadaceae* were found to have the ability to degrade various plant-derived organic compounds (Schellenberger et al. 2010; Yergeau et al. 2010; Stackebrandt and Schumann 2014; Huber et al. 2017) and their

high proportion was already reported in previous studies from the Arctic (Tveit et al. 2013; Gittel et al. 2014b). A higher proportion of complex SOM degrading oligotrophs in cryoOM could also be involved in the liberation of simple OM for copiotrophic microbes. We found copiotrophic families such as *Alicyclobacillaceae* and *Clostridiaceae* (phylum Firmicute) highly proportionate in cryoOM, the presence of these families (which possess genes for anaerobic metabolic pathways) in cryoOM points towards a high potential for anaerobic degradation.

Archaeal gene copies (per gram of soil, **paper I**) were not significantly different between cryoOM and regular soil layers (topsoil and subsoil). This was in contrast to Gittel et al. 2014b, who reported lower archaeal gene copies in cryoOM than in topsoil, but higher than in subsoil. The number of archaeal sequences acquired from our study sites was low in cryoOM, as described for regular soils, and mostly assigned to methanogenic Euryarchaeota (classes Methanobacteria and Methanomicrobia, Gittel et al. 2014b) which could indicate the higher potential for CH₄ production from this horizon, the implication of higher methanogens in cryoOM is discussed in Chapter 5.4.

The abundance of fungi expressed as fungal gene copies (**paper II**) was lower in cryoOM than in the topsoil and similar to the subsoil, consistent with a previous study (Gittel et al. 2014a, b). We, however, found differences in the proportions of fungi with specific lifestyles. The mycorrhizal genus *Russula* was more abundant in cryoOM compared to other horizons (**paper II**). The higher mean proportion of mycorrhizas in cryoOM can be explained by the fact that mycorrhizal fungi may possess the ability of multiple lifestyles (i.e., saprotrophic), as was observed in recent studies (Frey et al. 2021; Carteron et al. 2021). With the help of a co-occurrence network, we identified that the ectomycorrhizal species *Cortinarius flexipes* (genus *Cortinarius*) was a keystone taxon in the topsoil. Although the same species did not have an identical role in cryoOM, we found other ectomycorrhizal species *Tylospora fibrillosa* (genus *Tylospora*) to play a key role in cryoOM. Both these species were abundant in the other horizon, also, but as indicated by network parameters (i.e., among module connectivity and within module connectivity), they

switched their dominant role (**paper II**). *Cortinarius* spp. are plant root-associated C-demanding, rhizomorph-forming basidiomycetes that grow extensive mycelia in soil (Agerer 2001, 2006). The *C. flexipes*, specifically, was identified as a key mycorrhizal fungus that is involved in the transfer of C between individual Arctic plants (*Betula nana*) and the decomposition of SOM by mobilization of N in Arctic tundra (Deslippe et al. 2016). Cryogenic subduction of the topsoil to deeper soil layers likely forced the fungi of *C. flexipes* to change lifestyle, which was evidenced by the absence of an interaction of this species in cryoOM. Whereas ectomycorrhizal *T. fibrillosa* had positive interaction with *Cadophora* spp. (litter saprotroph) and *Pezoloma* spp. (root endophyte) in cryoOM. Therefore, we assume that *T. fibrillosa* could play an important role as SOM decomposer in cryoOM, as they have potential for oxidative enzyme activity (Chambers et al. 1999) and are involved in SOM decomposition in humus substrate (Bödeker et al. 2016). Altogether, some mycorrhizal fungi are likely to be able to switch their lifestyle to saprotrophic, but the extent of this ability differs between species (Promputtha et al. 2010; Schlegel et al. 2016; Lennon 2020).

5.3 Effect of different tundra vegetation on the composition of the microbial community

To reveal the specific effect of different tundra vegetation on the composition and activity of soil microbial communities soil samples were collected from hummocky tussock tundra (HT), disturbed upland tundra dominated by non-sorted circles (UT), wet polygonal tundra (WT), and hummocky tussock tundra dominated by nonsorted circles (HTNC). We found, in general, the strong effect of vegetation on microbial community composition, stronger than the effect of soil horizons (**paper I, II**). We will first discuss the effects of vegetation on distinct prokaryotic taxa and their functional guilds.

The HT harbored more oligotrophic microbes, such as Actinobacteria and Acidobacteria. This site was dominated by cotton-grass vegetation (*Eriophorum vaginatum*), which is known to have a large pool of bioavailable litter, but with very low quality C (Kaštovská et al. 2018) that appears to support slow-growing microbes such as Actinobacteria and

Acidobacteria (Weintraub and Schimel 2005; Wallenstein et al. 2007; Koyama et al. 2014).

Microbial community composition from deciduous shrubs (Arctic willow) dominated UT was mainly driven by soil pH and DN content and significantly enriched by the phyla Acidobacteria (Subgroup-6) and Epsilonbacteraeota (*Sulfurovaceae*). Alkaline soil pH and the higher DN content from UT could support the growth of Subgroup-6, since this class has a wide range of pH tolerance (Chan et al. 2006; Kim et al. 2014; Mukherjee et al. 2014) and thrives in nutrient-rich soils (Kielak et al. 2016). Whereas crucial role of family *Sulfurovaceae* in bio-weathering of rocks in the moraine systems and deglaciated region and therefore assisting in establishing vegetation structure in barren lands has been observed in previous study (Mapelli et al. 2011; Venkatachalam et al. 2021). Hence, we speculate that the higher proportion of *Sulfurovaceae* family in the UT could help to expand the vegetation cover, as this site was characterized by large bare soil in the center surrounded by shrubs grown at the edge. The expansion of shrub tundra is expected in the Arctic (Mekonnen et al. 2021) and *Sulfurovaceae* can play an important role in this process.

The WT site was dominated by *Carex* vegetation and characterized by high soil moisture, high DOC, and DN. We, therefore, identified several groups of strict anaerobic fermentative bacteria (i.e., *Clostridiaceae*, *Sphingomonadaceae*, and *Caldiseriaceae*). *Clostridiaceae* are involved in the degradation of cellulose and soluble C (Schellenberger et al. 2010), *Sphingomonadaceae* gain energy through anaerobic photosynthesis (Zhou et al. 2015) and are involved in extracellular polymeric substances (Gatheru Waigi et al. 2017) through which play important role in biofilms, and *Caldiseriaceae* were identified as anaerobic thiosulfate reducers (Mori et al. 2009). Furthermore, a Bacteroidetes vadin HA17 identified as anaerobic degraders of complex OM (Baldwin et al. 2015; Wei et al. 2019) was also found in WT in a significantly high proportion. They are also identified as important fermenters (Wieczorek et al. 2019), and fermentation products (CO₂ and H₂) can be used by methanogens. A previous study reported a higher proportion of Bacteroidetes vadin HA17 during rice straw degradation and mainly cooccurred with methanogens (Ji et al. 2018). In fact, a high-water content and enough energy (DOC) and

nutrients (DN) sources (**paper I, II, III**) can promote anaerobic microbial growth in WT and potentially act as a source of CH₄ emissions.

The archaeal community was dominated by methanogenic Euryarchaeota in HT and WT which could be attributed to significantly higher water content at both these sites in contrast to UT (**paper I**) which was dominated by ammonia oxidizing Thaumarchaeota. The higher proportion of Thaumarchaeota at the UT site could be attributed to the dominant vegetation type at this site, Arctic willow. This tundra vegetation was reported to have a lower amount of ammonium than tussock tundra (Wild et al. 2013), which could support the growth of Thaumarchaeota, because this phylum has lower affinity for ammonium and mostly thrive under low or moderate availability of ammonium (Verhamme et al. 2011; Prosser and Nicol 2012) and outcompetes its bacteria counterpart (ammonia oxidizing bacteria) when ammonium availability is low or moderate (Verhamme et al. 2011; Prosser and Nicol 2012). Not only the availability of ammonium, but also the form of available N could play important role in Thaumarchaeota distribution. For example, ammonia oxidizing archaeal activity dominated over ammonia oxidizing bacteria when N was supplied as mineralized organic N derived from SOM (Schleper and Nicol 2010). We found significantly higher content of DN from UT; however, our studies did not include ammonium analysis. But similar to our study site, a study from Greenland found a low concentration of ammonium (in DN) in health tundra compared to tussock tundra (Wild et al. 2013). Therefore, we speculate that a low concentration of ammonium (in DN) could support the growth of Thaumarchaeota in UT.

The fungal community was more diverse among different tundra types than among soil horizons and variability in fungal lifestyles was different in each of the tundra types (**paper II**). The fungal keystone taxon was specific to each type of tundra, for instance, HT- *Verrucaria* and *Bagliettoa* (lichenized), *Cadophora* (litter saprotroph); UT- *Bagliettoa* (lichenized) and *Pezoloma* (root endophyte); WT- *Laccaria* (ectomycorrhizal); and HTNC- *Scutellinia* (wood saprotroph). However, we found only one taxon that was keystone (generalist) for one tundra vegetation, but not for others (specialist). The litter saprotrophic *Cadophora* was identified as a keystone taxon from HT, but the same taxon was not the keystone in other tundra types. One of the potential reasons for this taxon to be the keystone could

be the large amount of litter shredded by moss and cotton grass, the most dominant vegetation in HT, providing abundant substrate for litter saprotrophs. Arctic vegetation cover is expected to change (Mekonnen et al. 2021), and so does the quality and quantity of plant litter entering in soil. These changes potentially enforce certain fungal taxa to change their role from generalist to specialist, vice versa.

Shrubification, the shift from shallow-rooting moss and grass communities toward shrubby vegetation with deeper rooting zone, has been currently documented in the Arctic (Mekonnen et al. 2021). The change in vegetation cover, and thus in litter composition, will also be accompanied by a shift in the dominance among the soil microbes, likely from slow-growing oligotrophs (Acidobacteria and Actinobacteria) and nonmycorrhizal (dominant lifestyle in grassy tundra) to fast-growing copiotrophs (Proteobacteria), ectomycorrhizal and ericoid mycorrhizal (dominant lifestyle in shrubby tundra) as implied by our data. The change can be traced even further to the level of ecosystem C turnover rates / SOM decomposition rates (Mekonnen et al. 2021). Therein, the author suggested that shrubification can lead to increases in C storage in the Arctic, but net ecosystem exchange is site-specific (dependent on changes in biomass vs SOC stocks).

5.4 Potential CH₄ production in cryoOM

Arctic cryosols can act as a source or sinks of CH₄ which can be decided by the balance between the upward diffusion of CH₄ produced by methanogens in deeper anaerobic soil layers and the oxidation of CH₄ by methanotrophs and methylotrophs in aerobic surface layers. Our data showed that the quantified abundance of methanogens was not significantly different between the horizons, but the detailed composition of the cryoOM microbiome revealed significant differences in the proportions of methanogenic genera from the Methanobacteria (*Methanobacterium*) and Methanomicrobia (*Methanosarcina*) classes (**paper I**). The functional annotation of the identified total genera (**paper I**) and active genera (**paper IV**) further evidence that the methanogenesis process is significantly higher in cryoOM compared to topsoil and subsoil. A higher proportion of hydrogenotrophic (*Methanobacterium*) and acetoclastic (*Methanosarcina*) methanogens in cryoOM compared to the

subsoil could imply higher CH₄ emission potential. However, we found hydrogenotrophic methanogens (using only H₂ and CO₂ as substrate) more proportional than the acetoclastic (broad range of substrate, for example, acetate, methanol, and H₂) in cryoOM which suggested that methanogens in cryoOM were more substrate specific because *Methanosarcina* has all major metabolic pathways to utilize the wide range of substrates, e.g., acetate, methanol, and hydrogen (Conrad 2005).

Total methylotrophs (**paper I**) and active methylotrophs (**paper IV**) were also significantly enriched in cryoOM compared to other horizons (mainly of the Methylophilaceae family). All representatives of the family *Methylophilaceae* have been reported to be obligate methylotrophs. They are capable of using methanol, methylamine, and methylated C compounds, but not CH₄ as sole sources of C and energy (Jenkins and Jones 1987; Doronina 2004; Kalyuzhnaya et al. 2006) that are released during demethylation of lignin by fungi (Filley et al. 2002). The fungal species *Tylospora fibrillosa* (Walker et al. 2014) was found in cryoOM, and this species was identified as a generalist (who has more connection with other microbes) in cryoOM (**paper II**). Therefore, we speculate that the methanol produced during the degradation of lignin by this fungal species can be used by methylotrophs for their growth in cryoOM. The oxidation of methanol was also significantly greater in cryoOM compared to that of the topsoil (**paper I**). Therefore, it is argued that even though cryoOM had a great proportion of methylotrophic families, they do not use CH₄ as a C source produced by methanogens (Kalyuzhnaya et al. 2006), and cryoOM may be a potential hotspot for CH₄ release as observed in previous studies (Ganzert et al. 2007; Jiang et al. 2010; Olefeldt et al. 2013; Lau et al. 2015b).

5.5 N₂ fixation potential in cryoOM

On the basis of the quantification of the *nifH* gene specific to diazotrophs, interestingly no significant differences were found between horizons (**paper I**). The reason was that the total number of bacteria decreased with depth and the ratio of total bacteria to diazotrophs in deeper horizons increased. This result implies that diazotrophs might play a more important role in cryoOM and could potentially drive an important flux of N into this

cryoturbated horizon of the active layer under favorable conditions (Jackson et al. 2017). By doing so, they could ultimately activate the entire N cycle in cryoOM (Takai 2019) and accelerate the decomposition of SOM by increasing N availability to other microbial decomposers (Wild et al. 2014).

In addition to the number of diazotrophs, the composition of diazotrophs based on metagenomic (**paper I**) and metatranscriptomics (**paper IV**) approaches also revealed the important difference between cryoOM and other horizons. For example, the actinobacterial genus *Arthrobacter* was the dominant diazotroph in cryoOM, while *Pseudomonas*, *Bradyrhizobium*, and *Sphingomonas* were the dominant diazotrophs in the topsoil. *Arthrobacter* is considered as copiotroph and was reported from cryoOM in the context of dark CO₂ fixation (Šantrůčková et al. 2018). They increased rapidly in proportion when easily available nutrients such as sucrose were added to cryoOM, and they also cooperated intensively in heterotrophic CO₂ fixation in anaplerotic reactions (Šantrůčková et al. 2018). They also possess the ability to survive long periods under stressful conditions (i.e., nutrient limitation, temperature shift, toxic chemicals), and they were even recovered from desert Antarctic soils after 3 years of drying (Zevenhuizen 1966; Boylen and Ensign 1970; Labeda et al. 1976; Mongodin et al. 2006). Moreover, they are known for their ability to use complex substrates that were found in large amounts in cryoOM (Dao et al. 2018). This finding suggested that a higher proportion of *Arthrobacter* could potentially have an impact on CO₂ storage through their metabolism in cryoOM. Hence, a higher proportion of *Arthrobacter* species (fast growers with the ability to utilize complex compounds) in cryoOM may have crucial implications for future vulnerability of OM decomposition in cryosols.

5.6 Microbial control on cryoturbated OM transformation

The cryoturbated organic matter horizons had a significantly higher amount of total C and N, dissolved C and N compared to the surrounding mineral subsoil (**paper I, II, III, IV**). Similar results were obtained in the previous study from permafrost-affected soil (Schnecker et al. 2014, 2015; Gittel et al. 2014a, b; Gentsch et al. 2015a, b). Therein, the authors also

found that cryoturbated SOM was poorly decomposed even with a composition of SOM and C and N content as topsoil (parent materials). The authors brought evidence that the cryoOM has gone through a long process of sequential degradation and is highly processed, but at a slower rate. The radiocarbon age of OC in cryoturbated organic horizons was found to be ~1300 years BP old (Kaiser et al. 2007; Bockheim 2007; Hugelius et al. 2010; Palmtag et al. 2015). Several factors have been hypothesized to be responsible for retarded (or slower) decomposition in cryoOM including high nutrient limitation of the soil microbial community (Wild et al. 2013, 2014) or inefficient enzyme performance (Schnecker et al. 2014; Čapek et al. 2015) or altered microbial community (Schnecker et al. 2014; Gittel et al. 2014a, b).

The microbial community composition revealed that Actinobacteria families *Intrasporangiaceae* and *Cellulomonadaceae* were significantly enriched in cryoOM (**paper I, IV**) which is consistent with a previous study (Gittel et al. 2014b). The role of these families in complex OM (i.e., cellulose, starch, xanthan) degraders has been reported in agricultural soil (Schellenberger et al. 2010; Stackebrandt and Schumann 2014) and in peatlands (Yergeau et al. 2010). They are well adapted to environments with low C availability (Fierer et al. 2003) and some of the members can solubilize and modify lignin and lignocellulose by that means gaining access to the associated polysaccharides (McCarthy 1987; Le Roes-Hill et al. 2011). Likewise, previous studies reported genes responsible for the degradation of lignin and cellulose in many bacterial genomes, also in Actinobacteria (Ausec et al. 2011b; Bugg et al. 2011). A higher proportion of Actinobacteria (family *Intrasporangiaceae* and *Cellulomonadaceae*) in cryoOM might be related to lower C availability (C limitation, **paper III**) for microbes in cryoOM. A previous study found that 70% of OC in cryoturbated soils was associated with mineral particles, therefore making it unavailable to the microbial community (Gentsch et al. 2015b, a). The retarded decomposition of cryoOM (Kaiser et al. 2007; Čapek et al. 2015) could be caused not only by low temperature and anaerobiosis but also partly by the protection of OM with minerals (Gentsch et al. 2015b, a). These conditions could favor fungi alike taxa from specific bacteria families *Intrasporangiaceae* and *Cellulomonadaceae*, which are able to

degrade this protected OM but probably with very low rates due to less efficient enzymes produced by bacteria than by the fungi (Godden et al. 1992). Fungi are more efficient than bacteria in producing an oxidative enzyme which is important for the initial breakdown of phenolic and aliphatic macromolecules (Talbot et al. 2008). However, in **paper III**, it was reported that bacterial gene copies in cryoOM were more closely correlated with hydrolytic and oxidative enzyme activities than fungal gene copies. This result further pointed toward the fact that bacteria may play a more important role in the transformation of OM in cryoOM.

There is increasing evidence that bacterial laccases and laccase-like enzymes are present in a broad diversity of bacteria and archaea (possibly due to horizontal gene transfer (Ochman et al. 2000)), including many anaerobic species (Nakamura et al. 2003; Ausec et al. 2011b, a; Freedman and Zak 2014). Possibly the expression of laccases by bacteria can be more efficient due to the lack of introns and post-translational modifications compared to fungi (Ausec et al. 2011b). Therefore, bacteria (anaerobic taxa) could outcompete saprotrophic fungi in deeper permafrost horizons (**paper II**), where anoxia together with a low temperature and high content of recalcitrant biopolymers (Dao et al. 2018) could create enormous selective pressure on the microbial community. Specific anaerobic taxa (e.g., Actinobacteria, Firmicutes) could therefore be responsible for higher production of laccases in deeper horizons (Kellner et al. 2008). A higher proportion of the active population of anaerobic microbes was observed in the cryoOM (**paper IV**). Moreover, in **paper III** it was found that only one fungal genus out of 14 genera (>1% relative proportion in the total community) had the laccase-like gene from all horizons (**paper III**), further supporting that bacterial laccase may be more important in permafrost soil.

Furthermore, the phospholipid-derived fatty acids-based study found that enzyme activity in cryoOM was mainly controlled by microbial community composition (bacteria and fungi) in the Siberian Arctic (Schnecker et al. 2014). Our amplicon sequencing data-based fungal co-occurrence network properties (lower path length and higher clustering coefficient) suggested that the cryoOM fungal community was more

vulnerable to environmental perturbation than the topsoil and subsoil (**paper II**). Hence, it was concluded that the bacterial community could play an essential role over fungi in cryoturbated OM transformation.

So far, it has been shown that fungal growth in cryoOM might be constrained by environmental factors than the bacteria, which could help fungi unlike bacteria to grow more vigorously in this cryoturbated OM (significantly lower fungal gene abundance, the **paper I, II**, Gittel et al. 2014a, b). In general, the microbial population in the soil is mainly controlled by its predators (protozoa and nematodes) (Gao et al. 2019; Thakur and Geisen 2019).

To better understand the interaction between bacteria and their predator (bacterivore), a holistic metatranscriptomics approach was used to study all three domains of life from the same samples simultaneously (**paper IV**). As described in Chapter 5.1, the abundance of predatory bacteria Myxococcales remained similar across all horizons, but the abundance of protozoa decreased with depth, especially in cryoOM. Changes in the active population of micropredators could influence the nutrient remobilization and consequently the C and N cycle in cryoOM.

Each identified bacterial genera were assigned to functional guilds related to biogeochemical cycles of C (chemoheterotrophs, cellulolysis, fermentation, methylotrophy) and N (nitrite oxidation, ammonia oxidation, nitrate reduction, N₂-fixation, ureolysis) (**paper IV**). A significant positive correlation was observed between C and N-related bacterial functional guilds and predatory bacteria, predatory protists. The correlation was, however, remarkably different between cryoOM and topsoil. In cryoOM, predatory myxobacteria regulated the activity and amount of the major bacterial N functional guilds (i.e., nitrifiers and denitrifiers); similar top-down control was previously observed (Griffiths 1989; Alpeh et al. 1996), but the active bacterial C guilds (i.e., methanotrophs, methanogens, lignin degraders, and hydrocarbon decomposers) were controlled mainly by the amount of available C (DOC). It is assumed that the C and N cycles in cryoOM are controlled by different mechanisms. The C cycle by bottom-up (e.g., by soil nutrient content), but the N cycle is controlled by top-down (e.g., predation by myxobacteria).

It is known that micropredators, during their bacterial grazing, release ammonia from the degradation of organic N. This higher amount of ammonia can be used either as an N source for heterotrophic microbes or autotrophic ammonia oxidizers (high proportion in cryoOM, Chapter 5.2) with ammonia (Treuner-Lange et al. 2017) and a strong correlation between ammonia oxidizers and predatory myxobacteria could form an important part of the microbial loop in cryoOM that holds N in the system and forming a closed loop. An older, more microbial processed N (more negative $\delta^{15}\text{N}$) was found in this layer than the parent topsoil and the surrounding mineral subsoil (**paper IV**), which was also reported in a previous study from Siberian permafrost (Gentsch et al. 2015b). Ammonia oxidizers, on the other hand, had a positive correlation with protists, and fungi in the topsoil. Therefore, the fundamental disconnection of the C and N cycles may be one of the reasons for the very slow decomposition of SOM from cryoOM, as found in a previous study (Kaiser et al. 2007; Čapek et al. 2015).

6 CONCLUSIONS

Our data showed significant differences in abundance, relative proportions, and activity of specific functional guilds between “regular” soil and cryoturbated SOM. We confirmed the lower fungi to bacteria ratio and a higher proportion of total and active members of Actinobacteria in cryoOM compared to the surrounding subsoil. In addition, extracellular enzyme activity and their correlation with bacterial 16S rRNA gene abundance suggested that bacteria had a more important role in extracellular enzyme activity than fungi in cryoOM.

The fungal community was dominated by the mycorrhizal genus *Russula* in cryoOM compared to other horizons, and some fungal taxa shifted their role from being a generalist in topsoil and subsoil to a specialist in cryoOM which could reflect unique chemical composition in cryoOM. Distinct plant community composition (shrubification) influenced with future climate change could harbor different belowground fungal communities (most likely dominated by saprotrophs) and with potential role shift in fungal lifestyle which can change the fluxes of C and N in cryoOM.

Potential CH₄ production determined by quantification of the *mcrA* gene was not significantly different between horizons but detailed taxonomical analysis showed higher proportion of methanogenic genera in Archaeal community in cryoOM. In addition, methylotrophs (utilizing single-C compounds but not CH₄ as sole sources of C and energy) also had higher significant proportion in cryoOM compared to other horizons. Collectively, a higher proportion of methanogens and methylotrophs but not obligate methanotrophs puts cryoOM in the position of a potential hotspot for CH₄ emissions in Arctic cryosols.

The higher ratio of diazotrophs to total bacteria (*nifH* / 16S rRNA gene) in cryoOM compared to topsoil implied the importance of N fixation potential in buried SOM. This can be an important biological N input to this specific horizon. Taxonomic annotation suggested that copiotrophic *Arthrobacter* (Actinobacteria phylum) was the dominant diazotroph fixing N in cryoOM. The members of *Arthrobacter* have versatile metabolisms

and through a higher flow of readily available C sources from deeper rooting shrubs can potentially increase N-flux to cryoOM

The metatranscriptomics approach showed that the functional guilds associated with C and N cycles in cryoOM were controlled by different combinations of biotic and abiotic factors. The C cycle (methanotrophs, methanogens, lignin degraders, and hydrocarbon decomposers) by bottom-up control (chemical composition of soil), whereas the N cycle (nitrite oxidation, ammonia oxidation, nitrate reduction, N₂-fixation, ureolysis) by top-bottom control (bacterial predation). This suggested that N in cryoOM is recycled mostly in prokaryotic biomass.

7 FUTURE IMPLICATION

The current research contributes to the understanding and identifying the microbiome and its potential to decompose SOM in different horizons (specifically emphasized in cryoturbated soil) and distinct tundra types. However, certain points need a more detailed study.

Previous studies have already elucidated the crucial role of temperature, soil moisture, oxygen availability, and availability of nutrients for SOM transformations. The vegetation change (shrubification) in the Arctic will change the composition, amount, and quality of root exudates that enter the soil, and as mentioned above, the different components of the root exudates potentially change the trophic interactions in the soil microbiome. Therefore, targeted laboratory incubations with isotopically labeled natural/simulated root exudates (e.g., different C/N ratio, the composition of root exudates) can help us to better understand the flux of C and N between plants, eukaryotes, and prokaryotes and identify important trophic interaction and their role in the C and N cycles in the Arctic cryosols.

Microbes interact with each other in the soil on a multitrophic level, forming a complex network. Therefore, a holistic view based on total RNA and protein analysis (quantitative metatranscriptomics and metaproteomic approaches) of prokaryotes and eukaryotes in the same study could improve our understanding of trophic interactions and better predict the rate of biogeochemical processes.

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9 ATTACHED PUBLICATIONS

Paper I

**Microbiome structure and functional potential in
permafrost soils of the Western Canadian Arctic**

Varsadiya, M., Urich, T., Hugelius, G., Bárta, J.

2021a

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RESEARCH ARTICLE

Microbiome structure and functional potential in permafrost soils of the Western Canadian Arctic

Milan Varsadiya^{1,*}, Tim Urich², Gustaf Hugelius³ and Jiří Bárta¹

¹Department of Ecosystems Biology, University of South Bohemia in České Budějovice, Branišovská 31, 370 05 České Budějovice, Czech Republic, ²Institute of Microbiology, University of Greifswald, Felix-Hausdorff-Str. 8 17487 Greifswald, Germany and ³Department of Physical Geography, Stockholm University, 106 91, Stockholm, Sweden

*Corresponding author: Department of Ecosystem Biology, Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31, 370 05 České Budějovice, Czech Republic. Tel: +00420 387772347; E-mail: mvarsadiya@prf.jcu.cz

One sentence summary: Composition, abundance, and functional potential of microbial communities in cryosols are mainly influenced by C/N ratio and distinct vegetation.

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¹Milan Varsadiya, <http://orcid.org/0000-0001-6311-6439>

ABSTRACT

Substantial amounts of topsoil organic matter (OM) in Arctic Cryosols have been translocated by the process of cryoturbation into deeper soil horizons (cryoOM), reducing its decomposition. Recent Arctic warming deepens the Cryosols' active layer, making more topsoil and cryoOM carbon accessible for microbial transformation. To quantify bacteria, archaea and selected microbial groups (methanogens – *mcrA* gene and diazotrophs – *nifH* gene) and to investigate bacterial and archaeal diversity, we collected 83 soil samples from four different soil horizons of three distinct tundra types located in Qikiqtaruk (Hershel Island, Western Canada). In general, the abundance of bacteria and diazotrophs decreased from topsoil to permafrost, but not for cryoOM. No such difference was observed for archaea and methanogens. CryoOM was enriched with oligotrophic (slow-growing microorganism) taxa capable of recalcitrant OM degradation. We found distinct microbial patterns in each tundra type: topsoil from wet-polygonal tundra had the lowest abundance of bacteria and diazotrophs, but the highest abundance of methanogens. Wet-polygonal tundra, therefore, represented a hotspot for methanogenesis. Oligotrophic and copiotrophic (fast-growing microorganism) genera of methanogens and diazotrophs were distinctly distributed in topsoil and cryoOM, resulting in different rates of nitrogen flux into these horizons affecting OM vulnerability and potential CO₂ and CH₄ release.

Keywords: arctic; climate change; permafrost; gene abundance; microbial community; vegetation

INTRODUCTION

Turbid Cryosols (FAO 2006) are unique soils because they are influenced by cryogenic processes such as cryoturbation (Bockheim and Tarnocai 1998), which move a substantial amount of the topsoil horizon into the deeper mineral subsoil (van Vliet-Lanoë 1991), forming randomly distributed organic carbon-rich pockets (cryoOM; Palmtag and Kuhry 2018). It has been estimated that the cryoOM store approx. A total of 470 Pg C out of

the total 1035 Pg C stored in Cryosols (Harden et al. 2012; Hugelius et al. 2014).

The burial of the topsoil during the cryoturbation processes is accompanied by the translocation of topsoil microbial communities into deeper horizons. During this translocation, microbes must adapt to extensive changes in crucial environmental factors influencing their survival (e.g. temperature, anoxia and nutrient availability). These abiotic factors exercise strong selective pressure on specific microbial groups, which can in turn

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affect the carbon (C) and nitrogen (N) cycling and can influence the stability/vulnerability of cryoOM (Schmidt et al. 2011; Dun-gait et al. 2012; Schnecker et al. 2014; Gentsch et al. 2018).

Recently, the bacterial and archaeal communities in the whole profile of the active layer of Turbic Cryosols from Greenland (Gittel et al. 2014a) and Eastern Siberia (Gittel et al. 2014b) have been described. These amplicon sequence-based studies highlighted several important features of microbial communities that were unique to different horizons of the active layer, including cryoOM. These cryoturbated horizons contain a comparable number of bacteria to topsoil, but the composition of the cryoOM microbial community showed a higher similarity to the surrounding mineral subsoil community (i.e. a higher proportion of *Actinobacteria*, *Chloroflexi*, *Firmicutes* and *Gemmatimonadetes*). This community shift can likely be attributed to (i) the mixing of topsoil with subsoil communities during the cryoturbation process and (ii) the migration of the subsoil community into a cryoOM as the result of the open niche caused by the extensive dieback of the original topsoil community. The activity of the microbial community in cryoOM was more influenced by its community composition (Schnecker et al. 2014). The authors used structural equation modeling and found that, unlike the topsoil, the activities of the extracellular enzymes responsible for the degradation of complex organic matter in cryoOM were strongly related to changes in the composition of the microbial community. However, this was not observed in the topsoil community where such activities depended more on the chemical composition of the SOM (i.e. C/N and microbial biomass). These findings might suggest that the microbial community in cryoOM is probably less functionally redundant and more sensitive to environmental changes and nutrient fluctuations (Wild et al. 2014, 2016; Čapek et al. 2015; Gentsch et al. 2018), which may lead to the slower decomposition of OM and lower vulnerability of cryoOM (Kaiser et al. 2007).

All of the above relationships were, however, based mainly on the relative abundances of microbial taxa, which rarely reflect real *in-situ* microbial processes (Söllinger et al. 2018). To predict OM vulnerability, there is a need for data on the quantification of specific functional guilds involved in the C and N cycle. In Turbic Cryosols, these mainly include methanogens (Ganzert et al. 2007; Barbier et al. 2012; Wagner et al. 2017) and diazotrophs (Izquierdo and Nüsslein 2006; Penton et al. 2016; Altshuler et al. 2019). The enumeration of methanogens is important to precisely predict CH₄ flux from anaerobic hotspots (Urbanová and Bárta 2020), while diazotrophs may accelerate the OM decomposition in N limited Turbic Cryosols (Schimel and Bennett 2004; Wild et al. 2013), because they can introduce additionally available N, which can be used by microbes for extracellular enzyme synthesis, accelerating the overall OM decomposition (Gittel et al. 2014a, b). The stimulation of decomposition influences the production rates of gasses, including CH₄ in anaerobic habitats (Roy and Conrad 1999; Kruger and Frenzel 2003). The fact that several methanogenic archaea have genes for N fixation has also been described (Brabban, Orcutt and Zinder 1999; Mizukami et al. 2006; Dang et al. 2013; Bae et al. 2018), which highlights the close connection between methanogenesis and N availability and utilization. The amount of available N in soil is also, to a large extent, controlled by the presence of vegetation (de Graaff, Van Kessel and Six 2009; Finzi et al. 2015) as it is the primary source of SOM either from aboveground litter, roots, or root exudates. Hence, to predict OM vulnerability and to better understand the associated microbial process, the quantification of specific functional guilds from distinct tundra types are essential.

Vegetation is one of the main driving forces of Arctic biogeochemical processes (Shi et al. 2015), including soil nutrient content, organic matter quality (Biasi et al. 2005) and microbial diversity (Gittel et al. 2014a, b). The amount of available SOM also controls the proportion of fast-growing microorganisms utilizing easily available SOM (e.g. copiotrophic, r-strategy bacteria) or slow-growing microorganisms utilizing more complex biopolymers (e.g. oligotrophic and K-strategy bacteria). The strength of the vegetation effect on the composition and functioning of the microbial community is influenced by the plants' physiology (i.e. depth of the rooting system, quality and quantity of rhizodeposits and litter). For example, a large difference in community composition at the phylum level was observed between tussock and shrub tundra soils from Alaska (Wallenstein, McMahon and Schimel 2007). Koyama et al. (2014) studying tussock tundra found that topsoil with low-quality C supported a large population of oligotrophic *Acidobacteria*. To the contrary, copiotrophic members of *Proteobacteria* were found to dominate shrubby soils (Wallenstein, McMahon and Schimel 2007), which contained a labile pool of available C (Weintraub and Schimel 2003). These studies revealed that the microbial community in the topsoil of Turbic Cryosols is to a large extent influenced by the quality and quantity of plant residues entering the soil environment governing the copiotrophic or oligotrophic nature of the microbial community (Fierer, Bradford and Jackson 2007; Eilers et al. 2010; Davis, Sangwan and Janssen 2011; Koyama et al. 2014). One disadvantage of these studies was the limiting resolution of sequencing technologies. Today, with longer lengths of sequences, we can examine microbial communities at better resolution and at lower taxonomic levels and even use specific bioinformatics pipelines such as FunGene (Fish et al. 2013), FAPROTAX (Louca, Parfrey and Doebeli 2016), PICRUST2 (Douglas et al. 2020), to predict the functional potential of the microbial community in C and N utilization. These tools can help us better predict potential hotspots for methane production or N₂ input into Arctic Cryosols.

In this study, we investigated four different soil horizons of the active permafrost layer from three distinct tundra types of the Western Canadian Arctic. Our main objectives were to (i) quantify ribosomal genes (bacteria and archaea) and functional guilds (i.e. methanogens and diazotrophs) in distinct horizons and tundra types, (ii) identify specific microbial taxa associated with individual horizons and tundra types and (iii) determine the functional potential of the microbial community (specifically methanogens and N₂ fixators). With this study, we expanded the current understanding of microbial communities inhabiting cryoOM (Gittel et al. 2014a, b). To the best of our knowledge, there is no detailed study available on microbial communities and their functional guilds from the Western Canadian Arctic, specifically focused on cryoOM. Hence, to achieve these goals, we analyzed soil samples from topsoil, cryoOM, subsoil and permafrost. We compared microbes using qPCR and Illumina gene-targeted sequencing. We used FunGene to identify specific microbial taxa possessing the methanogenic (*mcrA*) and diazotrophic genes (*nifH*) and FAPROTAX to predict the functional potential of microbial communities inhabiting different horizons and tundra types.

MATERIALS AND METHODS

Site description and soil sampling

The study area was located on the Qikiqtaruk (Herschel Island; 69°34'N, 138°55'W), in the Beaufort Sea, Canada. The mean

annual air temperature varies between -26.3°C in February and 8.7°C in July while the active surface experiencing big temperature difference ranging from -35°C in winter to 25°C during summer (Burn and Zhang 2009). The precipitation is 150–200 mm per year (Burn 2012). The active layer (the layer of soil which freezes during winter and thaw during summer) depth varied between 30–60 cm at our study site.

This location was chosen to represent the homogeneous properties of the ecological units, i.e. to be stationarity. These ecological units are the *Herschel* unit (Site 1), the *Komakuk* unit (Site 2) and the *Guillemot* unit (Site 3). Each ecological unit represented distinct vegetation and ground patterns (Smith et al. 1989). Site 1, Site 2 and Site 3 were characterized by hummocky tussock tundra (HT), slightly disturbed upland tundra dominated by non-sorted circles (UT) and wet polygonal tundra (WT), respectively. Tundra types from Site 1 included moss and cotton grass (*Eriophorum vaginatum*), Site 2 had vegetation of Arctic willow and *Dryas-Vetch*, and Site 3 had *Carex* and bryophytes as primary vegetation types. HT and UT were separated by ca 100 m, while the distance to WT was ca 1 km.

Soil samples were collected according to standard protocols (Schoeneberger et al. 2012), with additional adaptations for permafrost affected soils (Ping et al. 2013). The active layer was sampled, per soil genetic horizon, from open soil pits of 1 m width. This was done horizontally from the cleaned side of the soil pit, avoiding any mixing of material with depth. The permafrost section was sampled at the center of the soil pit by hammering a steel pipe at 5 or 10 cm depth increments into the frozen ground at the top of the permafrost table. In this way, no active layer soil could contaminate the permafrost samples. After each depth increment of coring into the permafrost, the frozen sample was retrieved from the steel pipe. Any contamination from the permafrost layers above the target depth was scraped away so that only intact frozen permafrost from each specific depth was sampled. The sampling environment was not strictly sterile, but cross-contamination between samples was avoided as much as possible. Samples were directly put into double plastic Ziploc bags and frozen within a few hours of sampling. They were then transported frozen and stored frozen (at -8°C) until analyses in the lab. Soil samples were acquired from four horizons and permafrost (PF): topsoil (O and A soil genetic horizons); cryoOM (Oj and Ajj soil genetic horizons); subsoil (BCg soil genetic horizon).

Soil properties

Soil water content was measured by drying the soil at 60°C for 24 h and reweighing the sample. Water dissolved pH was measured from the soil suspension with water at a solid to solution ratio of 1:2.5 using pH 3151i (Fisher Scientific, Germany). The 60°C dried soil samples were ground and used for determining the total carbon (C_{tot}) and total nitrogen (N_{tot}) content using NC 2100 soil analyzer and expressed in percentage. The dissolved organic carbon (DOC) and dissolved nitrogen (DN) were quantified by mixing soil: water at a 1:5 ratio (w/v) part of ultrapure water for an hour, and the filtered soil solution was used for LiquiTOCII (Elementar, Germany) and expressed in ug/g dry weight.

Soil DNA extraction and qPCR analysis of ribosomal RNA and functional genes

Total genomic DNA was extracted from 0.24 to 0.28 g of soil using a DNeasy PowerSoil DNA Isolation Kit (Qiagen, Germany). The final elution volume was 50 μL and eluted DNA was stored at

-20°C for further use. The ribosomal RNA genes of bacteria and archaea and the functional genes coding the alpha subunit of the methyl-coenzyme M reductase (*mcrA*) of methanogens and nitrogenase (*nifH*) of nitrogen-fixing prokaryotes were quantified by qPCR. Each reaction was performed with 20 μL of reaction mixture containing 3 μL of DNA from the soil samples. The primer sequences and PCR conditions for each of the four primer sets are shown in Table S1 (Supporting Information). Briefly, bacterial and archaeal 16S rRNA genes were amplified with the primer set of 341f/534r (Muyzer et al.) and ARC787F/ARC1059R (Yu et al. 2005), respectively. Other functional genes were quantified by amplifying ME1/MCR1R and IGK3/DVV for *mcrA* (Hales et al. 1996) and *nifH* (Gaby and Buckley 2012) genes, respectively. Melting curve analysis was used to confirm the product specificity, and the correct amplicon size was confirmed by agarose gel electrophoresis. Standards were made from 10-fold dilution of a known amount of purified PCR products obtained from *E. coli*, *Pyrococcus furiosus*, *Methanosarcina* and *Methylocystis heyeri* for bacteria, archaea, *mcrA* and *nifH*, respectively. The qPCR assay was performed with two technical replicates for each sample, standard and non-template control, and the efficiencies of qPCR reactions were 85%, 96%, 88% and 79% for bacteria, archaea, *mcrA* and *nifH*, respectively.

Barcoded amplicon sequencing of prokaryotic 16S rRNA genes

Aliquots of DNA extracts were sent to the SEQme company (the Czech Republic) for the preparation of a library and sequencing using the MiSeq2500 platform. The Earth Microbiome Project (EMP) protocol was used for library preparation with modified universal primers 515FB/806RB (Caporaso et al. 2011). Bacterial 16S rRNA raw pair-end reads (250 bp) were joined and quality filtered using USEARCH v. 10.0.240 to obtain reads of approx. 250 bp length (Edgar 2013). Amplicons were trimmed to equal lengths. Bacterial unique reads were grouped to zero-order OTUs (zOTUs) using a UNOISE 3.0 algorithm (Edgar and Flyvbjerg 2015; Edgar 2016). This step also included a chimera check and removal. The taxonomical assignment of each bacterial and archaea zOTUs performed using the BLAST algorithm (E-value = 0.001) using the curated ARB Silva 132 database (Quast et al. 2013). Only 22 zOTUs were not taxonomically classified to domain level, whereas the rest of the zOTUs were assigned at least to phylum level (99.6%). In the case of the UT site, only one sample of cryoOM had enough archaeal sequences for further analysis. Raw sequencing data were deposited in the European Nucleotide Archive (ENA) under the study of PRJEB38326.

Statistical analyses

Most of the analyses were performed in R 3.5.3 (R Development Core Team 2011). The Shapiro–Wilk test and Bartlett test were performed with log-transformed and non-log transformed data to verify the condition of normality and homogeneity, followed by one-way ANOVA (log transformation: gene abundance, square root transformation: zOTUs abundance data). One-way ANOVA was followed by Tukey's HSD to determine the significant difference between variables (physicochemical parameters and gene abundance). A significant difference was considered at P -values <0.05 unless indicated otherwise; however, we provided precise P -values wherever possible. Taxonomically assigned zOTUs were rarefied at 2000 even depth, one topsoil sample from the WT had a lower sequence and was

therefore removed. The rarified zOTUs table was used for all further analyses. The bacterial alpha diversity indices, Chao1 richness, Shannon and Simpson evenness were calculated in qiime2 software (Bolyen et al. 2019). Beta diversity was calculated based on the Bray–Curtis distance matrix. Redundancy analysis (RDA) was performed to relate measured soil parameters that differed with changes in relative zOTUs abundance, the forward selection was used to identify the most significant soil variable to explain the distribution of bacterial communities and the P-values were adjusted by Bonferroni. We employed permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) and distance-based RDA (dbrDA, Legendre and Andersson 1999) to find the effect of different horizons and distinct tundra types on the microbial community composition. Square root-transformed relative abundance data were used to perform PERMANOVA by the ‘adonis’ function from the ‘vegan’ package with 999 permutations in R (Oksanen et al. 2007). Whereas dbrDA was performed in CANOCO 5 software (Smilauer and Leps 2014). All prokaryotic genera with members having genes for methanogenesis (*mcrA* gene) and N₂ fixation (*nifH* gene) were manually searched in the FunGene database (<http://fungene.cme.msu.edu/>, Fish et al. 2013). We tested for consistent differences in taxon abundance among horizon and tundra types using the nonparametric Kruskal–Wallis sum-rank test and the unpaired Wilcoxon test. These analyses were followed with linear discriminant analysis (LDA) to estimate the effect size of taxonomical covariates driving the group difference procedure implemented in LEfSe (Segata et al. 2011). This tool allows the analysis of microbial community data at any clade; however, the analysis of the large number of zOTUs detected in this study would be computationally too complex, and therefore statistical analysis was performed only from the domain to the family level, and only significant zOTUs were used to plot the cladograms. For LEfSe analysis, statistically significant alpha values for the factorial Kruskal–Wallis test among classes and for the pairwise Wilcoxon test between subclasses were set to 0.05. The threshold on logarithmic LDA score for discriminative features was set to 4 to identify the bacterial family with a statistically significant difference. The all-against-all strategy was used for LEfSe. The STAMP bioinformatics package (Parks and Beiko 2010) was used to identify significantly different bacterial taxa between topsoil and cryoOM (Welch’s test, two-sided P-values <0.05). The functional annotation of prokaryotic taxa (FAPROTAX) was performed to predict bacteria functional potential (Louca, Parfrey and Doebeil 2016). FAPROTAX is a manually constructed database that maps prokaryotic taxa (e.g. genera or species) to putative functions based on the literature of cultured representatives. The output table from FAPROTAX was used to find the most significantly different functions between topsoil and cryoOM, and also for different tundra types through the LEfSe analysis (LDA effect size 2).

RESULTS

Physicochemical parameters of soil

A total of 83 soil samples were collected from three tundra types of the Western Canadian Arctic: HT (*n* = 26), UT (*n* = 30) and WT (*n* = 27). From each tundra type, samples were collected from four different horizons: topsoil (*n* = 40), cryoOM (*n* = 16), subsoil (*n* = 12) and permafrost (*n* = 15; Table 1). In general, topsoil had the highest moisture, DOC, Ctot and C/N, followed by cryoOM, permafrost and subsoil (Table 1).

Table 1. Selected soil physicochemical properties.

Tundra types	Horizon	N	Moisture (%)	pH	DOC (ug/g dw)	DN (ug/g dw)	Ctot (%)	Ntot (%)	C/N
HT	Topsoil	9	76.7 ± 7 (a)	6 ± 0.5 (a)	751.1 ± 618 (a)	2.6 ± 1.8 (ab)	40.1 ± 6.9 (a)	1.2 ± 0.5 (a)	44.8 ± 29.6 (a)
	CryoOM	8	52.9 ± 14 (b)	6.4 ± 0.4 (a)	370.1 ± 160.9 (a)	7.7 ± 6.6 (ab)	12.8 ± 7.8 (b)	0.8 ± 0.5 (ab)	15.9 ± 2.4 (b)
	Subsoil	5	30.5 ± 6.7 (c)	5.9 ± 0.1 (a)	206 ± 127.4 (a)	10 ± 2.5 (a)	4.7 ± 2.3 (b)	0.4 ± 0.2 (b)	13.5 ± 1.3 (b)
UT	Permafrost	4	48.5 ± 11.7 (bc)	6.3 ± 0.5 (a)	453.1 ± 369.4 (a)	10 ± 6 (a)	7.9 ± 1 (b)	0.6 ± 0.1 (ab)	14.9 ± 0.4 (b)
	Topsoil	10	53.7 ± 12.5 (a)	7.7 ± 0.7 (a)	810.7 ± 1235.9 (a)	4.3 ± 3.8 (b)	28.5 ± 11 (a)	1.3 ± 0.4 (a)	22.9 ± 4.9 (a)
	CryoOM	3	45.7 ± 15.3 (ab)	8.2 ± 0.2 (a)	760.4 ± 882.9 (a)	62.7 ± 104 (a)	12.7 ± 6.4 (b)	0.8 ± 0.5 (a)	16.2 ± 1.5 (b)
WT	Subsoil	7	23.9 ± 7.8 (c)	8.2 ± 0.5 (a)	326.2 ± 131 (a)	19.6 ± 13.6 (ab)	4.2 ± 1.5 (b)	0.3 ± 0.2 (b)	21.3 ± 4.4 (b)
	Permafrost	10	29.5 ± 8.6 (bc)	8.3 ± 0.6 (a)	359.8 ± 257.6 (a)	30 ± 21.8 (ab)	3.1 ± 0.3 (b)	0.2 ± 0.1 (b)	29.4 ± 3.4 (b)
	Topsoil	21	78.4 ± 7.3 (a)	6 ± 0.7 (a)	5104.1 ± 4917.8 (a)	14.6 ± 12.9 (a)	30.2 ± 8.3 (a)	1.8 ± 0.5 (a)	18 ± 5.6 (a)
	CryoOM	5	54.2 ± 17.1 (b)	6.2 ± 0.7 (a)	1066.5 ± 1246.7 (a)	22.1 ± 17.3 (a)	14.6 ± 5.7 (b)	1.1 ± 0.4 (b)	13.8 ± 2.8 (a)
	Permafrost	1	60.4	5.7	452	6.9	14.7	1.3	12

Averages and standard deviation were shown. The significant difference between horizons was calculated by One-Way ANOVA and followed by Tukey’s HSD test. Different letters in the brackets indicate a significant difference between horizons within the individual tundra type.
N, number of replicates; DOC, Dissolved Organic Carbon; DN, Dissolved Nitrogen; Ctot, total Carbon; Ntot, total Nitrogen; C/N, Carbon to Nitrogen ratio.
na, no sample available.

CryoOM from UT had a greater concentration of DN. The pH did not significantly change in different horizons but was significantly higher (P -value <0.05) in the UT compared to other tundra types. All horizons from WT had the highest concentration of DOC and Ntot (Table S2, Supporting Information). Principal component analysis on soil property data placed the cryoOM at an intermediate position between the topsoil and subsoil. It supported the finding that the cryoOM horizon was highly variable in soil properties, resulting in a shared ordination space with the unburied topsoil and subsoil horizons (Figure S1, Supporting Information).

The gene abundance of distinct microbial groups

The microbiomes in the four horizons significantly differed in the abundance of bacteria (16S rRNA gene copies per gram of dry soil) and diazotrophs (*nifH* gene), in contrast, archaea and methanogens (*mcrA* gene) did not show any significantly different abundance. In general, bacterial abundance decreased in the order topsoil > cryoOM > subsoil > permafrost (Fig. 1). Bacterial abundance was significantly higher in topsoil samples ($2.3 \pm 2.3 \times 10^{11}$ 16S rRNA gene copies per g dry soil, one-way ANOVA, P -values <0.001), compared to other horizons and decreased with depth. In contrast, cryoOM samples ($8.6 \pm 2.29 \times 10^{10}$, P -values <0.01) had significantly higher bacterial abundance than the surrounding subsoil ($8.6 \pm 4.9 \times 10^8$). Permafrost samples ($1.99 \pm 4.4 \times 10^7$) had the lowest bacterial abundance.

The average archaeal gene abundance was $7.9 \pm 23.2 \times 10^7$, $1.4 \pm 1.8 \times 10^7$, $5.8 \pm 3.8 \times 10^5$ per gram of dry soil, while methanogen abundance ranged from $1.3 \pm 4.7 \times 10^{11}$, $5.9 \pm 12.6 \times 10^5$, $1.0 \pm 80.2 \times 10^4$ per gram dry soil for topsoil, cryoOM and subsoil, respectively (Fig. 1). However, they were not significantly different between horizons. We found three samples from UT which had higher *mcrA* gene abundance (ranged from 9.5×10^{11} to 2.3×10^{12} per gram dry soil), which caused a high average *mcrA* abundance when all tundra types were considered together. Archaea and methanogens in permafrost were under the detection limit of qPCR assay.

Diazotrophs gene abundance ranged from $1.5 \pm 3.5 \times 10^7$ to $7.6 \pm 4.2 \times 10^2$ per gram of dry soil, with topsoil having the highest abundance and permafrost the lowest. In contrast to other genes, the diazotrophs were similar when comparing cryoOM and subsoil except for the UT, where cryoOM was significantly lower than subsoil (P -values <0.01). The general trend described above was also apparent for the individual tundra types, including higher gene copy numbers in topsoil samples and followed by cryoOM, subsoil and permafrost. However, this trend was only valid for bacteria, whereas no significant difference between horizons was found for archaea and *mcrA* in HT and WT and HT and UT, respectively. The WT had no significant difference for diazotrophs (Fig. 1). We also plotted the microbial groups' gene copies of the individual tundra types within individual horizons, and only topsoil samples displayed a significant difference (P -values <0.05) among different tundra types (Figure S2, Supporting Information).

Microbiome composition

DNA from 77 soil samples were subjected to Illumina MiSeq amplicon sequencing. In total, 909810 16S rRNA gene amplicon tag sequences (bacteria-906761; archaea-3049) were acquired after quality filtering (Table 2). Sequences were clustered in 5893 zOTUs, where bacteria and archaea comprised 5858 and 35 zOTUs before rarefaction, respectively. In total, one sample from

the topsoil of the WT was removed after rarefaction. The bacterial zOTUs were assigned to 44 different phyla, 432 families and 781 genera. The most dominated phyla consisted of Proteobacteria (33%), Actinobacteria (28%), Acidobacteria (8%), Chloroflexi (8%) and Bacteroidetes (7%; Fig. 2). Less abundant phyla (12% in total) were the Verrucomicrobia, Firmicutes, Planctomycetes, Gemmatimonadetes and Epsilonbacteraota. We also found 29 bacterial phyla with an overall relative abundance of less than 1%. The relative abundances of individual bacterial taxa were highly variable across the collected samples (Table S3 and Figure S3, Supporting Information) and differed among different horizons (Fig. 2) and tundra types (Figure S4, Supporting Information). Actinobacteria (26%), Alphaproteobacteria (17%) and Gammaproteobacteria (16%) dominated in the topsoil. In contrast, the cryoOM horizon was dominated by Actinobacteria (34%), Gammaproteobacteria (14%) and Chloroflexi (10%). We also compared different tundra types (Figure S4, Supporting Information), the HT was dominated by Actinobacteria (30.6%), whereas the UT had a higher relative abundance of classes Alphaproteobacteria (16.9%) and Bacteroidetes (8.5%) compared to other tundra types. The WT samples had a greater relative abundance of the phylum Firmicutes (9.1%).

Archaea comprised on average 0.08% of the prokaryotic communities and were represented by four phyla with seven classes. In general, the topsoil and cryoOM samples were dominated by Euryarchaeota, whereas subsoil and permafrost samples had a higher relative abundance of Thaumarchaeota (Fig. 2). At the class level, Methanomicrobia had a high relative abundance in topsoil and cryoOM, whereas Methanobacteria were dominant in topsoil and subsoil samples (Table S3, Supporting Information).

Averaged archaeal relative abundances varied between tundra types and were 0.01%, 0.008% and 0.21% for HT, UT and WT, respectively. The more archaeal sequence in the WT was in line with the SSU rRNA archaeal gene abundance (Figure S2, Supporting Information). Methanogenic Euryarchaeota (mainly Methanomicrobia and Methanobacteria) dominated in the HT (88%) and WT (89%), while the UT was dominated by Thaumarchaeota (52%). Different horizons from individual tundra types also harbored distinct archaeal communities (Fig. 2). The topsoil from HT and UT exhibited a co-dominance of Euryarchaeota and Thaumarchaeota, on the contrary, the WT was mostly dominated by Euryarchaeota. The cryoOM horizon also showed a relatively greater abundance of Euryarchaeota from tundra types; HT and WT. Overall, we found a greater relative abundance of Thaumarchaeota in subsoil and permafrost samples; however, this apparent pattern was due to the higher relative abundance of Thaumarchaeota in the UT.

Alpha diversity, assessed by Chao1, Shannon and Simpson evenness, was highest in topsoil samples independent of tundra type. The significant differences (P -value <0.05) among different horizons were observed only for the UT (Table 2). Simpson evenness was lower in the cryoOM sample compared to the topsoil and subsoil, showing the dominance of several taxa in cryoOM. The Chao1, Shannon, Simpson evenness diversity indexes values were similar in the HT and UT, and both were higher than the WT (Table 2).

The relationship between the prokaryotic community and soil physicochemical parameters

Permutational multivariate analysis of variance (PERMANOVA) confirmed that both horizons (F -value = 3.5, P -value = 0.001) and

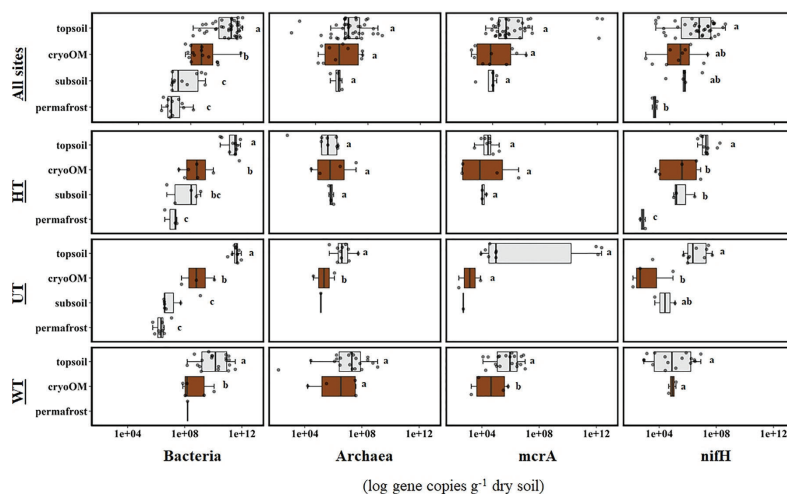


Figure 1. Box-and-whisker plot showing log-transformed gene copies per gram dry soil of individual soil horizons from each tundra types determined for bacteria, archaea, *mcrA*-methanogens and *nifH*-diazotrophs by qPCR. Different letters depicted significant differences between soil horizons ($P < 0.05$, Tukey's HSD test). The bottom and top of each box indicate the first and third quartiles, and the horizontal line inside the box shows the second quartile (median). Whiskers indicate maximum and minimum values. The black-colored points indicated the distribution of the sample. HT, Hummocky tussock tundra; UT, Upland tundra; WT, Wet polygonal terrain.

Table 2. Alpha diversity indices of prokaryotic communities.

Tundra types	Horizon	N	Number of reads	Number of OTUs	Chao1	Shannon	Simpson evenness
HT	Topsoil	9	189 342	1921 ± 730 (a)	931.8 ± 213.8 (a)	5.4 ± 0.6 (a)	0.17 ± 0.08 (a)
	CryoOM	7	97 367	1307 ± 410 (a)	755.5 ± 181.3 (a)	4.6 ± 0.8 (a)	0.10 ± 0.07 (a)
	Subsoil	5	115 916	1689 ± 662 (a)	779 ± 210.3 (a)	4.9 ± 0.5 (a)	0.11 ± 0.04 (a)
	Permafrost	2	16 944	929 ± 113 (a)	927.3 ± 596.2 (a)	4.8 ± 1.6 (a)	0.15 ± 0.16 (a)
UT	Topsoil	8	132 647	1974 ± 528 (a)	1151.4 ± 172.3 (a)	5.8 ± 0.4 (a)	0.28 ± 0.08 (a)
	CryoOM	2	17 300	1006 ± 77 (ab)	664 ± 8.4 (b)	5.1 ± 0.1 (ab)	0.16 ± 0.02 (ab)
	Subsoil	7	120 853	1355 ± 528 (ab)	634.1 ± 108.7 (b)	5.4 ± 0.2 (a)	0.24 ± 0.05 (ab)
	Permafrost	9	96 789	894 ± 432 (a)	621.2 ± 140(b)	4.6 ± 0.5 (b)	0.14 ± 0.11 (b)
WT	Topsoil	21	97 716	722 ± 369 (a)	734.4 ± 222 (a)	4.7 ± 0.8 (a)	0.13 ± 0.09 (a)
	CryoOM	5	18 602	501 ± 191 (a)	531.8 ± 46.2 (a)	4.2 ± 0.6 (a)	0.08 ± 0.06 (a)
	Permafrost	1	6379	683	626	3.9	0.05

Means and standard deviation were calculated from each sample. A significant difference between horizons was calculated by One-Way ANOVA and followed by Tukey's HSD test and indicated by different letters in the brackets.

N, number of replicates; na, no sample available.

tundra types (F -value = 6.0, P -value = 0.001) were significant factors for the variation of bacterial community structure; however, the effect of different tundra types was stronger than for different horizon (Table S4, Supporting Information). To visualize the difference in bacterial community structure across all samples, the dbRDA analysis based on the weighted Bray–Curtis distance matrix was conducted and revealed that the HT and WT clustered close together apart from the UT (Fig. 3A). In the HT and UT, the topsoil samples were clustered close together, whereas cryoOM and subsoil communities were close to each other. We found two separate clusters of topsoil microbial communities from the WT (Fig. 3A). We used seven parameters, including moisture, pH, DOC, DN, Ctot, Ntot and C/N for RDA analysis. The most significant variables shaping the soil bacterial community were determined by forward selection, which included pH (9.0%, $F = 7.2$, P -values = 0.001) and Ctot (6.4%, $F = 5.5$, P -values = 0.001; Fig. 3B). The topsoil samples were positively correlated

with moisture, Ctot and C/N, while the cryoOM samples were positively correlated with pH and DN but negatively correlated with moisture, Ctot and C/N (Fig. 3B).

Metabolic potential of microbiomes in Cryosols horizons and tundra types

We employed FAPROTAX and FunGene pipelines to predict the metabolic potential of the prokaryotic communities. With the help of these bioinformatics tools, we identified the differentially abundant prokaryotic functional groups between different horizons and tundra types by LefSe analysis (Figure S6, Supporting Information).

FAPROTAX identified a total of 56 different prokaryotic functional groups, which comprised 20.4% of the total community (1004 out of 4915 with at least one functional group). Among significantly different functions (more than 2-fold changes and

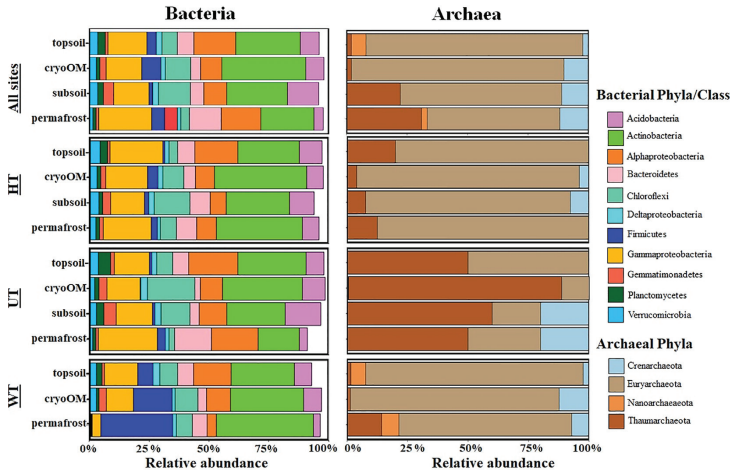


Figure 2. Relative distribution of bacterial and archaeal phyla/classes between different horizons based on targeted 16S rRNA sequencing. Left panels: Only those bacterial phyla with >1% relative abundance was shown, phylum *Proteobacteria* were shown at respective class levels. Right panels: Relative abundance of archaeal phyla were shown.

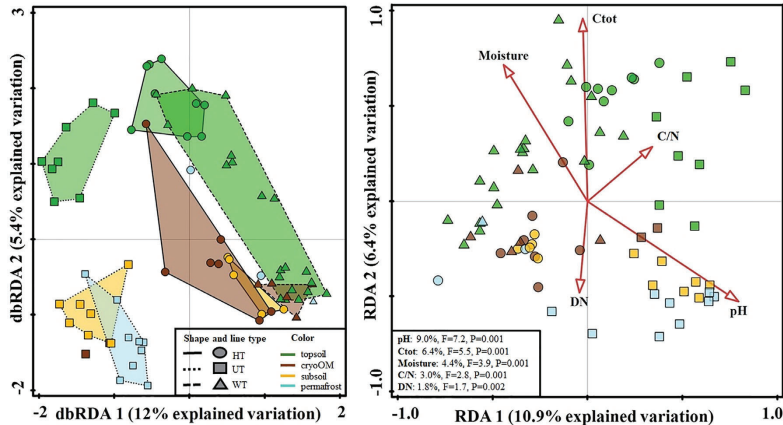


Figure 3. The phylogenetic dissimilarity between soil horizons and tundra types. (A) Distance-based RDA (dbRDA) of microbial communities of different horizons from distinct tundra types. Different colored envelopes represented different horizons, whereas the shape of points and line type represented different tundra types (B) Results of RDA ordination diagrams of main soil characteristic interactions with changes in the relative abundance of zOTUs. The red arrows indicate the soil parameters that had a significant impact on the microbial communities' structure identified by the forward selection method, and the corresponding explained the proportion of variability given in the lower-left corner. DOC, Dissolve Organic Carbon; DN, Dissolve Nitrogen; C/N, Carbon to Nitrogen ratio.

P -values <0.05), nitrogen fixation and ureolysis were more dominant in topsoil. On the contrary, methanol oxidation and fermentation were significantly different in cryoOM compared to other horizons (Figure S6A, Supporting Information). For the different tundra types, the HT was dominated by dark iron reduction, whereas UT had a significantly higher proportion of bacteria capable of aromatic compound degradation, chitinolysis, denitrification and nitrogen fixation. The WT was a hotspot for anaerobic processes like methanogenesis as compared to other tundra types (Figure S6B, Supporting Information).

We specifically focused on the composition of diazotrophs and methanogens, which were also the main groups quantified

by qPCR (see above). Using the FunGene database, we identified 70 distinct genera predicted to contain the *nifH* gene and 12 genera containing the *mcrA* gene and evaluated the N_2 fixation and methanogenic potential in distinct horizons and tundra types (Table 3). The proportion of diazotrophs in the microbial community did not differ significantly between different tundra types and represented on average approximately 25%. On the contrary, we found a significant difference (P -values <0.05) between soil horizons in individual tundra types. Diazotrophs in general decreased in the order topsoil (12.3%) > subsoil (6%) > cryoOM (4.7%) > permafrost (4%). We identified the most abundant genera of diazotrophs (>1% relative abundance):

Table 3. The proportion of methanogenic archaea and diazotrophic bacteria in the different horizons and tundra types. Data were obtained based on the taxonomic classification and FunGene database (<http://fungene.cme.msue.edu/>, Fish et al. 2013).

Class	Genus	ARN	All sites						HT			UT			WT									
			topsoil	cryoOM	subsoil	permafrost	Topsoil	cryoOM	subsoil	permafrost	topsoil	permafrost	subsoil	permafrost	topsoil	permafrost	subsoil	permafrost	topsoil	permafrost	subsoil	permafrost		
Methanogens (<i>mcA</i>)	All methanogens	NA	8.3	2.5	2.5	2.3	3.0	6.0	4.0	2.0	2.0	1.0	4.0	20.0	4.0	4.0	4.0	20.0	4.0	1.0				
	Methanobacteria		3.5	1.3	1.2	0.6	2.0	4.7	2.3	0.3	1.0	1.0	1.0	1.0	7.4	0.6	1.0	7.4	0.6	0.5				
	Methanomicrobia	2*	3.2	0.9	1.3	1.0	1.0	1.1	1.7	1.7	1.0	1.0	1.0	7.7	2.3	1.0	7.7	2.3	0.2					
	Methanomicrobia	3	1.0	0.1				0.1							3.0	0.2		3.0	0.2	0.1				
	Methanomicrobia	1	0.5	0.1											1.4	0.4		1.4	0.4	0.1				
Diazotrophs (<i>nifH</i>)	All diazotrophs	NA	12.3	4.7	6.0	4.0	9.0	7.0	5.0	2.0	2.0	8.0	2.0	2.0	9.0	7.0	2.0	20.0	5.0	1.0				
	<i>Pseudomonas</i>	5	2.5	0.6	0.9	0.5	3.0	0.7	0.6	0.4	1.4	1.4	0.2	1.1	3.1	0.9	1.1	3.1	0.9					
	<i>Rhodiferax</i>	3	0.8	0.6	1.2	0.5	0.3	1.2	1.9	0.2	0.3	0.2	0.2	0.5	1.4	0.2	0.5	1.4	0.2					
	<i>Actinobacteria</i>	5	0.9	1.0	0.4	0.4	0.7	1.9	0.3	0.6	0.3	0.3	0.3	0.6	1.8	0.4	0.6	1.8	0.4	0.3				
	<i>Bradyrhizobium</i>	1	1.9	0.5	0.5	0.1	1.9	0.8	0.6	0.1	0.7	0.1	0.7	0.1	0.4	0.2	0.1	0.4	0.2	0.1				
	<i>Alphaproteobacteria</i>	2	1.9	0.4	0.5	0.4	0.6	0.4	0.2	0.1	0.9	0.3	0.9	0.2	0.9	0.2	0.1	0.9	0.2	0.6				
	<i>Sphingomonas</i>	2	1.9	0.4	0.5	0.4	0.6	0.4	0.2	0.1	0.9	0.3	0.9	0.2	0.9	0.2	0.1	0.9	0.2	0.6				
	<i>Actinobacteria</i>	3	0.6	0.1	0.3	0.1	0.4	0.1	0.3	0.1	1.2	0.3	0.6	0.3	0.3	0.3	0.6	0.3	0.3	0.3				
	<i>Nocaritoides</i>	1	0.1	0.2	0.1	0.4	0.1	0.3	0.1	0.1	0.2	0.1	0.2	0.1	1.3	0.1	1.3	0.1	1.3	0.1				
	<i>Polaromonas</i>	2	0.1	0.1	0.1	0.4	0.1	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1				
<i>Brevundimonas</i>	2	0.1	0.1	0.1	0.4	0.1	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1					
other	NA	3.5	1.1	1.9	1.2	2.0	1.5	1.3	0.4	2.9	0.5	4.6	0.5	5.5	1.4	0.5	5.5	1.4	0.5					

ARN, Average ribosomal copy number.
 *Based on Methanobacteriales ARN.
 NA—not analyzed.

Bradyrhizobium, *Sphingomonas* (Alphaproteobacteria); *Pseudomonas*, *Rhodiferax* (Gammaproteobacteria); and *Arthrobacter* (Actinobacteria), and their relative proportions differed between soil horizons and tundra types. In topsoil, *Pseudomonas*, *Bradyrhizobium* and *Sphingomonas* were the most dominant diazotrophs, *Arthrobacter* dominated in cryoOM and *Rhodiferax* in the subsoil. Diazotrophs dominated in topsoil in WT, while in HT and UT, they were more shifted to lower horizons. Contrary to diazotrophs, methanogens significantly differed (P -values <0.05) for tundra types (i.e. *Methanobacterium* and *Methanosarcina*). They comprised 15%, 7% and 25% of the archaeal community in HT, UT and WT tundra, respectively. The WT also had more methanogenic genera than the other two tundra types. Topsoil again harbored the highest proportion of methanogenic genera and was followed by cryoOM, subsoil, and permafrost; however, they were not significantly different.

To validate the above-mentioned pattern of decreasing methanogen and diazotrophs from topsoil to permafrost and increasing trend from HT to WT, we plotted the average gene abundance and the relative mean proportion of methanogen and diazotrophs identified by qPCR and the FunGene database, respectively (Figure S7, Supporting Information). We found that the gene abundance and relative mean proportion of methanogens and diazotrophs showed a similar trend for the horizon, decreasing from topsoil to permafrost. In contrast to horizons, tundra type displayed an opposite trend for diazotrophs. The relative proportion suggested that WT had the highest mean proportion of diazotrophs, whereas diazotrophs gene abundance showed the lowest number for WT compared to other tundra types.

We also evaluated average copy numbers (ACN, Thompson et al. 2017) of ribosomal genes per genome of each methanogen and diazotroph (Table 3). The higher the ACN number the higher the copiotrophic/oligotrophic nature of the microbe (Klappenbach, Dunbar and Schmidt 2000). In topsoil, the diazotroph community in WT contained a higher proportion of taxa with copiotrophic lifestyle, while HT and UT communities had more oligotrophic taxa. We did not find a major difference between copiotrophic and oligotrophic methanogen from our study site (Table 3).

Unique microbiomes of distinct cryosols horizons and tundra types

We additionally used linear discriminant analysis effect size (LEfSe) to identify zOTUs differentially abundant in each horizon (Fig. 4A) and tundra type (Fig. 4B). The order *Rhizobiales* and the family *Pseudomonadaceae* both from the phylum *Proteobacteria* were significantly enriched in topsoil (Fig. 4A and Figure S5a, Supporting Information), the cryoOM community was enriched in *Firmicutes* (classes *Bacilli*, *Clostridia*), *Verrucomicrobia*, *Chloroflexi* and *Actinobacteria* (order *Gaiellales* and family *Intrasporangiaceae*), the subsoil was enriched in *Acidobacteria* and *Gemmatimonadetes*, and the permafrost was enriched in the phylum *Epsilonbacteraeota* and *Bacteroidetes*, and the class *Gammaproteobacteria* (Fig. 4A and Figure S5a, Supporting Information). The differential distribution of bacteria for different tundra types was depicted in Fig. 4B and their LDA histogram was available in Figure S5b (Supporting Information). The HT was significantly enriched in the class *Actinobacteria*, *Acidobacteria*, and a member of these classes. The most differentially abundant microbial taxa from the UT belonged to the phylum *Epsilonbacteraeota*, the class *Acidimicrobiia*, *Subgroup 6* (within the phylum *Actinobacteria*), and

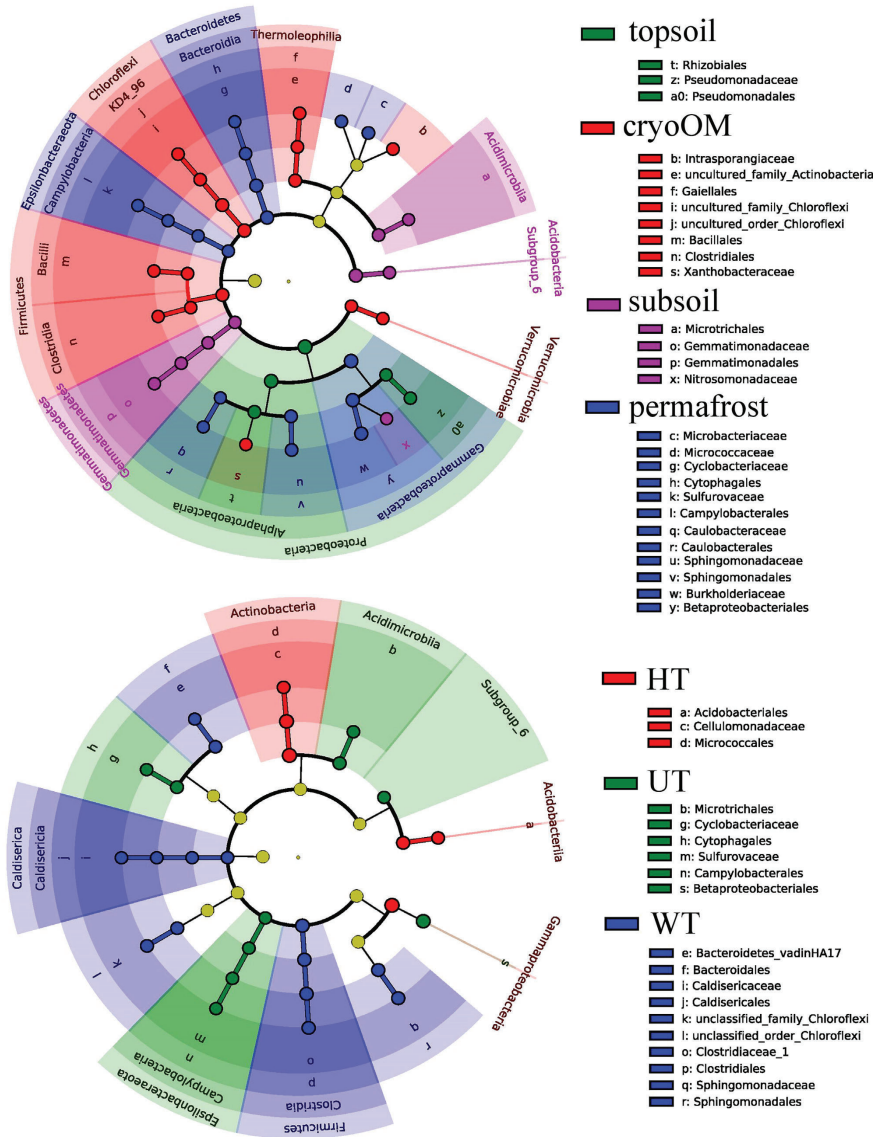


Figure 4. Cladogram reporting results from the LEfSe analysis for horizons (A) and tundra types (B) at Phylum/Class/Order/Family level. In the figure, the edges closest to the center represent the zOTUs at the phylum level, whereas the edges on the outer circle represent the zOTUs at the family level. The color of the dots and sectors indicate which phyla/classes/orders/families respective were significantly differentially abundant in each horizon or tundra type (LDA score >4). Light-colored sectors mean phyla level and darkest sectors mean families' level. Yellow-colored edges show non-significantly abundant phyla/classes/orders/families between soil horizons and tundra types.

the order Betaproteobacteriales (within the phylum Proteobacteria). Whereas the WT exhibited the highest number of significantly differentiated taxa compared to the HT and UP. The phylum *Caldiserica* and *Firmicutes* were the most significantly enriched from the WT.

We compared topsoil with cryoOM to see the difference in the mean proportion of zOTUs. We found that the phylum *Actinobacteria* had the highest difference between the two horizons (Figure S8, Supporting Information). The zOTUs, which had a more significant mean proportion from topsoil compared to cry-

oOM, were affiliated to the families *Xanthobacteraceae*, *Burkholderiaceae*, *Chitinophagaceae*, *Microbacteriaceae*, *Caldiseriaceae*, *Sphingomonadaceae* and *Solirubrobacteraceae*. In contrast, zOTUs affiliated with *Intrasporangiaceae*, *Cellulomonadaceae* and an uncultured family (affiliated to the order *Gaiellales*) had a greater mean proportion within the prokaryotic community in cryoOM than the topsoil (Welch's test, two-sided, P -values <0.05). This finding was in line with the cladogram results at the family level (Fig. 4A).

DISCUSSION

This study provides a detailed structural and functional characterization of microbial communities in different horizons and tundra types of Arctic Cryosols. Our results showed a greater number of bacteria in cryoOM compared to the surrounding subsoil, on the contrary, archaea, methanogens and diazotrophs abundances were similar. The distinct tundra types displayed different numbers of bacteria and archaea; however, the significant and most prominent differences were found mainly for topsoil. We found a significant effect for both different horizons and tundra types on microbial community composition (Fig. 3, Table S4, Supporting Information). In general, our data showed that the cryoOM community was enriched with specific taxa reflecting the quality of SOM (Dao et al. 2018). These were specific oligotrophic taxa capable of complex and recalcitrant biopolymer degradation (i.e. lignin and lignocellulose), mainly members of *Actinobacteria* families *Intrasporangiaceae*, *Cellulomonadaceae* and uncultured members of *Gaiellales* (Fig. 4, Figure S8, Supporting Information). Additionally, the wet polygonal tundra might be at putative hot spot for these anaerobic microbial communities and possess a higher potential for CH_4 production.

Abundance and composition of methanogens and diazotrophs in different horizons

The cryoOM harbored a greater number of bacteria (Gittel et al. 2014a, b) than the surrounding mineral subsoil (Fig. 1). In contrast, archaea and methanogens (*mcrA*) were less abundant and more comparable between cryoOM and subsoil (Gittel et al. 2014a). Detailed taxonomic analysis revealed that genera from the classes *Methanobacteria* and *Methanomicrobia* dominated in both topsoil and cryoOM (Gittel et al. 2014a). The cryoOM communities were significantly enriched also with methylotrophs (mainly family *Methylophilaceae*, Figure S6, Supporting Information). All representatives of the family *Methylophilaceae* have been reported as obligate methylotrophs capable of utilizing methanol or methylamine but not CH_4 as a sole source of carbon and energy (Jenkins and Jones 1987; Doronina 2004, 2005; Kalyuzhnaya et al. 2006). Methanol oxidation was also significantly greater in cryoOM compared to topsoil (Figure S6, Supporting Information). Therefore, we argue that even though the cryoOM had a great proportion of methylotrophic families, they do not use CH_4 as a carbon source produced by methanogens (Kalyuzhnaya et al. 2006), and cryoOM may be a potential hotspot for CH_4 release (Olefeldt et al. 2013; Lau et al. 2015).

Based on the specific quantification of the *nifH* gene, we were able to estimate the abundance of N_2 fixators (diazotrophs) in different horizons and tundra types. We found a significantly higher abundance of diazotrophs in the topsoil (except WT tundra type, Fig. 1). However, the ratio of diazotrophs to the total

number of bacteria was significantly greater in cryoOM and subsoil compared to topsoil. This highlights their greater importance in the microbial community in lower soil horizons. The higher proportion of diazotrophs in the cryoOM and subsoil can drive an important flux of N into these deeper horizons of the active layer (Jackson et al. 2017), which can turn on the whole N cycle (Takai 2019) and accelerate OM decomposition by increasing N availability (Wild et al. 2014). Additionally, with the help of next-generation sequencing of 16S rRNA gene amplicons and the FunGene database (Fish et al. 2013), we were able to specifically identify bacterial genera belonging to the diazotrophs (having the *nifH* gene, Table 3). We found a distinct distribution of diazotrophic genera in different horizons, mainly *Pseudomonas*, *Bradyrhizobium* and *Sphingomonas* were the most dominant diazotrophs in the topsoil, whereas in cryoOM the members of the *Actinobacteria* genus *Arthrobacter* were the main N_2 fixators. The important role of the genus *Arthrobacter* in the subsoil of the active layer has already been described (Šantrůčková et al. 2018). The members of *Arthrobacter* are known for their ability to use complex substrates which were present in high amounts in cryoOM (Dao et al. 2018), they rapidly increased in abundance when easily available nutrients like sucrose were added to Cryosols and they also cooperated intensively in heterotrophic CO_2 fixation in anaerobic reactions (Šantrůčková et al. 2018). The high abundance of copiotrophic and complex biopolymer degrading *Arthrobacter* species in cryoOM may have crucial implications in the future vulnerability of OM decomposition in Turbic Cryosols. Through their versatile metabolic capabilities, they can pump additional N to cryoOM, and degrade complex compounds, making them available for the whole microbial community.

Specific microbial communities and functional guilds in distinct tundra types

In addition to distinct horizons the distinct tundra types also exhibited varying microbial communities' (Fig. 4 and Figure S4, Supporting Information). The HT showed significant enrichment of oligotrophic classes of *Actinobacteria* and *Acidobacteria*, this finding can be attributed to low C availability in the tussock tundra (Koyama et al. 2014). Whereas the UT was dominated by the class *Acidimicrobiia* and *Subgroup_6* from the phylum *Acidobacteria* and microbial communities from the UT were mainly driven by pH (Fig. 3). In contrast, the WT was characterized by high moisture and high DOC content, which may be connected to higher fermentative families such as *Clostridiaceae* and *Caldiseriaceae*. The family *Clostridiaceae* is involved in cellulose degradation under anoxic conditions (Schellenberger, Kolb and Drake 2010). Whereas another study suggested that the member of the *Sphingomonadaceae* family is considered as a phototrophic organism and gains a major fraction of their metabolic energy via anoxygenic photosynthesis (Gupta and Mok 2007) and also efficiently ferments sugar to ethanol (Seo et al. 2005). The anaerobic thiosulfate-reducing family *Caldiseriaceae* from *Caldiserica* phylum was also found at WT (Mori et al. 2009). This family comprised a single species, thermophilic *Caldisericum exile* (Ström, Mastepanov and Christensen 2005), and was previously reported in permafrost thaw pond (Perner et al. 2014). Indeed, significantly high moisture content and labile nutrient source in the form of DOC can promote anaerobic microbial growth at the WT.

Different tundra types also showed a difference in the abundance of methanogens and diazotrophs, but these differences were most pronounced and significant in the topsoil. The HT and

UT had a lower number of methanogens compared to WT, suggesting the greater potential of CH₄ emission from the Cryosols of WT (Fig. 1 and Figure S2, Supporting Information). These results were not surprising, as anoxia created by high moisture content from WT compared to the other two tundra types potentially favored the growth of methanogens (Barbier et al. 2012). Microbial communities from WT were also found to be driven mainly by moisture content (Fig. 3). Moreover, we also found a high relative abundance of the hydrogenotrophic methanogen genus *Methanobacterium* (Conrad 2007) and the metabolically versatile methanogens genus *Methanosarcina* (Thauer et al. 2008) in WT (Table 3). In support of this, a higher anaerobic process (i.e. hydrogenotrophic and acetoclastic methanogenesis) was also observed in WT (Figure S8b, Supporting Information). Collectively, these results suggested that WT was a potential hotspot for CH₄ emission.

In contrast to methanogens, the total numbers of diazotrophs were the lowest in WT (Fig. 1, Figures S2 and S7, Supporting Information); however, the proportion of diazotrophs were highest in topsoil in WT, whereas they were more shifted toward lower soil horizons in HT and UT (Table 3). A higher water table can explain this distinct distribution of diazotrophs in the microbial community in WT, where diazotrophs favor more aerated topsoil, while in HT and UT, the soil is also more aerobic in deeper layers. Moreover, diazotrophs in HT and UT can be more associated with the rooting system of the vegetation. As mentioned previously, some methanogenic archaea have the ability to fix N₂ (Brabban, Orcutt and Zinder 1999; Mizukami et al. 2006; Dang et al. 2013; Bae et al. 2018). This may explain the lesser abundance of bacterial diazotrophs in WT where the methanogenic community may have sufficient N for their metabolic needs in anoxic conditions by their own ability to fix N₂.

We found a distinct pattern of microbial groups distribution for relatively proportionated amplicon sequencing data (FunGene) and quantified gene abundance data (qPCR; Figure S7, Supporting Information). The methanogens relative mean proportion and mean gene abundance were decreased from topsoil to permafrost and from HT to UT. In contrast, the relative mean proportion of diazotrophs increased but mean gene abundance decreased from HT to WT. These results showed the crucial difference in using different molecular techniques to identify specific microbial groups. The relative proportion gives information about how well the microbial community composed together and the reliability of such an estimate in reflecting the actual abundance of the community is insufficient (Zhou et al. 2015, 2016). On the other hand, the quantification of certain microbial groups provides exact information for one species regardless of whether its population is declining, growing, or stable along with spatial and temporal shifts (Molles 2001). Therefore, we argue that special consideration needs to be given when interpreting data based on these molecular analyses.

CONCLUSION

The present study provides comprehensive information about the abundance of bacteria and archaea and their composition in the microbiomes of different horizons and tundra types of Arctic Cryosols in Qikiqtaruk (Hershel Island, Western Canada). The number of microbial groups varied markedly between different horizons. Bacterial numbers decreased from topsoil to permafrost. They were significantly enriched in cryoOM compared to the surrounding subsoil and also showed specific bacterial composition patterns. This was mainly caused by the higher

proportion of individual taxa (*Intrasporangiaceae* and *Cellulomonadaceae*) capable of recalcitrant OM degradation. The numbers of methanogens and diazotrophs were lower in cryoOM and in the case of diazotrophs even lower than in the surrounding subsoil, suggesting lower N availability and the possible N limitation of the cryoOM microbial community. This is one of the reasons for the lower OM vulnerability of cryoOM. We, however, confirmed a higher proportion of copiotrophic bacteria from the *Arthrobacter* genus. Using their versatile metabolic capabilities (including N₂ fixation), they are one of the potential taxa which can boost cryoOM decomposition when more available nutrients flow into this buried horizon (e.g. by root exudation). Significant differences in the number of functional guilds were also observed between the topsoil of different tundra types. The methanogens were more frequent in WT, and the WT community also had a more significant proportion of methanogenic taxa, suggesting a potential for CH₄ emission. On the other hand, WT had the lowest number of diazotrophs, which is probably the result of a sufficient amount of N in the system indicated by the lowest C/N among the three tundra types

FUNDING

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DATA AVAILABILITY

The raw sequence generated for this study can be found in the European Nucleotide Archive (ENA) under the study of PRJEB38326.

AUTHOR CONTRIBUTIONS

GH completed fieldwork and collected samples from Qikiqtaruk (Hershel Island), Canada. MV and JB analyzed and evaluated soil physicochemical parameters, molecular, and sequencing data. MV, JB wrote the manuscript, and TU, GH contributed to and have approved the final manuscript.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

Conflicts of interest. None declared.

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Paper II

**Fungi in Permafrost-Affected Soils of the Canadian
Arctic: Horizon- and Site-Specific Keystone Taxa
Revealed by Co-Occurrence Network**

Varsadiya, M., Urich, T., Hugelius, G., Bárta, J.

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Article

Fungi in Permafrost-Affected Soils of the Canadian Arctic: Horizon- and Site-Specific Keystone Taxa Revealed by Co-Occurrence Network

Milan Varsadiya ¹, Tim Urich ², Gustaf Hugelius ^{3,4} and Jiří Bárta ^{1,5,*}

- ¹ Department of Ecosystems Biology, University of South Bohemia, 37005 České Budějovice, Czech Republic; mvarsadiya@prf.jcu.cz
- ² Institute of Microbiology, University of Greifswald, 17487 Greifswald, Germany; tim.urich@uni-greifswald.de
- ³ Department of Physical Geography, Stockholm University, 10691 Stockholm, Sweden; gustaf.hugelius@natgeo.su.se
- ⁴ Bolin Centre for Climate Research, Stockholm University, 10691 Stockholm, Sweden
- ⁵ Centre for Polar Ecology, Faculty of Science, University of South Bohemia, 37005 České Budějovice, Czech Republic
- * Correspondence: jiri.barta@prf.jcu.cz

Abstract: Permafrost-affected soil stores a significant amount of organic carbon. Identifying the biological constraints of soil organic matter transformation, e.g., the interaction of major soil microbial soil organic matter decomposers, is crucial for predicting carbon vulnerability in permafrost-affected soil. Fungi are important players in the decomposition of soil organic matter and often interact in various mutualistic relationships during this process. We investigated four different soil horizon types (including specific horizons of cryoturbated soil organic matter (cryoOM)) across different types of permafrost-affected soil in the Western Canadian Arctic, determined the composition of fungal communities by sequencing (Illumina MPS) the fungal internal transcribed spacer region, assigned fungal lifestyles, and by determining the co-occurrence of fungal network properties, identified the topological role of keystone fungal taxa. Compositional analysis revealed a significantly higher relative proportion of the litter saprotroph *Lachnum* and root-associated saprotroph *Phialocephala* in the topsoil and the ectomycorrhizal close-contact exploring *Russula* in cryoOM, whereas Sites 1 and 2 had a significantly higher mean proportion of plant pathogens and lichenized trophic modes. Co-occurrence network analysis revealed the lowest modularity and average path length, and highest clustering coefficient in cryoOM, which suggested a lower network resistance to environmental perturbation. Zi-Pi plot analysis suggested that some keystone taxa changed their role from generalist to specialist, depending on the specific horizon concerned, *Cladophialophora* in topsoil, saprotrophic *Mortierella* in cryoOM, and *Penicillium* in subsoil were classified as generalists for the respective horizons but specialists elsewhere. The litter saprotrophic taxon *Cadophora finlandica* played a role as a generalist in Site 1 and specialist in the rest of the sites. Overall, these results suggested that fungal communities within cryoOM were more susceptible to environmental change and some taxa may shift their role, which may lead to changes in carbon storage in permafrost-affected soil.

Keywords: arctic; permafrost; keystone taxa; co-occurrence network; Zi-Pi plot



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1. Introduction

Fungi are ubiquitous and one of the most species-rich groups of organisms in the Arctic soil ecosystem [1]. Our knowledge of their role in soil organic matter transformation is continually increasing, still, there are many unanswered questions regarding the relationship between different taxa with distinct lifestyles (i.e., saprotrophs, mycorrhizae) as they are thought to be the key players of elemental and energy flow in carbon (C) and nitrogen (N) cycles. They also influence the occurrence of other microbes, such as bacterial decomposers, pathogens, and symbiotrophs [2–4]. Despite the ubiquitous distribution of fungi in

the soil, our knowledge of their biodiversity and functional traits in permafrost-affected soil (PAS) remains limited to relatively few studies. Nevertheless, the number of fungal studies from PAS is continually increasing, including studies from Svalbard [5,6], Alaska [7,8], and Greenland [9,10]. However, only a few specific studies, from Eastern Siberia [11] and the Northern American Arctic transect [12], have studied the fungal community from the buried organic matter (cryoOM) pocket, which stores a significant amount (approx. 470 Pg C) of organic C due to cryoturbation of the top organic layer.

Most ecological studies have focused on functional diversity, as opposed to biodiversity only, due to the fact that individual species can have several functions in an ecosystem [13,14]. It is well-known that many fungal species play redundant roles by altering or manipulating the distribution of the same soil resource [15]. Several sequence-based studies have parsed operational taxonomic units (OTUs) into more ecologically meaningful groups [16–18]. These groups would have a similar function in the ecosystem and can be divided into symbiotrophs, pathotrophs, and saprotrophs, collectively called trophic modes [19]. These trophic modes of fungi play critical roles in the Arctic, for example, symbiotrophs help plants to uptake nutrients, especially N, which is considered growth-limiting in the Arctic tundra [20]. On the other hand, saprotrophic fungi are essential for decomposing dead plant biomass and, therefore, crucial for nutrient and carbon cycling in the Arctic soil [21–23]. Pathotrophic fungi are known to infect other fungi to gain organic carbon and by doing so, they control other trophic modes [24]. There is still a lack of data on the occurrence and potential interactions of these trophic guilds in Arctic PAS.

Microbes in the soil create a complex ecological network by interacting with each other [25]. This interaction includes predation, competition, parasitism, and mutualism [26–30]. To predict the composition of ecological microbial networks, it is especially important to understand the microbial assembly, the potential interactions of keystone taxa, and the resulting ecological function [29,31,32]. Despite the importance of these interactions in ecological functions, the direct detection and investigation of these interactions are difficult [25,33]. Several studies have demonstrated that the specific properties of ecological species networks can, at least to some extent, explain the real response of the microbial community to environmental changes [34–36]. For example, a study of experimental warming from Alaskan tundra soil evidenced that warming conditions had a more complex and denser bacterial co-occurrence network compared to the control site, while the opposite was observed for the fungal network [36]. The authors suggested that the environmental changes were associated with a distinct response by microbial communities [36].

The specific properties (topological properties) of the co-occurrence networks include (1) the degree distribution, which determines how many other taxa in the network are connected with the given taxa; (2) the clustering coefficient, which describes how well a taxon is connected to its neighboring taxa (analogy to human society, the clustering coefficient is a measure of an “all-my-friends-know-each-other” property); (3) the average path length, which is the shortest path between the two most distant taxa in the network (a short average path length facilitates the quick transfer of information and reduces costs leading to the concept of a small world where everyone is connected to everyone else through a very short path); (4) modularity, which measures the degree to which the network was organized into clearly delimited modules. Networks with high modularity have dense connections between the taxa within modules but sparse connections between taxa in different modules [37].

Another aspect of the ecological network is the identification of keystone species, connectors and modular hubs [38,39]. The connectors are defined as those taxa or nodes which have more connections among different modules, in contrast, module hubs are those taxa or nodes which have more connections within their own modules [27]. These keystone taxa play a key role in modulating network structure and function, as they often have dominant relationships and interactions among other taxa [27]. The network analysis may also provide information about the importance of low abundant taxa for supporting the structure and functions of microbial communities. Most soil ecosystem studies have

concentrated on the most abundant microbial species [11,40–42]. However, low abundance taxa play a significant role in maintaining ecosystem functions, despite their low proportion. Therefore, some of them are also considered as keystone taxa [31,43]. Herren and co-authors [44] suggested that keystone taxa can explain microbiome compositional turnover better than the most abundant taxa combined. The keystone species are most important to protect since their absence might lead to network fragmentation [45]. For instance, the disappearance of a keystone species from a network of bacterial wilt-susceptible soil made it more loose and unstable compared to a network of healthy soil that had more keystone species [46].

To understand the complexity of these interactions in fungal communities in Arctic PAS, we addressed the following questions: (1) Does each horizon type (topsoil, cryoOM, subsoil, and permafrost) contain exclusive/unique fungal genera and lifestyles? (2) Do network topological properties significantly differ between different horizons and tundra sites? (3) Which are the keystone species in different horizons and tundra sites? (4) Is there any correlation between network modules, keystone species, and environmental factors?

To address these questions, we collected 122 soil samples from four different horizons of four distinct tundra sites from Herschel Island, Canada. We used Illumina MiSeq sequencing data of the fungal ribosomal internal transcribed spacer (ITS) to analyze the change in the fungal community composition and intertaxa interaction. We implemented sequencing data to infer fungal community composition, functional guild distribution, and microbial ecological network analysis. Our central objective was to characterize and understand the microbial ecological network pattern of sequencing data obtained from Illumina MiSeq sequencing and specific emphasis was given to cryoOM.

2. Materials and Methods

2.1. The Site Description and Soil Sampling

The study area is located on Herschel Island (Qikiqtaruk; 69°34' N, 138°55' W, Beaufort Sea, Canada). The mean annual air temperature is $-9\text{ }^{\circ}\text{C}$ with the mean monthly air temperature varying between $-26.3\text{ }^{\circ}\text{C}$ (February) and $8.7\text{ }^{\circ}\text{C}$ (July). The mean annual precipitation ranges between 150 and 200 mm [47].

During late summer, a total of 122 samples were collected from four tundra sites and three different types of soil horizons of the active layer. These horizons represented upper topsoil, cryoOM, and mineral subsoil based on field description. We also collected samples from the permafrost. The four sites had a landscape of hummocky tussock tundra (Site 1), slightly disturbed upland tundra dominated by non-sorted circles (Site 2), wet polygonal tundra (Site 3), and hummocky tussock tundra dominated by nonsorted circles (Site 4). The main vegetation types were from Site 1, moss and cotton grass; Site 2, Arctic willow and *Dryas-Vetch*; Site 3, *Carex* and bryophytes as primary vegetation types; and Site 4, *Ledum palustre* and *Betula nana*. The different landscape types and the variability of soil properties in the landscape are described in detail by Siewert et al. [48].

Soil samples were collected from four horizons of permafrost-affected soil which included topsoil; cryoOM; subsoil; and permafrost. We collected samples according to protocol described by Schoeneberger et al. [49] and we employed additional methods to acquire soil samples from permafrost [50,51]. A detailed description of the sampling location and sampling protocol was described in our previous study [52].

2.2. Measurement of Environmental Factors

We dried and reweighed soil samples at $60\text{ }^{\circ}\text{C}$ for 48 h to determine the moisture content. Soil pH was measured by pH meter 3151i (Xylem incorporation GmbH, Hessen, Made in Germany) in soil suspension with a ratio of 1:2.5 (*w/v*). Total carbon (C_{tot}) and nitrogen (N_{tot}) content were determined from $60\text{ }^{\circ}\text{C}$ dried soil sample (8–10 mg) using an Elementar Vario Micro cube (Elementar, Langensfeld, Germany) and expressed in percentage. The carbon to nitrogen ratio (C/N ratio) was calculated by dividing C_{tot} with N_{tot}. The dissolved organic carbon (DOC) and dissolved nitrogen (DN) were analyzed

by mixing soil: water in a 1:5 ratio (w/v) and shaking on an orbital shaker (150 rpm) for an hour and the filtered soil solution (10–15 mL) was used for LiquiTOCII (Elementar, Germany) and expressed in $\mu\text{g g}^{-1}$ dry weight of soil.

2.3. Extracellular Enzymes Activities

Hydrolytic enzymes involved in degradation of organic molecules like cellulose, chitin, protein, and lignin were measured by microplate fluorometric assays according to Barta et al. [53]. We used a half gram of sieved soil suspended in 50 mL of distilled deionized nuclease-free water (ddH_2O) and ultrasonicated at low energy (120 W) for 4 min. Potential activities of β -glucosidase (BG), 1, 4- β -cellobiohydrolase (CBH), chitinase (NAG), and leucine aminopeptidase (LAP) were measured fluorometrically using 4-methylumbelliferyl-(MUF) and aminomethylcoumarin (AMC) as substrates (50–300 μM), respectively [54]. A 200 μL sample of the soil suspension and 50 μL substrate (β -D-glucopyranoside, N-cellobiopyranoside, phosphate, N-acetylglucosaminide, and L-leucine-7-amido-4-methyl coumarin, respectively) were pipetted into black microtiter plates in 3 analytical replicates. For each sample, a standard curve with methyl umbelliferyl was used for the calibration of β -glucosidase, cellobiohydrolase, chitinase, whereas aminomethylcoumarin was used for the calibration of leucine amino-peptidase. Plates were incubated in the dark for 30 min and the first fluorescence was measured at 465 nm emission at an excitation of 360 nm (Tecan Infinite F200 fluorimeter, Schoeller instruments, Prague-Kunratice, Czech Republic). Fluorescence was measured again after 60 and 120 min. Enzyme activities were measured nmol g^{-1} dry weight of soil h^{-1} .

2.4. DNA Extraction and Quantitative Assessment of Fungal Community by qPCR

We extracted total genomic DNA from all collected soil samples (approx. 0.25 g) using a DNeasy PowerSoil™ DNA Isolation Kit (Qiagen, Düsseldorf, Germany). Extracted DNA was stored at $-20\text{ }^\circ\text{C}$ for further use. The 18S rDNA was used to amplify total fungal abundance in the sample, each reaction was performed with 20 μL of reaction mixture containing 3 μL of DNA from soil samples. The fungal ribosomal gene was amplified using a nu-SSU-0817-5'/nu-SSU-1196-3' primer set [55]. We used melt curve and gel electrophoresis analysis to confirm the product specificity and amplicon size, respectively. Standards were made from 10-fold dilution of a known amount of purified PCR product obtained from *Aspergillus niger*. The qPCR assay was performed in two replicates for each sample, along with standard and control (non-template ddH_2O water).

2.5. Barcoded Amplicon Sequencing

Aliquots of DNA extracts were sent to the SEQme Company (Dobříš, Czech Republic) for the preparation of a library and sequencing using the MiSeq2500 platform. The Earth Microbiome Project (EMP) protocol was used for library preparation with modified universal primers ITS1F/ITS2 [56]. The fungal ITS1 region was extracted from reads using the ITSx algorithm [57]. Amplicons were trimmed to equal lengths (150bp) and fungal unique reads were grouped to zero-radius OTUs (zOTUs) using a UNOISE 3.0 algorithm [58,59], which also included the removal of potential chimeric sequences. The taxonomic assignment of each fungal zOTUs was performed using the BLAST algorithm (E-value = 0.001) in UNITE [60]. Raw sequencing data were deposited in the European Nucleotide Archive (ENA) under the PRJEB44296 study.

Species richness (Chao1), diversity (Shannon), and evenness (Simpson) were calculated using the “microbiome” package [61] in R 3.5.3 [62]. To determine if the specific functional groups of fungi differed between different horizons and tundra sites, we classified each zOTU into trophic modes and lifestyles using the fungal functional database FungalTraits [63].

2.6. Network Construction

To better understand the fungal communities' interaction across different horizons and tundra sites, we constructed the fungal ecological network by calculating all possible Spearman correlation coefficients between zOTUs. To increase the robustness of the ecological network, we used only those zOTUs that were present in more than 30% of the sample (each horizon and tundra sites), and relative proportions of less than 0.1% were also excluded from the analysis. Spearman's Rho between the pairwise zOTUs matrices were constructed using the "Hmisc" package [64] in R. The false discovery rate (FDR) controlling procedure was used to calculate the p -values for multiple testing [65]. A valid co-occurrence was considered to be robust if the absolute value of the Spearman correlation coefficient was either equal or greater than 0.6 or -0.6 and statistically significant if p -values < 0.01 . The cut-off correlation of 0.6 or -0.6 was chosen to increase the confidence for strong fungal interactions. Network images were generated in R with the help of the "igraph" package [66]. In the network, nodes represented zOTUs, whereas edges represented the correlation between nodes. We used the undirected network (where the edge has no direction) and the Fruchterman–Reingold layout. The topology properties of the co-occurrence networks, positive edge, negative edge, total node, average path length (APL), degree distribution (DD), average closeness (AC), average betweenness (AB), edge density (ED), diameter (D), clustering coefficient (CC), number of modules, and modularity (M) were calculated using the "igraph" package [66] in R. We also constructed a random network with the same node and edges from a real biological network to determine whether our biological networks were not random networks and represented the actual fungal interactions in soil. We used the "erdos.renyi.game" function from the igraph package to generate a thousand random networks and calculated APL, CC, and M.

Different nodes in the network play different topological roles. These topological roles can be described by two parameters. First is the within-module connectivity (Z_i) which describes how well a node is connected with other nodes within its own module. The second parameter is connectivity between modules (P_i) which suggests how well a node is connected to different modules. The threshold values of Z_i and P_i for categorizing nodes into different topological roles are 2.5 and 0.62, respectively, according to previous studies [67–70]. In general, the topological role of each node subdivides into four categories according to pollination networks [70]. These categories are: (1) peripheral nodes (specialist), which have low Z_i (< 2.5) and P_i values (< 0.62) (i.e., they have only a few edges that are always connected to the node within their modules); (2) connectors (generalist), which have a low Z_i (< 2.5) but a high P_i value (> 0.62) (i.e., these nodes tend to have more connections with several modules); (3) module hubs (generalist), which have a high Z_i (> 2.5) but a low P_i value (< 0.62) (i.e., these are the nodes which have more connections with other nodes but within their own modules); (4) network hubs (supergeneralist), which have both high Z_i (> 2.5) and P_i (> 0.62) values (i.e., they are connector and module hubs). The generalists (connectors, module hubs) and supergeneralist (network hubs) are considered the key microorganisms (keystone), which maintain network stability and play pivotal roles [71].

2.7. Statistical Analyses

The difference in environmental factors, fungi gene copies, and α -diversity indices were assessed using one-way ANOVA and followed by Tukey's HSD post hoc test. A significant difference was considered at $p < 0.05$ unless indicated otherwise. However, we provide precise p -values wherever possible. We performed Spearman correlation of the log-transformed environmental factors with network modules (top five) and keystone taxa (identified from the Z_i - P_i plot) using the "Hmisc" package [64] in R. A permutational analysis of variance (PERMANOVA) test was used to evaluate the linkage between fungal community composition and environmental factors using the Bray–Curtis dissimilarity matrix. The PERMANOVA test was performed by the "adonis" function in the R package "vegan" [72]. The best environmental factors explaining the fungal community composition

were determined by the forward selection method. STAMP software was used to identify the difference in the mean proportion of genera and lifestyle between different horizons and tundra sites [73].

3. Results

3.1. Environmental Variables

In general, the soil samples from the topsoil had significantly greater moisture, DOC, Ctot, Ntot, and C/N ratio and followed the order topsoil > cryoOM > subsoil > permafrost. In contrast, the DN was significantly lower in the topsoil compared to other horizons. The soil samples from cryoOM had significantly greater moisture, Ctot, and Ntot compared to those from the surrounding mineral subsoil. In comparison to other horizons, the permafrost samples had the highest values for pH and DN (Table 1). The enzymatic activity of BG and LAP was significantly greater in the topsoil and decreased in the order of topsoil > cryoOM > subsoil > permafrost. The CBH and NAG activities were similar between topsoil and cryoOM, and both horizons had significantly greater activities of these enzymes than subsoil and permafrost (Table 1).

The individual horizon also had significant differences between each tundra site, the topsoil from Site 2 had significantly lower moisture, but significantly higher pH, BG, CBH, and LAP (Table S1). For cryoOM, the only significant difference between different tundra sites was found for pH and C/N ratio, Site 2 significantly had the highest pH value whereas Site 4 had, significantly, the highest C/N ratio. The lower mineral subsoil had a significant difference between the tundra sites for pH, DN, C/N ratio, CBH, and NAG.

3.2. Fungal Gene Abundance, Community Composition, and Diversity Differed between Horizons and Sites

Fungal 18S rRNA gene abundance was determined by quantitative PCR (qPCR), in total 104 samples were successfully amplified from 122 soil samples (Figure 1a,b). Average fungal SSU gene copies per gram of dry soil per individual soil horizon decreased in order: topsoil ($5.7 \pm 11.5 \times 10^9$) > subsoil ($2.2 \pm 8.3 \times 10^8$) > cryoOM ($1.9 \pm 9.2 \times 10^8$) > permafrost ($1.7 \pm 2.0 \times 10^6$), whereas Site 1 had a significantly higher fungal gene abundance ($9.1 \pm 8.2 \times 10^9$) compared to the other sites.

The complete data set of fungal composition contained 858,309 filtered sequences, in which 3199 zero radius OTU (zOTUs) were affiliated to 11 fungal phyla (Table S2). Those phyla which had at least 1% of relative proportion were: Ascomycota, Basidiomycota, Mortierellomycota, and Rozellomycota.

In total, we identified 366 genera, 24 of which had more than 1% relative proportion (Figure 1c,d, Table S2). The most dominant genera belonged to the phyla Ascomycota, Basidiomycota, and Mortierellomycota. The root-associated genus *Lachnum* and endophytic fungus genus *Phialocephala* had a significantly greater mean proportion in topsoil, whereas the ectomycorrhizal genus *Russula* had a greater mean proportion in cryoOM compared to all other horizons (Welch's *t*-test, two-sided, $p < 0.05$, Figure S1). Individual tundra sites also differed significantly at genera levels (Figure 1d). For example, the genus that had the greatest mean proportion included ectomycorrhizal genus *Amphinema* from Site 1, soil saprotrophic genus *Oidiodendron* from Site 2, unspecified saprotrophic genus *Rhodotorula* from Site 3, and root endophytic genus *Meliniomyces* from Site 4 (Welch's *t*-test, two-sided, $p < 0.05$, Figure S1).

Table 1. Soil environmental factors in each horizon. Averages and standard deviation were shown. The significant difference between different horizons within all tundra sites were calculated using One-Way ANOVA and followed by a Tukey's HSD test. Different letters in the brackets indicated a significant difference between tundra sites.

Site	Horizon	N	Moisture (%)	pH	DOC (ug/g dw)	DN (ug/g dw)	Ctot (%)	Ntot (%)	C/N ratio	BG (nmol MUF g ⁻¹ dw h ⁻¹)	CBH (nmol MUF g ⁻¹ dw h ⁻¹)	LAP (nmol MUF g ⁻¹ dw h ⁻¹)	NAG (nmol MUF g ⁻¹ dw h ⁻¹)
Site 1	Topsoil	9	76.6 ± 3.45 (a)	6 ± 0.22 (a)	751.08 ± 308.98 (a)	2.56 ± 0.9 (b)	40.06 ± 3.43 (a)	1.15 ± 0.22 (a)	44.76 ± 14.8 (a)	1624.9 ± 294.78 (a)	239.86 ± 76.91 (a)	186.85 ± 79.3 (a)	400.23 ± 83.42 (a)
		7	50.89 ± 6.91 (b)	6.35 ± 0.19 (a)	382.47 ± 84.75 (a)	8.3 ± 3.38 (ab)	11.87 ± 3.95 (b)	0.77 ± 0.24 (ab)	15.11 ± 0.57 (a)	441.33 ± 375 (b)	101.23 ± 107.23 (a)	74.09 ± 66.4 (a)	144.61 ± 40.54 (b)
	Subsoil	5	30.44 ± 3.31 (c)	5.81 ± 0.05 (a)	205.97 ± 63.69 (a)	9.92 ± 1.22 (a)	4.63 ± 1.11 (b)	0.33 ± 0.07 (b)	13.48 ± 0.64 (b)	75.32 ± 26.19 (b)	13.47 ± 3.88 (a)	15.39 ± 3.48 (a)	76.08 ± 22.59 (b)
		2	55.2 ± 5.89 (ab)	5.93 ± 0.18 (a)	695.72 ± 187.2 (a)	7.68 ± 1.56 (ab)	8.2 ± 0.33 (b)	0.56 ± 0.03 (ab)	14.78 ± 0.25 (b)	115.51 ± 7.6 (b)	13.16 ± 3.89 (a)	10.67 ± 4.41 (a)	73.9 ± 10.39 (b)
Site 2	Topsoil	8	54.51 ± 4.08 (a)	7.49 ± 0.29 (b)	466.72 ± 142.64 (a)	3.19 ± 1.46 (b)	29.57 ± 4.66 (a)	1.27 ± 0.13 (a)	22.93 ± 2.25 (b)	1595.35 ± 381.81 (a)	268.63 ± 72.32 (a)	704.2 ± 140.03 (a)	389.05 ± 174.6 (a)
		2	54.26 ± 2.53 (a)	8.05 ± 0.11 (ab)	250.78 ± 11.86 (a)	2.64 ± 0.48 (b)	16.13 ± 1.29 (b)	1.03 ± 0.03 (a)	15.6 ± 0.75 (b)	263.6 ± 96.89 (b)	36.63 ± 18.79 (b)	128.23 ± 24.4 (b)	109.24 ± 2.79 (ab)
	Subsoil	7	23.86 ± 3.87 (b)	8.12 ± 0.23 (ab)	326.18 ± 65.47 (a)	19.58 ± 6.76 (ab)	4.15 ± 0.71 (c)	0.22 ± 0.06 (b)	21.22 ± 2.2 (b)	21.58 ± 17.59 (b)	1.56 ± 1.33 (b)	77.66 ± 45.09 (b)	6.66 ± 5.14 (b)
		9	29.04 ± 4.5 (b)	8.24 ± 0.3 (a)	374.77 ± 134.22 (a)	31.19 ± 11.31 (a)	3 ± 0.13 (c)	0.1 ± 0.01 (b)	29.52 ± 1.76 (a)	1.02 ± 0.47 (b)	0.15 ± 0.12 (b)	25.35 ± 4.21 (b)	0.58 ± 0.37 (b)
Site 3	Topsoil	21	78.32 ± 4.17 (a)	5.94 ± 0.38 (a)	5104.09 ± 2839.27 (a)	14.5 ± 7.45 (a)	30.2 ± 4.74 (a)	1.74 ± 0.25 (a)	17.93 ± 3.19 (a)	690.13 ± 399.39 (a)	90.1 ± 75.82 (a)	94.67 ± 73.1 (a)	241.01 ± 116.29 (a)
		5	54.11 ± 9.86 (b)	6.11 ± 0.39 (a)	1066.41 ± 719.77 (a)	22.09 ± 9.95 (a)	14.57 ± 3.26 (b)	1.06 ± 0.2 (b)	13.72 ± 1.6 (a)	175.59 ± 108.25 (a)	20.53 ± 13.51 (a)	16.18 ± 4.58 (a)	119.29 ± 31.8 (a)
	Permafrost	1	60.4	5.61	451.92	6.87	14.64	1.22	11.97	206.35	25	12.02	46
		12	75.57 ± 6.73 (a)	5.35 ± 0.42 (b)	2023.25 ± 628.9 (a)	6.86 ± 3.88 (c)	37.97 ± 6.07 (a)	1 ± 0.26 (a)	44.73 ± 12.06 (a)	1336.83 ± 371.99 (a)	204.45 ± 77.38 (a)	59.27 ± 40.06 (a)	271.86 ± 110.15 (a)
Site 4	CryoOM	16	59.85 ± 9.11 (a)	5.93 ± 0.23 (b)	1588.26 ± 1028.78 (a)	13.9 ± 4.18 (bc)	17.44 ± 3.81 (b)	0.87 ± 0.12 (a)	19.68 ± 2.59 (b)	473.34 ± 422.09 (b)	110.9 ± 134.8 (a)	53.8 ± 25.98 (a)	316.64 ± 104.08 (a)
		10	29.9 ± 6.03 (b)	5.91 ± 0.37 (b)	442.22 ± 138.73 (a)	38.54 ± 10.32 (b)	4.23 ± 0.98 (c)	0.22 ± 0.04 (b)	18.65 ± 2.12 (b)	99.28 ± 55.15 (b)	11.69 ± 4.79 (a)	35.73 ± 9.39 (a)	37.7 ± 23.6 (b)
	Permafrost	8	38.1 ± 11.77 (b)	7.32 ± 0.56 (a)	671.93 ± 477.72 (a)	100.06 ± 24.95 (a)	3.17 ± 0.64 (c)	0.21 ± 0.04 (b)	15.22 ± 0.88 (b)	60.28 ± 52.38 (b)	7.04 ± 7.78 (a)	27.92 ± 9.69 (a)	36.17 ± 33.42 (b)

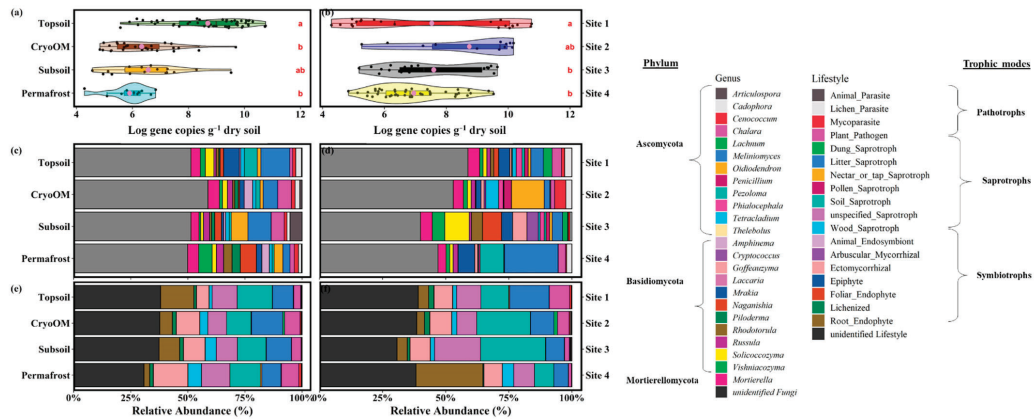


Figure 1. Fungi gene abundance and community composition. Log-transformed fungal gene copies per gram dry weight of soil are shown for (a) horizon and (b) tundra site. Based on Tukey’s HSD post hoc tests, gene abundance that differed between horizons and tundra sites was represented by different letters. The lavender point inside the bar plot suggested a mean value. The relative fungal taxonomic composition at genus level and fungal lifestyle for (c,e) horizon and (d,f) tundra sites were shown, respectively. Only those genera which had >1% relative proportion and filled to 100% were shown, whereas all fungal lifestyles were depicted.

Using the fungal functional database FungalTraits, we were able to assign those zOTUs that were classified into genera to trophic modes (i.e., pathotrophs, saprotrophs, and symbiotrophs) (Figure 1e,f, and Table S2). In total, we were able to assign 56.6% of zOTUs to trophic modes. Of these, roughly one-third of the assigned zOTUs, pathotrophic, saprotrophic, and symbiotrophic fungi accounted for approximately 8.4%, 29.7%, and 18.4%, respectively, on average. The pathotrophs were mainly dominated by the plant pathogens and their proportion was significantly lower in topsoil (Welch’s *t*-test, two-sided, $p < 0.05$, Figure S2). The root endophytes had a greater mean proportion in topsoil compared to other horizons, however, this difference was nonsignificant. We found a significantly greater mean proportion of ectomycorrhizal and wood saprotrophs in cryoOM compared to topsoil (data not shown). We did not find any significant difference in fungal trophic modes between cryoOM and subsoil. The relative proportion of plant-pathogen and litter saprotrophs decreased from Site 1 to Site 4, whereas the relative proportion of soil saprotrophs increased from Site 1 to Site 3 (Figure 1f). We found a significantly greater mean proportion of litter saprotrophs, plant pathogen, and lichenized trophic modes in Site 1 compared to all other sites (Welch’s *t*-test, two-sided, $p < 0.05$, Figure S2). On the other hand, Site 2 and Site 3 had a significantly greater mean proportion of plant-pathogen and lichenized and soil saprotrophs, respectively.

The alpha diversity index suggested that fungal communities from topsoil were more rich (nonsignificant chao1 index) but significantly less evenly (Simpson evenness index) distributed compared to other horizons and the opposite was true for cryoOM (Table S3). Tundra sites also significantly differed for alpha diversity indices, Site 1 had significantly higher richness and diversity whereas Site 4 had the lowest.

We performed a permutational multivariate analysis of variance (PERMANOVA) to determine the effect of different horizon and tundra sites, both had a significant effect on fungal community composition (Figure 2). We found a stronger site (F-Model = 6.9, $R^2 = 0.15$, p -value = 0.001) effect on fungal beta diversity than the horizon effect (F-Model = 1.9, $R^2 = 0.05$, p -value $< 1 \times 10^{-4}$). Topsoil samples were clustered close to

The number of nodes (zOTUs) that were significantly correlated was the highest in the topsoil samples (476), however, the number of significant correlations between zOTUs was greatest in the subsoil samples (3426). The number of total zOTUs after the abundance filtration was highest from Site 1 and lowest from Site 4. The higher number of zOTUs from Site 1 also accounted for a more connected co-occurrence network. We found Site 2 had a greater number of zOTUs compared to Site 4 but a considerably smaller number of significant correlations between zOTUs.

Co-occurrence network complexity is generally measured by DD and CC indexes. We found considerably different DD and CC indexes from the individual horizons. The higher the DD value, the more complex the network. Hence, the DD value suggested that the ecological network became more complex from the topsoil to the subsoil. The CC was highest in the cryoOM network compared to other horizons which suggested that the node's neighbors were also connected in the cryoOM network. The DD values implied that the co-occurrence network from Site 1 was more complex, whereas the CC value indicated that the Site 4 network was more connected.

All generated networks were modular, as suggested by their modularity values which were higher than the suggested threshold value of 0.4 for modular structure [37] and higher than the corresponding random network (Table 2). A total of 31, 21, and 12 modules were obtained for topsoil, cryoOM, and subsoil, respectively, and 21, 40, 27, and 18 modules for Site 1, Site 2, Site 3, and Site 4, respectively. The relative proportion of the top five modules for each horizon and tundra site network at the trophic mode's level is given in Figure 3. The top two modules (T1 and T2) from topsoil had a higher relative proportion of ectomycorrhizal, whereas C3 and C1 modules from the cryoOM co-occurrence network had a great relative proportion of litter saprotrophs and root endophytes, and dung and soil saprotrophs, respectively. The biggest module (97 nodes) in all horizons, S3, had a high relative proportion of soil saprotrophs, ectomycorrhizal, and root endophytes. In comparison to the horizons, the modules from the tundra site co-occurrence network were relatively smaller, except for Site 1 which had bigger modules. The biggest modules from individual tundra sites (S1-1, S3-1, and S4-2) had a greater relative proportion of ectomycorrhizal. Overall, the network structure was dramatically different between each horizon and tundra site, and also the shared nodes between them.

The shared nodes (zOTUs, identified from the co-occurrence network only) between horizons were lower compared to the unique nodes for the individual horizon networks (Figure S3). Whereas there were only four nodes shared between the individual tundra sites, Site 1 had the highest number of unique nodes (311).

We observed significant correlations between the network modules (top five only) and environmental variables (Figure 4). In topsoil modules, modules T1 and T12 which had a high relative proportion of ectomycorrhizal and litter saprotrophs, respectively, had a strong positive correlation with moisture and a significant negative correlation with NAG. Other than moisture, dissolved nutrients (DOC and DN) had a positive correlation and C enzyme (BG and CBH) activity had a negative correlation with topsoil module T1, whereas modules C1 and C2 from cryoOM had a strong positive correlation with pH. Ectomycorrhizal, which had a high relative proportion in module C4, was positively affected by NAG and negatively affected by BG and CBH. In the subsoil, soil saprotrophs, ectomycorrhizal, and root endophytes had a great relative proportion in module S3, and a significant positive correlation with pH and negative with DOC. The total number of significant correlations was highest in permafrost. A strong significant positive correlation was found between ericoid mycorrhizal comprised module P4 and both moisture and C/N ratio, whereas a negative correlation was observed with Ntot, BG, CBH, and NAG.

Table 2. Major topological properties of the empirical networks of soil fungal communities in different horizons and tundra sites, and their associated random network.

	Horizon	Topsoil	CryoOM	Subsoil	Permafrost	Site 1	Site 2	Site 3	Site 4
Total zOTUs ^a		558	413	479	479	688	458	324	212
Abundance (%) ^b		61.13	68.22	63.22	71.06	75.97	67.33	65	71.36
Total significant correlations		3054	2454	3426	1110	6886	666	1152	814
Total node		476	340	437	281	643	267	246	130
Total edge		1527	1227	1713	555	3443	333	576	407
Positive edge		1527	1216	1699	553	3277	331	568	407
Negative edge		0	11	14	2	166	2	8	0
Average path length (APL)		7.12	5.3	5.42	7.61	5.72	7.2	5.4	5.23
Degree distribution (DD)		6.42 ± 7.88	7.22 ± 7.05	7.84 ± 6.05	3.95 ± 3.3	10.71 ± 9.83	2.49 ± 1.89	4.68 ± 4.66	6.26 ± 7.64
Average closeness (AC)		-4.42 ± 0.28	-4.16 ± 0.28	-3.82 ± 0.21	-4.5 ± 0.24	-4.33 ± 0.25	-4.67 ± 0.16	-4.23 ± 0.27	-3.82 ± 0.23
Average betweenness (AB)		1225.6 ± 2195.08	606.04 ± 1405.7	923.8 ± 1432.28	494.75 ± 1148.79	1404.42 ± 1920.9	246.18 ± 597.43	353.79 ± 728.76	153.46 ± 332.88
Edge density (ED)		0.0135	0.0213	0.018	0.0141	0.0167	0.0094	0.0191	0.0485
Diameter (D)		20	16	15	20	17	19	17	17
Clustering coefficient (CC)		0.24	0.34	0.23	0.26	0.18	0.13	0.23	0.3
Number of modules		31	21	12	34	21	40	27	18
Modularity (M)		0.72	0.68	0.73	0.82	0.69	0.85	0.65	0.38
Average path length (APL)		3.52 ± 0.008	3.16 ± 0.007	3.18 ± 0.005	4.2 ± 0.042	2.44 ± 0.001	3.64 ± 0.022	2.7 ± 0.005	2.17 ± 0.005
Clustering coefficient (CC)		0.01 ± 0.002	0.02 ± 0.003	0.02 ± 0.002	0.01 ± 0.004	0.03 ± 0.001	0.02 ± 0.004	0.04 ± 0.003	0.1 ± 0.005
Modularity (M)		0.32 ± 0.01	0.3 ± 0.011	0.28 ± 0.01	0.44 ± 0.016	0.15 ± 0.004	0.38 ± 0.014	0.26 ± 0.011	0.21 ± 0.012

^a total zOTUs left after filtration, ^b total abundance of filtrated zOTUs, ^c same number of nodes and edges from empirical networks were used to calculated random network.

We found only three significant correlations between the network’s module and environmental factors from Site 1. These correlations included modules S1-5 and S1-7, which had a great relative proportion of unspecified saprotrophs and litter saprotrophs, negatively correlated with pH and positively correlated with Ctot and CBH, respectively. Modules S2-1 and S2-2 from Site 2 had a significantly negative correlation with Ctot, Ntot, BG, CBH, LAP, and NAG and a significantly positive correlation with pH and DN. We found a significant positive correlation between ectomycorrhizal comprised module S3-1 and pH and C/N ratio. Module S4-2, S4-3, S4-5, and S4-4 from Site 4 had a significant negative correlation with C/N ratio, BG, and CBH and the same environmental factor had a positive correlation with module S4-1 which had a great relative proportion of root endophytes.

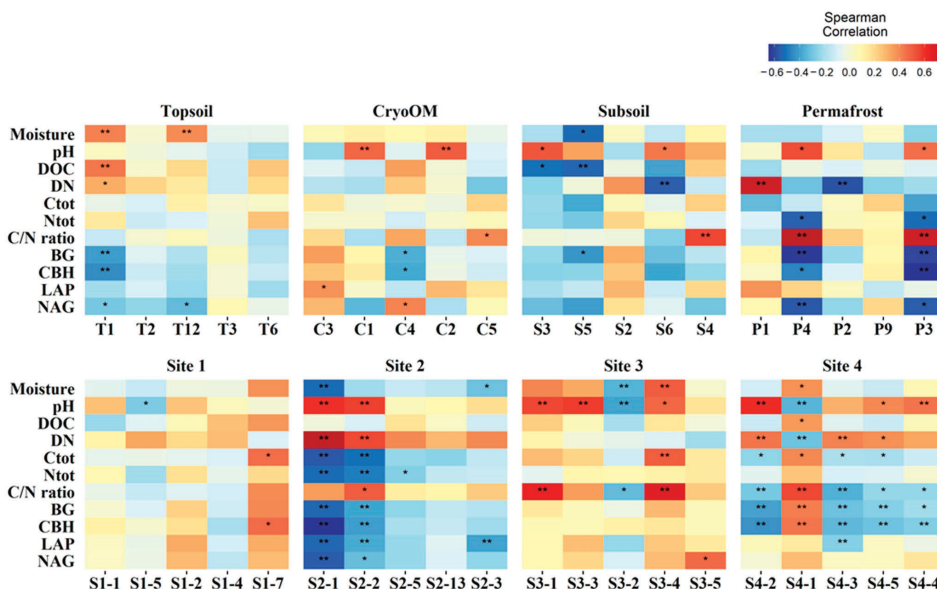


Figure 4. Spearman rank correlation coefficients of soil environmental factors and network modules (top five) for individual horizons and tundra sites. The reds represented a positive correlation and the blue represented a negative correlation. The heatmap cells marked by “*” or “**” were statistically significant: * p -value < 0.05 and ** p -value < 0.01.

3.4. The Topological Roles of Nodes and Generalist-Specialist Shift

The topological roles of the nodes in networks were identified from the Zi-Pi plot (Figure 5), by plotting the within-module connectivity (Zi) and among-module connectivity (Pi) proposed by [74] and simplified by [70]. All nodes fell into four categories (peripherals, module hubs, network hubs, and connectors). We found that most nodes (97.2%, 98.2%, 98.1%, 100% for topsoil, cryoOM, subsoil, and permafrost, respectively) were peripherals that had a connection to other nodes but only in their own modules. Among them, 77.3% (topsoil), 76.1% (cryoOM), 65.4% (subsoil), and 57.3% (permafrost) of the peripherals had no edge outside of their own module (i.e., $P_i = 0$). In total, we found 11 nodes as connectors and 17 nodes as module hubs. A total of 4, 1, and 6 connectors and 9, 5, and 2 module hubs were found for topsoil, cryoOM, and subsoil networks, respectively. We did not find supergeneralists in any of the horizon’ networks. Detailed taxonomic information for the topological role is given in Table S4.

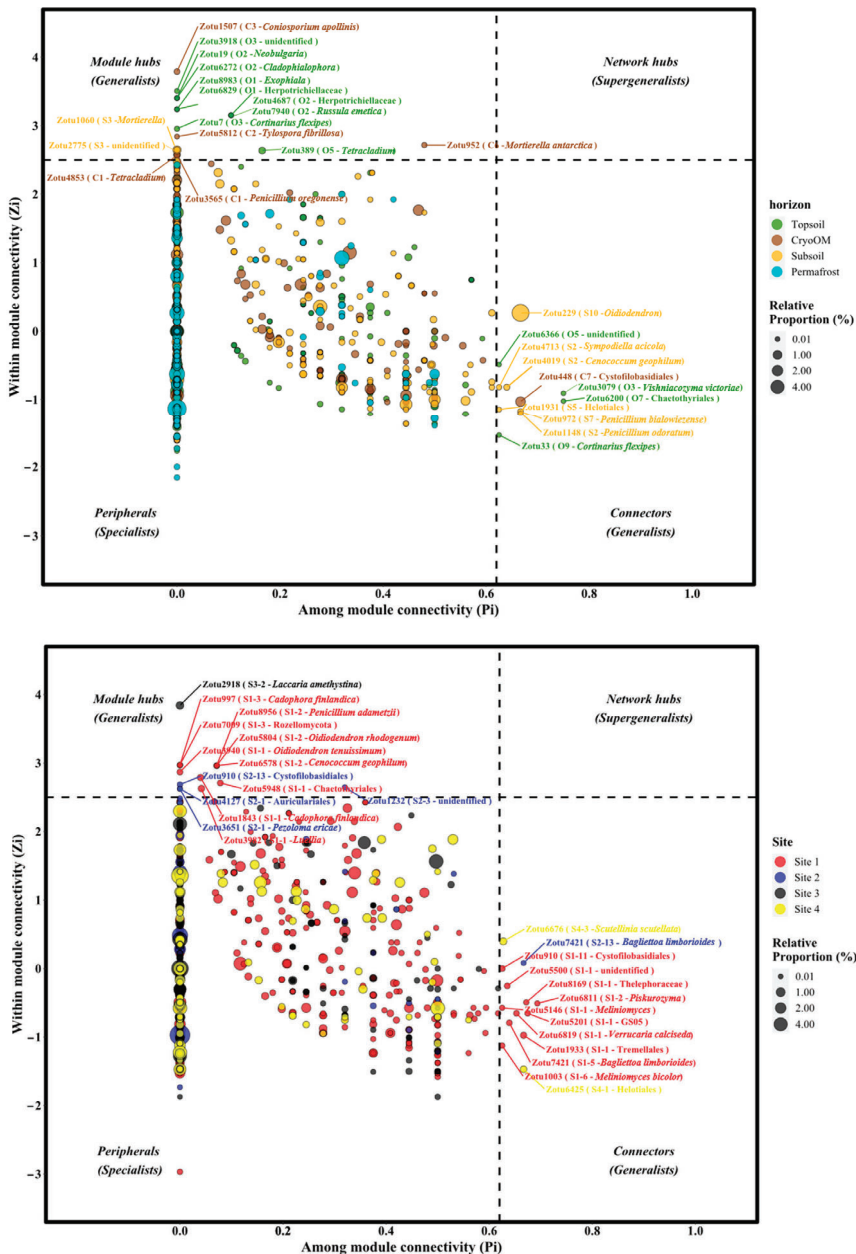


Figure 5. Zi-Pi plot showing topological roles of nodes in different horizons and tundra sites. The threshold values of Zi and Pi for categorizing nodes were 2.5 and 0.62, respectively. Generalists (connectors and module hubs) were labeled with zOTU IDs, module number, and maximum assigned taxonomy (bolded letters). Generalists were colored according to horizons and tundra sites and the size of each node represented the abundance of that node.

Similar to the horizon's Zi-Pi plot, the Zi-Pi plot from the tundra site identified most of the nodes as a peripheral and a great number of those nodes (57.5%, 92.5%, 75.2%, and 68.4% for Site 1, Site 2, Site 3, and Site 4, respectively) did not have an edge outside their own module (Figure 5). Site 1 had the highest, and Site 3 had the lowest number of generalists (module hubs and connectors). In total, we found 10 connectors and 9 module hubs from Site 1.

It is also worth mentioning that some nodes were identified as generalists in one horizon but played the role of specialist (peripheral) in other horizons (Table 3 and Table S5). For instance, in topsoil, generalists included zOTU6200 (unidentified Chaetothyriales), zOTU4687 (unidentified Herpotrichiellaceae), and zOTU6829 (unidentified Herpotrichiellaceae), however, these zOTUs were found as specialists in other horizons. Similarly, generalists from cryoOM included zOTU952 (*Mortierella antarctica*), zOTU3565 (*Penicillium oregonense*), and zOTU4853 (unidentified *Tetracladium*), but these were observed as specialists in other horizons. Subsoil generalists included zOTU1148 (*Penicillium odoratum*) and zOTU2775 (unidentified Fungi), while these zOTUs were identified as specialists in other horizons. Similar to the horizon, we also found some zOTUs that identified as a generalist for one site but specialist for other sites, for instance, zOTU1843 (*Cadophora finlandica*) was identified as a generalist from Site 1 (module hubs) but specialist from all other sites (Table 4 and Table S5).

Table 3. Topological role shift between different horizons. Module hubs and connectors were considered as generalists whereas peripheral as specialists.

zOTUs ID	Genera	Species	Lifestyle	Topsoil	CryoOM	Subsoil	Permafrost
Zotu6272	<i>Cladophialophora</i>	unidentified	Soil_Saprotroph	Module hubs	Peripheral	Peripheral	-
Zotu7	<i>Cortinarius</i>	<i>Cortinarius flexipes</i>	Ectomycorrhizal	Module hubs	-	-	Peripheral
Zotu952	<i>Mortierella</i>	<i>Mortierella antarctica</i>	Soil_Saprotroph	Peripheral	Module hubs	Peripheral	Peripheral
Zotu1060	<i>Mortierella</i>	unidentified	Soil_Saprotroph	Peripheral	Peripheral	Module hubs	-
Zotu19	<i>Neobulgaria</i>	unidentified	Wood_Saprotroph	Module hubs	-	Peripheral	Peripheral
Zotu229	<i>Oidiodendron</i>	unidentified	Soil_Saprotroph	-	Peripheral	Connectors	Peripheral
Zotu972	<i>Penicillium</i>	<i>Penicillium bialowiezense</i>	unspecified_Saprotroph	Peripheral	-	Connectors	Peripheral
Zotu1148	<i>Penicillium</i>	<i>Penicillium odoratum</i>	unspecified_Saprotroph	Peripheral	Peripheral	Connectors	Peripheral
Zotu3565	<i>Penicillium</i>	<i>Penicillium oregonense</i>	unspecified_Saprotroph	Peripheral	Module hubs	Peripheral	Peripheral
Zotu7940	<i>Russula</i>	<i>Russula emetica</i>	Ectomycorrhizal	Module hubs	-	Peripheral	-
Zotu4713	<i>Sympodiella</i>	<i>Sympodiella acicola</i>	Litter_Saprotroph	-	-	Connectors	Peripheral
Zotu389	<i>Tetracladium</i>	unidentified	Litter_Saprotroph	Module hubs	-	-	Peripheral
Zotu4853	<i>Tetracladium</i>	unidentified	Litter_Saprotroph	Peripheral	Module hubs	Peripheral	Peripheral
Zotu5812	<i>Tylospora</i>	<i>Tylospora fibrillosa</i>	Ectomycorrhizal	-	Module hubs	Peripheral	-
Zotu3079	<i>Vishniacozyma</i>	<i>Vishniacozyma victoriae</i>	Soil_Saprotroph	Connectors	-	-	Peripheral

Table 4. Topological role shift between different tundra sites. Module hubs and connectors were considered as generalists whereas peripheral as specialists.

zOTUs ID	Genera	Species	Lifestyle	Site 1	Site 2	Site 3	Site 4
Zotu1843	<i>Cadophora</i>	<i>Cadophora finlandica</i>	Litter_Saprotroph	Module hubs	Peripheral	Peripheral	Peripheral
Zotu6578	<i>Cenococcum</i>	<i>Cenococcum geophilum</i>	Ectomycorrhizal	Module hubs	Peripheral	-	-
Zotu3982	<i>Luellia</i>	unidentified	Wood_Saprotroph	Module hubs	Peripheral	Peripheral	-
Zotu5146	<i>Meliniomyces</i>	unidentified	Root_Endophyte	Connectors	Peripheral	Peripheral	-
Zotu6819	<i>Verrucaria</i>	<i>Verrucaria calciseda</i>	Lichenized	Connectors	Peripheral	-	-

The fungal zOTU role shift may be attributed to different environmental factors. We found distinct correlation patterns between generalists and specialists with environmental factors from different horizons (Table S6) and tundra sites (Table S7). The distinct correlations, which to a certain extent indicated that the dominant factors shaping fungal networks were specific to each horizon and tundra site, and potentially change or shift the role of generalist–specialist (topological role shift).

In general, the fungal ecological networks in the topsoil, subsoil, and Site 1 contained more keystone taxa (generalists) than those in other horizons (cryoOM and permafrost) and sites (Site 2, Site 3, and Site 4), which may lead to a more effective organization of taxa connections in the network as they are regulated by more connectors and module hubs.

4. Discussion

Several studies have reported that high fungal diversity has a positive effect on ecosystem functioning, and a loss of fungal diversity can alter the ecosystem functioning, with changes such as lower enzyme activities and litter decomposition rates [75,76]. Additionally, soil fungi have specific substrate preferences and acquisition strategies. Hence, each of the soil fungi comprises different lifestyles and functions [77], and ultimately they form complex interactions with each other (i.e., competition, mutualism, predation, parasitism). These complex interactions determine the overall fungal community structures and stability [27,70,78]. In this study, we constructed a fungal co-occurrence network of different horizons and tundra sites based on high-throughput sequencing data of the fungal ITS region. Previous studies have reported differences between the fungal community structure of organic and mineral soils, for instance, a study from the high Arctic found a more diverse fungal community from the organic horizon than the mineral subsoil [8,79]. Only one previous study has focused on the fungal community composition from cryoOM soil [11]. Studies on the co-occurrence of microbial networks, on the other hand, provide essential information regarding the interaction between species in complex soil ecosystems. In this study, we constructed an ecological network of fine-scale taxonomy and identified important fungal interactions in the PAS.

4.1. Horizon and Tundra Specific Fungal Lifestyle

Differences in the soil fungal community across distinct horizons and tundra vegetation were apparent at the genera and lifestyle level, which suggest significant changes occur in the entire fungal community with depth and supports the theory that at least some degree of ecological coherence exists among different fungal lifestyles [80].

Our study showed that symbiotrophs are the most abundant functional lifestyle in PAS which is in agreement with studies from other ecosystems [22,81,82]. We also found that root endophytes had a greater relative proportion in topsoil compared to other horizons, however, not significant (Figure 1 and Figure S4). Root endophytes are plant-associated fungi that reside within plant tissues or grow inside roots, stems, or leaves, and they have been previously studied and isolated from Arctic vascular plants [83–85]. They have been shown to play an important role in the nutrient cycle of the other natural ecosystem, including the decomposition of Norway spruce needles [86,87]. Some species of endophytes exhibit functions morphologically and phylogenetically similar to saprotrophs and produce leaf degrading enzymes [88]. We, for instance, found the dark septate endophytic genus *Phialocephala* to have a significantly greater mean proportion in topsoil compared to other horizons (Figure 1 and Figure S2). Members of this genus utilize proteins as a sole nitrogen source [89], mineralize organic nitrogen in the rhizosphere [90], and potentially decompose SOM [91]. On the other hand, their relatively significant presence in the deeper layers of the PAS shows that endophytic fungi are not strictly tied to life inside plant tissues, but instead can migrate over relatively long distances in the soil, where they can participate in the decomposition of complex organic matter.

Recent studies show that other symbiotrophs such as mycorrhizal fungi can be viable competitors for saprotrophic fungi [92,93], but only under certain conditions. Due to their symbiotic plant friends, they gain a greater competitive advantage under C-limiting conditions in which the plant “pumps” its own C to them, which is used in part by the mycorrhizal fungus for the synthesis of extracellular enzymes [94,95]. These will help it “win” over the saprotrophic fungus. In this fight, mycorrhizal fungi have a competitive advantage where roots are present, but in deeper soil where roots are absent and mostly recalcitrant SOM dominates, it can be inhabited by litter saprotrophs. This seemingly minor

battle can have a major impact on soil organic matter transformation in PAS. In our study, however, we found a significantly greater proportion of mycorrhizas in deeper soil of PAS in comparison to upper topsoil (Figure 1 and Figure S2). This can be explained either by the fact that plants root deeper on Herschel Island, which has not been confirmed, or that fungi in temperate ecosystems, known as mycorrhizal fungi, have multiple life strategies in the Arctic and can survive without a host in deeper horizons and feed saprotrophically. The litter saprotrophs are more efficient than mycorrhizal in colonizing and utilizing fresh, energy-rich compounds [96,97]. However, as the C/N ratio and available energy decrease with soil depth [98,99], saprotrophs might become less competitive, and be replaced by mycorrhizal fungi that do not depend on litter-derived energy in deep soil horizons [100]. We hypothesize that the energy and nutrient-demanding extracellular enzymes synthesized by mycorrhizal fungi utilize nutrient-rich compounds (mainly organic N), but because a large part of this N is then transported to plant symbiont, they remove N from the soil and leave C-rich and nutrient-poor substrates behind. Therefore, it may result in inadequate nutrient availability for the saprotrophic fungi in deeper soil, reducing SOM decomposition and potentially increasing C in PAS soil [101,102]. A previous study also reported that the C in cryoOM was thousands of years old and the decomposition process rate was slower and was three times older than the C in topsoil horizons [103]. CryoOM in soils is considered highly N-limited [99,104] and the greater proportion of ectomycorrhizal fungi which are known to have a less efficient enzyme activity [100,105] compared to saprotrophs can exacerbate this limitation and potentially increase C storage in PAS. We argue that the “role shift” of mycorrhizal lifestyle in topsoil to a more saprotrophic lifestyle in deeper soil horizons can affect the vulnerability of C in PAS.

4.2. Co-Occurrence Networks Reveal More Complex Interactions in Deeper Soil Horizons

The analysis of co-occurrence patterns can provide a vivid and simplified version of the interactions in complex fungal communities. Moreover, it offers an in-depth insight into ecological assembly from different horizons and sites.

We found that fungal assemblages in topsoil formed a less complex network compared to those in the subsoil, even with the highest number of nodes and significantly higher fungal gene abundance among all horizons. Topsoil in the Arctic is experiencing extreme changes (i.e., a higher fluctuation temperature and nutrient cycling) compared to deeper soil horizons. This may have forced selective pressure on the fungal communities, which was also evidenced by the high fungal richness but unevenly distributed fungal communities (Table S3). This was reflected in a less connected network in topsoil compared to the subsoil. A relationship between species richness, diversity, and network connectivity has been previously observed [30,106]. It was suggested that microbial diversity decreases as network size and connectivity increase. Furthermore, an increased network complexity with increased soil depth (in subsoil) for bacterial and fungi were previously observed in a grassland study [107]. We hypothesize that the more densely connected network of fungal communities in deeper soil horizons is due to the oligotrophic environment of these horizons, where different groups of fungi must compete or cooperate to obtain nutrients that are in short supply. This may be due to the decreased direct input of root exudates and possible metabolic recalcitrant byproducts that remain in the lower soil horizons. These conditions could generate more competition or co-metabolism due to the lower quality and quantity of substrate available in deeper soil horizons. In support of this idea, negative correlation, which suggests co-exclusion between two taxa, increased from the topsoil to the subsoil network (Table 2). This trend may indicate a more competitive (negative correlation) relationship between fungal species in deeper soil horizons compared to topsoil [108]. Moreover, APL and CC were lowest and highest, respectively in cryoOM compared to all other horizons. Networks that have smaller APL and higher CC are considered a “small world” which means every species is connected to every other species through a very short path and an “all-my-friends-know-each-other” relationship [109,110]. The networks termed “small worlds” are generally vulnerable to the rapid changes of an

ecosystem perturbation [111]. Therefore, fungal communities from the cryoOM may be more sensitive to environmental changes compared to other horizons. It may also reflect a less fluctuating environment compared to that experienced by topsoil.

We found a great difference in the co-occurrence network for different tundra sites too, Site 1 was more complex, whereas nodes from Site 4 were more connected. The potential reason was that the fungi had a higher richness and Shannon index from Site 1 compared to other sites, thus causing more complex fungal interaction [30,106]. Whereas Site 4 had the lowest richness and diversity which made it a less complex but more connected co-occurrence network as suggested above for the topsoil horizon. Moreover, APL and CC values suggested that Site 4 is a “small world” and vulnerable to environmental changes.

4.3. Greater Connectivity but Lower Specialization

The modules in ecological networks play a critical role in maintaining overall microbial community structure and stability, hence, the majority of ecosystem studies have focused on identifying modules in ecological networks [27,70,112,113]. Modules, by definition, are densely connected nodes that have more edges inside the module than outside. From our study, we found an average modularity higher than 0.4 which suggested a modular structure in all horizons and tundra sites [37].

The modularity value was lowest in buried cryoOM and Site 4 and highest in the mineral subsoil and Site 2 (Table 2). This highly modular network means that the fungal community is stable with an ordered structure with high efficiency at nutrient and information exchange [68]. Previous studies have interpreted modules as niches [114,115], and we found higher modularity values within subsoil and Site 2 which linked to stronger niche separation compared to other horizons and tundra sites.

4.4. Environmental Condition Associated with Topological Role Shift

In the present study, connectors and module hubs were considered as generalists and peripherals (taxa in the network which have only a few connections and only within their own module) as specialists [27]. Generalists are the key fungi that promote the exchange of nutrients and information among different taxa in network and hence play a pivotal role in maintaining the balance between different microbial taxa. In a natural ecosystem, generalists uptake nutrients from a broad range of sources and grow well in many habitats, whereas specialists have very specific nutrient requirements and therefore their growth is restricted to some habitats only [27,45,112,116]. In total, we found 13, 6, and 8 generalists within the topsoil, cryoOM, and subsoils, respectively. Additionally, the role of some taxa shifted in different horizons, topsoil (zOTU6200, unclassified Chaetothyriales; zOTU4687, *Cladophialophora*; and zOTU6829, *Cladophialophora*), cryoOM (zOTU952, *Mortierella antarctica*; zOTU3565, *Penicillium oregonense*; and zOTU4853, *Tetracladium*), and subsoil (zOTU1148, *Penicillium odoratum* and zOTU2775, unidentified fungi) were found as generalists in the respective horizons but a specialist in other horizons (Table 3). The role shifts of a generalist to a specialist in cryoOM probably occurred as a result of major events whereby the topsoil community was buried into deep soil horizons and surrounded by mineral subsoil with low nutrient availability and higher competition pressure between taxa. Two lines of evidence supported this generalization. Firstly, the generalist taxa in the topsoil network were found to be specialist taxa in the cryoOM network, suggesting their role shift (Figure 5 and Table S5). Secondly, the majority of taxa identified in the cryoOM network were not shared with topsoil network taxa (Figure S3), but with subsoil network taxa [11,117].

The number of generalists identified from each tundra site was 19, 5, 1, and 2 from Site 1, Site 2, Site 3, and Site 4, respectively. The taxa which were identified as generalists from Site 1 but specialists from other sites were litter saprotrophic zOTU1843 (*Cadophora finlandica*) (Table 4). This taxon has shown the ability to degrade various polysaccharides including cellulose, starch, and xylem [89], and previously detected from the Canadian High Arctic [118]. We speculate that the reason for this taxon being a generalist from Site 1 was mainly because Site 1 was mostly dominated by vascular plant vegetation (cotton

grass) which has more above-ground biomass compared to the other sites' vegetation. Tussock cotton grass generally has more dead leaves and culms than the living which may also nourish the higher proportion of litter-decomposing saprotrophic fungi. The role shift of key fungal taxa can be attributed to the different environmental conditions experienced in different horizons (Table S6) and tundra sites (Table S7).

The generalist taxa from topsoil but specialists from other horizons belonged to the order Chaetothyriales. Fungi from this order are dark septate root endophytes and as described above, they commonly interact with plant roots which may explain their role as generalists in topsoil. In contrast, in deeper soil horizons where plant roots are less abundant and also the availability of nutrients is scarcer, they might have different roles to play. For example, members of *Cladophialophora* were found as mycoparasites [119,120], and due to the lower nutrient availability in deeper soil, they might feed on other fungal species. This is in support of the fact that overall fungal gene abundance was found to be lower in cryoOM and subsoil compared to upper topsoil from this study and a previous study [11]. The generalists from cryoOM, had a negative correlation with LAP activity from topsoil but no such correlations were observed from cryoOM, we found a positive correlation with NAG activity instead. We speculated that high LAP production by other taxa (i.e., *Articulospora*) might have a negative effect on these taxa and potentially change their role to specialists. Furthermore, significantly higher DN content in subsoil and permafrost compared to the other two horizons (Table 1), potentially contributed to shifting the role of generalist from cryoOM to specialist in subsoil and permafrost.

Collectively, these co-occurrence data suggest that role shifts probably happen when the top layer becomes buried in the deep soil layer, and more connectors being shared between cryoOM and subsoil may suggest that most of these changes in cryoOM were driven by the different environmental conditions in surrounding mineral subsoil and the resident fungi [11,117].

5. Conclusions

In conclusion, our data showed that different horizons and tundra sites of the active layer of cryosols harbored not only distinct fungal communities with diverse lifestyles but also specific co-occurrence patterns along with changes in topological role (from generalist to specialist and vice-versa). The interactions of distinct microbial taxa can be more important to soil processes than species richness and their abundance, more importantly in the ecosystem where extreme changes happen in a short time. The inference of microbial networks allows us to find key microbes which are pivotal in maintaining the overall community structure and perform key roles.

Ultimately, such co-occurrence network analysis will be able to predict the outcome of community alterations (topological role shift) and the effects of environmental perturbations. For example, members of *Cladophialophora* were found as generalists in upper PAS where microbes are not limited by nutrients, but in the deeper soil layer, where nutrients are scarcer, they shifted their role from being a generalist to a specialist (mycoparasite) due to nutrient constraints. The topological indexes, average path length, and clustering coefficient suggested that the fungal network from cryoOM is a small world where everyone is connected to each other with a short path and every taxon is known to each other. The small world (cryoOM) is suggested to be more vulnerable to environmental changes than the bigger world (topsoil), thus, perturbation may lead to change in the overall carbon storage in PAS. The taxon *Cadophora finlandica* (litter saprotrophs) was identified as a generalist from Site 1 where litter is in ample amounts, however, for the other site its role shifted to a specialist due to environmental constraints.

Although exploring such an ecological network improves our understanding of microbial ecology, more investigations are needed to overcome methodological limitations such as the prediction of a relationship between two taxa by interpreting the correlation. For instance, the incorporation of techniques that will not only take into account the relationship between two taxa but also third-party microorganisms and random soil processes. In

addition, limited information on biotic and abiotic factors that covary in different horizons demands further investigation to determine the exact drivers and mechanisms of topological role shifts (generalist to a specialist), the number of which increased from topsoil to permafrost.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/microorganisms9091943/s1>, Figure S1: Significantly different in mean proportion of fungal genera for the different horizon and tundra sites. Only >1% mean proportion and significant difference between one horizon or site to rest of the horizons or sites are shown. Different colored bars represent topsoil, green; cryoOM, brown; subsoil, yellow; permafrost, cyan; Site 1, red; Site 2, blue; Site 3, black; Site 4, yellow; Figure S2: Significantly different in mean proportion of fungal lifestyle for different horizon and tundra site. Only >1% mean proportion and significant difference between one horizon or site to rest of the horizons or sites are shown. Different colored bars represent topsoil, green; cryoOM, brown; subsoil, yellow; permafrost, cyan; Site 1, red; Site 2, blue; Site 3, black; Site 4, yellow; Figure S3: Venn diagram of the total number of nodes (zOTUs) overlapping between different horizons and site networks and percentages of overlapping are given in the brackets; Table S1: Soil environmental factors in each tundra site. Averages and standard deviation are shown. The significant difference between tundra sites within all horizons together and individual horizons were calculated using one-way ANOVA and followed by a Tukey's HSD test. Different letters in the brackets indicate a significant difference between tundra sites; Table S2: Fungal relative proportion at the genera level for individual samples; Table S3: Fungi α -diversity indices. Averages and standard deviation are shown. The significant difference between all horizons and site together and within site were calculated using one-way ANOVA and followed by a Tukey's HSD test. Different letters in the brackets indicate a significant difference between horizons and sites; Table S4: Taxonomy of zOTUs identified by Zi-Pi plot; Table S5: zOTUs identified either module hub or connect (generalist) for one horizon or tundra site, but peripheral (specialist) for other horizons and tundra sites; Table S6: Correlation between keystone taxa (connectors and module hubs) identified from the Zi-Pi plot and environmental factors. Only significant correlations are shown. zOTUs ids were followed by letters in brackets which denote T, topsoil; C, cryoOM; S, subsoil; and P, permafrost. These letters mean that zOTU was identified as specialists from other horizons as well; Table S7: Correlation between keystone taxa (connectors and module hubs) identified from the Zi-Pi plot of tundra site and environmental factors. Only significant correlations are shown. zOTUs ids were followed by the number in brackets which denote 1, Site 1; 2, Site 2; 3, Site 3; and 4, Site 4. These numbers mean that zOTU was identified as specialists from other sites as well.

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Paper III

Extracellular enzyme ratios reveal locality and horizon-specific carbon, nitrogen, and phosphorus limitations in Arctic permafrost soils

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Under major revision in Biogeochemistry

1 **Extracellular enzyme ratios reveal locality and horizon-specific carbon, nitrogen, and phosphorus limitations**
2 **in Arctic permafrost soils**

3 Milan Varsadiya¹, Patrick Liebmann², Sebastian Petters³, Gustaf Hugelius^{4,5}, Tim Urich³, Georg
4 Guggenberger^{2,6}, Jiří Bárta^{*1,7}

5 ¹Department of Ecosystems Biology, University of South Bohemia, České Budějovice, Czech Republic; ²Institute of
6 Soil Science, Leibniz University Hanover, Germany; ³Institute of Microbiology, University of Greifswald,
7 Germany; ⁴Department of Physical Geography, Stockholm University, Stockholm, Sweden; ⁵Bolin Centre for
8 Climate Research, Stockholm University, Stockholm, Sweden; ⁶VN Sukachev Institute of Forest, SB RAS,
9 Krasnoyarsk, Russia; ⁷Centre for Polar Ecology, Faculty of Science, University of South Bohemia, Na Zlaté Stoe 3,
10 370 05 České Budějovice, Czech Republic

11 *Correspondence: jiri.barta@prf.jcu.cz

12 **1. Abstract**

13 Permafrost affected soils are highly vulnerable to climate change. These soils store huge amounts of organic carbon
14 (C), and a significant proportion of this carbon is stored in subsoil horizons where it might become available to
15 microbial decomposition under global warming. An important factor in understanding and quantifying the C release
16 from soils include the limitation of resources for microbes. Microbes decompose soil organic matter (SOM) by
17 secreting extracellular enzymes into the soil, thus enzyme activity and their ratios are considered important
18 indicators of soil nutrient availability and microbial substrate limitation. To evaluate nutrient limitation and the
19 limitation of microbial substrate utilization, we investigated the potential enzyme activity from whole soil profiles,
20 including topsoil, cryoturbated organic matter, mineral subsoil, and permafrost of Herschel Island (Canada) and
21 Disko Island (Greenland). We included seven enzymes (five hydrolytic and two oxidative) and related them to
22 bacterial and fungal gene abundance. The results showed hydrolytic enzymatic activity was strongly influenced by
23 soil type, whereas oxidative enzymes varied between different localities. The enzyme ratios indicated that the topsoil
24 microbial communities were C and phosphorus (P) co-limited in both localities, whereas the subsoil communities
25 were nitrogen (N) limited from HI locality and C, P limited from DI locality. A strong positive correlation between
26 all measured enzymes and bacterial gene abundance compared to that of fungi suggested that bacteria might play a
27 more important role in SOM decomposition in permafrost soil horizons. This study suggests that Arctic permafrost

28 microbial communities were not only limited by N, but also by C, P, and their co-limitation under specific
29 conditions (i.e., higher abundance of bacteria and lower abundance of fungi).

30

31 **Keywords:** enzyme stoichiometry; nutrient limitation; climate change; permafrost; microbial abundance

32

33 2. Introduction

34 The microbial breakdown of complex soil organic matter (SOM) is governed by the activity of extracellular
35 enzymes, and this process occurs across different depths of the soil profile. Since carbon (C) in Arctic permafrost
36 soils is unevenly distributed, this also applies to the activity of enzymes in the soil profile. The uneven distribution
37 of C in permafrost soils is mainly due to cryoturbation, a process which is driven by repeated freezing/thawing
38 periods of an active soil layer. During this process, parts of the C-rich topsoil horizons are translocated into the
39 deeper mineral subsoil (van Vliet-Lanoë 1991), thereby forming pockets containing a high amount of cryoturbated
40 organic matter (cryoOM) (Kaiser et al. 2007; Palmtag and Kuhry 2018). Cryoturbated OM represents one of the
41 main pools of soil organic carbon (SOC) in the northern permafrost region, accounting for ca. 400-500 Pg C of the
42 total 1,035 PgC in the upper 3 m of soil (Hugelius et al. 2014). Deepening of seasonally thawed active layer (soil
43 layer which freeze during winter and thaw during summer (Dobiński 2020)) is expected to promote the microbial
44 degradation by deeper plant rooting in this huge reservoir of SOC in permafrost soils (Keuper et al. 2020).

45 Extracellular enzymes produced by soil microbes play an important role in the decomposition of SOM and its
46 transformation and are thus relevant in maintaining biogeochemical cycles (Sinsabaugh et al. 2009). However, the
47 enzymes produced by microbes are highly dependent on total microbial biomass and microbial activity and both of
48 them are limited by the availability of soil nutrients (N and P) and energy (C) (Ekblad and Nordgren 2002;
49 Sinsabaugh et al. 2005). Most studies of Arctic soil ecosystems have suggested that the microbial community is
50 limited by N availability (e.g. (Schnecker et al. 2014; Wild et al. 2014)). In addition, a recent incubation study
51 indicated that the limitation of the microbial community changed with the depth of the soil (Wild et al. 2014).
52 Therein, the authors found that the mineral subsoil was co-limited by C and N, while cryoOM was limited by N.
53 Along with C and N, P is also a crucial soil nutrient for microbial growth and limits enzymatic activity in many soil
54 systems (Schimel 2003), but P limitation of microbial processes has rarely been observed in Arctic ecosystems.
55 Since microbes play an important role in the biogeochemical cycling of Arctic SOM, improved knowledge on the

56 nutrient limitation to microbial growth can help us to understand ecosystem functions and to better predict the
57 ecosystem response to global changes.

58 The enzymes that are most widely studied are those involved in the degradation of cellulose, chitin, protein,
59 phosphate, lignin, and polyphenolics. The most common enzymes that catalyze the production of bioavailable
60 terminal monomers includes β -1,4-glucosidase (BG), 1, 4- β -cellobiohydrolase (CBH), β -1,4-N-
61 acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and phosphatase (PME); these enzymes represent the
62 metabolic activities in degradation of organic C, N, and P, respectively (Allison et al. 2007). The decomposition of
63 more complex SOM molecules such as lignin occurs by the oxidation of aliphatic and aromatic hydrocarbons by
64 peroxidase (PER) and the oxidation of phenolic compounds by phenol oxidase (POX) (Sinsabaugh 2010). However,
65 the ratios of C, N, and P enzymes reflect the actual limitation of available resources for microbial metabolism
66 better than their absolute activities. The analyses of C, N, and P enzyme ratios provide insight into the limitation of
67 nutrients (N and P) and energy (C) in the soil by assessing the shift in microbial metabolism from energy to nutrient
68 flow or vice versa (Sinsabaugh et al. 2009). In general, the so-called enzyme stoichiometry is calculated from the
69 ratio of different enzymes acquiring C, N, and P from the decomposition of OM (Sinsabaugh et al. 2009). The use of
70 enzyme stoichiometry has gained attention, and apart from the simple individual enzyme ratio, a new approach of
71 vector analysis of enzyme stoichiometry based on (Sinsabaugh et al. 2008) and modified by (Moorhead et al. 2016)
72 has been widely used to study nutrient cycles in different ecosystems (Sinsabaugh et al. 2008, 2011; Hill et al. 2012;
73 Waring et al. 2014; Peng and Wang 2016; Chen et al. 2019a). Vector analysis provides information regarding the
74 limitation of C, N, and P based on the activities of C and N acquiring enzymes versus C and P acquiring enzymes.

75 The soil enzyme activity and stoichiometry can be affected by many abiotic and biotic factors. For example,
76 environmental conditions such as soil moisture, available dissolved nutrients, total C, total N, and pH could alter soil
77 enzyme stoichiometry by changing microbial growth efficiency (Schnecker et al. 2014; Peng and Wang 2016; Zuo
78 et al. 2018; Chen et al. 2019b; Liu et al. 2020). In addition to abiotic factors, biotic factors such as microbial
79 biomass, gene abundance, or microbial community inhabiting different depths of a soil profile also have a
80 significant influence on soil enzyme activity and stoichiometry (Schnecker et al. 2014; Kivlin and Treseder 2014; Li
81 et al. 2020). Although the impact of soil abiotic and biotic factors have received much attention, most of these
82 studies were carried out in temperate ecosystems, but only a few studies from permafrost soil provided information

83 on enzyme activity (Schnecker et al. 2015; Varsadiya et al. 2021a) and stoichiometry (Schnecker et al. 2014),
84 despite their important role in global C cycling (Schuur et al., 2015). Therefore, to better understand the
85 transformations of complex OM in permafrost soils, studies are needed that focus on microbial activities, including
86 extracellular enzymes involved in the utilization of C, N, and P and their relation to the availability of resources in
87 permafrost soils.

88 The goals for our study were (1) to determine the limitation of microbial C, N, and P in different horizons and
89 localities, and (2) to unravel the relative contribution of abiotic factors to the potential enzymatic activity and its
90 stoichiometry. To achieve these goals, we investigated the potential activity of five hydrolytic enzymes and two
91 oxidative from the topsoil (O and A soil genetic horizons), cryoturbated soil (O_{jj} and A_{jj} soil genetic horizons),
92 mineral subsoil (B, BC, BC_g soil genetic horizons), and permafrost layer from the western Canadian Arctic
93 (Herschel Island, HI) and Western Greenland (Disko Island, DI). We selected these localities due to the unique soil
94 formation processes leading to distinct soil horizons. For example, the soil profile of the HI locality was mainly
95 influenced by cryoturbation (Smith et al. 1989), while the DI locality is dominated by solifluction, i.e. “slow
96 gravitational downslope movement of water-saturated, seasonally thawed materials” (Thomas and Goudie 2000), as
97 the main soil process by which SOM can be buried in the deep soil. (Palmtag et al. 2015) describe systematic
98 differences in the composition of buried cryoOM from Eastern Greenland and Siberia, suggesting that slope
99 processes are slower and lead to a higher degree of decomposition of the buried OM before it becomes incorporated
100 into the permafrost. We speculate that microbial communities originating from these soil processes can be different
101 and potentially have different microbial resource limitations. To determine microbial resource limitation and co-
102 limitation we calculated C, N, and P enzyme stoichiometry. We also analyzed the abundance of bacterial and fungal
103 genes using quantitative PCR as markers of microbial activity and related it to the activities of measured enzymes.

104 Based on the great heterogeneity in the soil physicochemical properties of the different horizons within the soil
105 profiles and between different sites (locality), we hypothesized that

- 106 1) The activity of hydrolytic and oxidative enzymes will be higher in the upper organic topsoil due to
107 ample availability of fresh microbial resources.

- 108 2) As N is considered main limiting nutrient in Arctic soils (Sistla et al. 2012), the activity of N enzymes
109 will be higher than that of C enzymes and will increase with depth, where the availability of N is
110 lower.
- 111 3) As the limitation of N and P increases from topsoil to permafrost, microbial nutrient acquisitions ratio
112 decreases but more complex C compound increases.

113

114 3. Materials and Methods

115 1.1 Study sites

116 Soil samples were collected from Herschel Island (Qikiqtaruk; 69 ° 34 ' N, 138 ° 55 ' W, Beaufort Sea, Canada) and
117 Disko Island (Blæsedalen valley; 69°16'16.6"N 53°28'20.2"W, Qeqertarsuaq, Greenland). In total, 136 and 103
118 samples were collected from the active layer down to the permafrost table, which includes the topsoil (O and A soil
119 genetic horizons), cryoturbated soil (Ojj and Ajj soil genetic horizons), mineral subsoil (B, BC, BCg soil genetic
120 horizons) from HI and DI localities, respectively. We also collected soil samples from the permafrost layer (PF). We
121 stored all acquired sample to -20 °C until further analysis. More detailed sampling schemes were given in a previous
122 study from HI (Varsadiya et al. 2021a); a similar method for soil sampling was used for DI locality.

123 1.2 Soil physico-chemical analysis

124 As soil physicochemical parameters we analyzed soil pH, water content, water-extractable organic carbon (WEOC),
125 water-extractable total nitrogen (WETN), total carbon (Ctot) and total nitrogen (Ntot). The soil pH was determined
126 with water (ultra-pure water) at a solid to solution ratio of 1:25 using a 3151i pH meter (Fisher Scientific GmbH,
127 Schwerte Germany). The soil water content was determined gravimetrically by drying field fresh soil at 60°C for 48
128 h and weighing the sample before and after. The Ctot and Ntot content were determined from dried soil samples at
129 60 °C using an Elementar Vario Micro cube (Elementar, Langensfeld, Germany). WEOC and WETN were
130 quantified by mixing soil and water (ultra-pure) in 1: 5 ratios (w/v) and subsequent shaking for one hour (2.5 rad s⁻¹
131 on a horizontal shaker). Water extracts were filtered through 0.45 µm polyether sulfone (PES) filters (Macherey-
132 Nagel GmbH & Co. KG, Düren, Germany) and measured by a LiquiTOCII (Elementar, Langensfeld, Germany),
133 and expressed in µg gram⁻¹ dry weight. Each measured soil physico-chemical parameter is given in **Table S1**.

134 **1.3 Soil enzyme analysis**

135 In this study, the potential activities of five hydrolytic and two oxidative enzymes were measured. Those included β -
136 glucosidase (BG), 1, 4- β -cellobiohydrolase (CBH), chitinase (NAG), leucine aminopeptidase (LAP), phosphatase
137 (PME), phenol oxidase (POX), and peroxidase (PER).

138 The assay method consisted of microplate protocols as described in (Varsadiya et al. 2021a). In summary, soil
139 suspensions were prepared by homogenizing 0.5 g of soil sieved to 2 mm in 50 ml of ultra-pure water. The
140 suspension aliquots were distributed into 96-well black microtiter plates in 3 analytical replicates. After incubation
141 in the dark at 20 °C (4 h for BG, CBH, LAP, NAG, PME and 18 h for POX and PER), fluorescence was measured
142 using 465nm excitation and 450 emission filters. Plates were incubated in the dark for 30 min and the first
143 fluorescence was measured at 465 nm emission at an excitation of 360 nm (Tecan Infinite F200 fluorimeter, City,
144 State). Fluorescence was measured again after 60 and 120 min. Enzyme activities were calculated per gram of dry
145 soil and natural log-transformed before statistical analysis. The function of individual enzymes, their Commission
146 Number, and substrate used for the assay were given in **Table S2**.

147 **1.4 Indicator of microbial resource limitation**

148 Three approaches were used to investigate the microbial resource limitation. The first was the ratios of enzymes
149 involved in the acquisition of C, N, and (Ec:En, Ec:Ep, and En:Ep, respectively) and carbon recalcitrant index (CRI)
150 which were calculated by the following formulas (Sinsabaugh et al. 2008, 2009; Waring et al. 2014; Hill et al.
151 2018).

152
$$Ec:En = \ln(BG + CBH) / \ln(LAP + NAG) \quad (1)$$

153
$$Ec:Ep = \ln(BG + CBH) / \ln(PME) \quad (2)$$

154
$$En:Ep = \ln(LAP + NAG) / \ln(PME) \quad (3)$$

155
$$CRI = \ln(POX) / (\ln(BG) + \ln(CBH) + \ln(POX)) \quad (4)$$

156 Higher Ec:En than Ec:Ep indicates P-limitation, otherwise it points towards N-limitation (Sinsabaugh et al. 2008,
157 2009; Waring et al. 2014). Further, higher CRI values indicate a great proportion of recalcitrant carbon (Hill et al.
158 2018).

159 In the second approach, enzyme stoichiometry vector analysis was carried out (Moorhead et al. 2013, 2016; Chen et
160 al. 2019b). The vector analysis is based on plotting the activities of C and N acquiring enzymes versus C and P
161 acquiring enzymes and then calculating the vector angle from the origin (vector A) and the vector length as a
162 distance (vector L), finally determining the acquisition of C vs. nutrients and the relative P vs. N.

163 Vector L (length, unitless) and vector A (angle, °) were calculated with natural logarithmic transformation of the
164 enzymatic activities.

$$165 \text{ Vector } L (\text{unitless}) = \sqrt{x^2 + y^2} \quad (5)$$

$$166 \text{ Vector } A (\text{degree}) = \text{Degrees} (\text{Atan2}(x, y)) \quad (6)$$

167 Where $x = \ln(BG + CBH) / \ln(LAP + NAG)$ and $y = \ln(BG + CBH) / \ln(PME)$

168 A longer vector L suggests a greater limitation of C and an increasing vector A (>45°) indicates the limitation of P
169 relative to N based on metabolic and stoichiometric theories (Moorhead et al. 2016).

170 The third approach was based on the method described by (Hill et al. 2012), where a scatter plot of enzymatic
171 stoichiometry was drawn with N/P enzymes as the x-axis and C/N as the y axis. Due to deviation from the expected
172 N/P (1:1) or C/N (1:1) ratios (Sinsabaugh et al. 2008), different constraints on microbial resources were shown in
173 the scatter plot; these constraints can be related to N, P, C plus P-, and N plus P-limitations (Schmidt et al. 2016).

174 **1.5 Genomic DNA extraction and gene quantification**

175 Total genomic DNA was extracted from all the soil samples using a DNeasy PowerSoil DNA isolation kit (Qiagen,
176 Germany) and concentrations of extracted DNA were given in **Table S1**. We used total extracted DNA as a proxy
177 for microbial biomass in this study. The obtained genomic DNA extracts were used to quantify bacteria and fungi by
178 quantitative PCR. A detailed description of PCR conditions and primers were given elsewhere (Varsadiya et al.
179 2021b, a). We calculated the fungi to bacteria ratio (F/B ratio) by dividing fungal gene copies with bacterial gene
180 copies. Gene copies number were expressed in gram dry weight and log-transformed for further analysis.

181 **1.6 Statistical analysis**

182 Most of the analyzes were performed in R 3.5.3 (R Development Core Team 2011). Two- way analysis of variance
183 (ANOVA) was used to test the main effect of the horizon, locality, and their interactive effects on enzyme activity,

184 stoichiometry, and vector analysis. ANOVA was followed by Tukey's multiple comparison test (HSD) to determine
185 the statistical significance of the soil horizon and locality types in potential enzymatic activity and stoichiometry.
186 Type II standard major axis regression (SMA) was used to test the significant difference between the microbial
187 acquisition ratios and the 1:1 line using the R package, 'smatr'(Warton et al. 2012). Redundancy analysis (RDA)
188 was performed using Canoco 5.0 software (Smilauer and Leps 2014) to test which soil physico-chemical parameters
189 drive the enzyme activities and stoichiometry. Pearson's correlation was used to find relationships of potential
190 enzyme activity and stoichiometry with a single soil physico-chemical parameter. Pearson's correlation was
191 performed using the 'psych' package (Revelle 2021) in R. The significance level was established at $p < 0.05$.

192

193 4. Results

194 1.7 Potential enzyme activities and stoichiometry

195 We determined seven extracellular enzymes activities from different horizon types and localities and based on two-
196 way ANOVA results we found that the horizon, the locality, and their interaction had a significant influence on the
197 enzymatic activity (**Table 1**). The horizon topsoil had significantly higher BG, CBH, LAP, NAG, and PME enzymes
198 activities, while POX and PER had significantly higher activities in permafrost samples (**Fig. 1**). The horizon type
199 had a significantly greater influence on the hydrolytic enzymes (BG, CBH, LAP, NAG, and PME), while the
200 activities of the oxidative enzymes (POX and PER) were significantly influenced by the locality.

201

202 In HI, the potential activities of BG, CBH, LAP, and NAG enzymes showed a similar trend, decreasing from topsoil
203 to permafrost (**Fig. 1**). On the contrary, POX activity was significantly greater in cryoOM compared to other
204 horizons, while no significant differences were observed for PER enzyme activity. In addition, there were no
205 significant differences in the activity of NAG enzymes in the topsoil and cryoOM, but both horizons were
206 significantly higher than those of the subsoil and permafrost.

207 In DI, the subsoil had the lowest activities of BG, CBH, and NAG, compared to all other horizons (**Fig. 1**). LAP and
208 PME had the highest activity in the topsoil followed by cryoOM. Oxidative enzymes had significantly higher
209 activities in permafrost samples. The subsoil had the lowest PER activity, whereas the topsoil had the lowest POX
210 activity.

211 When enzyme activities were normalized to total soil C and microbial biomass (DNA), we detected an increase in
212 enzyme activity from topsoil to permafrost, irrespective of locality (**Fig. S1**).

213 **1.8 Indicators of microbial resource limitation**

214 We used three different approaches to determine the microbial resource limitation (see Materials and Methods for
215 details) and all the approaches indicated similar results. For the first approach, potential enzyme activity ratios
216 showed that Ec:En, Ec:Ep, and CRI were significantly affected by the horizon, location, and their interaction, while
217 En:Ep was not significantly affected by localities (**Table S3, $p < 0.05$**). The horizon effect was stronger than the
218 locality and their interaction. Ec:En was significantly higher in the topsoil of HI compared to DI. However, this ratio
219 was significantly lower in cryoOM and the subsoil of HI compared to DI (**Fig. 2**). In HI locality, Ec:En ratio was
220 significantly higher in topsoil samples, while Ec:Ep ratio was similar for both topsoil and cryoOM (**Fig. 2**). In
221 contrast, topsoil had a significantly lower En:Ep ratio in comparison to other horizons. Increasing values of CRI
222 with soil depth indicate a decrease of carbon quality (organic matter is present in more complex structures) from
223 topsoil to permafrost (**Fig. 2**). In DI locality, C to N (Ec:En) and C to P (Ec:Ep) acquisition ratios were significantly
224 lowest in topsoil, but highest in cryoOM, while N to P (En:Ep) acquisition ratio was significantly higher for
225 permafrost samples. Similar to HI locality, higher CRI values at DI locality indicate more complex organic matter in
226 deeper soil horizons.

227 The second approach was based on vector analysis, vector L and vector A. The vector L and vector A significantly
228 differed between different horizons and localities (**Fig. 3A and Table S4**). The horizon had a stronger effect on
229 vector L (length) that indicates a C limitation, while vector A (angle) was similarly affected by the horizon and
230 locality and hints towards a P-limitation for angles $>45^\circ$ and N-limitation for angles $<45^\circ$.

231 At both localities, vector L was significantly longer in topsoil and cryoOM samples and shorter in permafrost and
232 topsoil, respectively (**Fig. 3A**). The longer vector L suggested a greater C limitation in DI locality than HI locality,
233 except for the topsoil where vector L suggested greater C limitation in HI locality in comparison to DI locality. The
234 vector A results showed that topsoil samples from both localities had $>45^\circ$ which suggested more P limitation than
235 the N limitation (**Fig. 3**). With increasing depth (from organic topsoil to deeper mineral subsoil), P limitation was

236 decreased and N limitation was gradually increased for HI locality, on contrary, deeper soil samples from DI locality
237 showed stronger P limitation.

238 Our third approach also suggested a similar result as the second approach, this approach provided information also
239 about the co-limitation of resources (**Fig. 3B**). Accordingly, the topsoil horizons of both HI and DI localities were
240 limited or co-limited by C and/or P, but not by N. This limitation was shifted toward N limitation in deeper soil of
241 HI locality, whereas deeper soil samples from DI locality hold similar C and/or P limitation or co-limitation as upper
242 topsoil.

243 Collectively, all three approaches suggested that HI locality had significantly greater N limitation, except topsoil
244 samples, which were co-limited by P and C. The soil samples from DI locality had great variability for energy (C
245 limitation) and nutrient (P and N co-limitation) and topsoil had P limitation, while cryoOM and the subsoil had P
246 and C co-limitation.

247 In addition, the SMA regression showed that the microbial acquisition deviated from the 1:1:1 line (**Fig. 4 and**
248 **Table S5**).

249 **1.9 Bacterial and fungal abundance**

250 Quantification of bacteria and fungi by quantitative PCR of marker genes revealed that the abundance was
251 significantly higher for DI than HI, except for bacteria in the topsoil. Overall, the fungi to bacteria (F/B) ratios were
252 <1, showing that bacteria were more abundant than fungi. We found decreasing microbial gene abundance from
253 topsoil to permafrost soil. This trend was stronger for bacterial gene abundance at HI locality and fungal gene
254 abundance at DI locality (**Fig. 5**). We did not find significant differences between the F/B ratio in the horizons of HI.
255 In contrast, at DI significantly higher ratios were found in the topsoil than in the soil underneath.

256 **1.10 Key factors affecting soil enzyme activity and stoichiometry**

257 We performed Pearson's correlation analysis between enzyme activity and enzyme ratio with abiotic and biotic
258 factors including both localities together (**Table 2**) and individual (**Table S6, S7**), the result of correlation suggested
259 a strong significant correlation between them. The activity of some C enzymes (BG and CBH) had a significant
260 positive correlation with all measured abiotic and biotic factors, except pH and WETN. The activity of N enzymes

261 (LAP and NAG) was significantly positively correlated with soil moisture, WEOC/WETN ratio, Ctot, Ntot, bacteria
262 and fungi gene abundance. In contrast, the activity of the N enzyme NAG decreased with increasing pH and WETN
263 values (only true for HI locality, **Table S6**). Similar to NAG, PME activity also decreased with pH and WETN,
264 whereas it increased with the rest of the parameters. Interestingly, the F/B ratio had only one significant positive
265 correlation, and that was with PME. The activity of oxidative enzymes (POX and PER) increased with higher
266 moisture content, WEOC content, WEOC/WETN ratio, and decreased with pH, C/N ratio, and abundance of fungal
267 genes. We found that the Ec:En and Ec:Ep ratios increased with moisture, the WEOC/WETN ratio, Ctot, and Ntot.
268 On the contrary, the En:Ep ratio decreased with the increase of the same abiotic factors (WEOC/WETN ratio, Ctot,
269 and Ntot). This suggests that SOM compounds were more complexed when pH and WETN were high and less
270 complexed (easy to decompose) with high moisture, WEOC, WETN, WEOC/WETN ratio, Ctot, Ntot, and bacterial
271 and fungal gene abundance. The vector L, which suggested C limitation, increased with increasing moisture (only
272 true for HI locality), the WEOC/WETN ratio, the abundance of bacterial and fungal genes, while pH and WETN
273 responded oppositely. Based on vector angle analysis, the P limitation increased with the WEOC/WETN ratio, and
274 the N limitation increased with pH and WETN.

275

276 The RDA indicated 59.1% and 48.1% of the total explained variation in potential enzymatic activities and enzyme
277 stoichiometry, respectively (**Fig. 6**). Soil pH had the highest explanatory power for both enzyme activity and
278 stoichiometry. All C, N, and P enzymes were strongly positively correlated with Ctot, Ntot, and moisture, while
279 negatively correlated with pH and WETN. The oxidative enzymes PER and POX had a strong positive correlation
280 with WEOC. The enzyme ratios Ec:En, Ec:Ep, and vector L were positively correlated with soil moisture, Ctot,
281 Ntot, and the WEOC/WETN ratio. Further, CRI was correlated with WETN and En:Ep with pH.

282

283 5. Discussion

284 Our potential enzyme activity data from Arctic field samples in two contrasting permafrost soil systems showed
285 remarkable differences in the microbial nutrient acquisition ratios specific to each horizon and locality. For instance,
286 the topsoil was C and P limited at both localities, while the subsoils (mineral subsoil and cryoOM) were N limited at
287 HI locality and C and P co-limited at DI locality. Furthermore, all measured enzyme activities were found to

288 increase with a higher abundance of bacterial genes, suggesting that bacteria might play a significant role in the
289 decomposition of SOM in Arctic cryosols.

290 **1.11 Horizon-specific enzyme activity**

291 Soil extracellular enzymes fractions are a mixture of enzymes that use different mechanisms to cleave covalent
292 bonds in biopolymers and thus, play a key role in the degradation of complex organic matter (Sinsabaugh 2010;
293 Sinsabaugh et al. 2014). Hydrolytic enzymes destabilize complex biopolymers such as cellulose, chitin, and proteins
294 by the incorporation of water molecules into these substances. On the other hand, metalloenzymes from the class of
295 oxidoreductases (e.g., laccases and peroxidases) destabilize aromatic rings, which are the main components of lignin
296 and polyphenolics. The activity of soil enzymes generally (per gram dry soil) decreases with increasing depth due to
297 a decrease in SOM availability (Sinsabaugh et al. 2005). Our data from two permafrost locations showed a similar
298 trend, except for oxidative enzymes, which increase with depth (Fig. 1). We found that the abundance of bacteria but
299 not the abundance of fungi (as the main producer of oxidative enzymes, Schneider et al. 2012) correlated more
300 closely with the activity of oxidative enzymes (Table 2 and Fig. S2). There is increasing evidence that bacterial
301 laccases and laccase-like enzymes are present in a broad diversity of bacteria and archaea (possibly due to horizontal
302 gene transfer, (Ochman et al. 2000)), including many anaerobic species (Nakamura et al. 2003; Ausec et al. 2011b,
303 a; Gittel et al. 2014; Freedman and Zak 2014). The relative proportion of anaerobes in the total bacterial community
304 increased with depth in permafrost affected soils of HI locality (Varsadiya et al. 2021b). Possibly the expression of
305 laccases by bacteria can be more efficient due to the lack of introns and post-translational modifications as compared
306 to fungi (Ausec et al. 2011b). Furthermore, they might outcompete saprotrophic fungi in deeper horizons of
307 permafrost where anoxia together with low temperature and high content of recalcitrant biopolymers create
308 enormous selective pressure on the microbial community. Specific anaerobic taxa (e.g., Actinobacteria, Firmicutes)
309 can be responsible for higher production of laccases in deeper horizons and can therefore play a bigger ecological
310 role than fungi (Kellner et al. 2008). In support of this, we found that out of fourteen most proportionated genera
311 only one fungal genus had the laccase-like gene from all samples (data not shown), which further supports that
312 bacterial laccase may be more important. Therefore, we postulate that not the fungi, but the higher bacterial
313 abundance might lead to significantly higher oxidative enzyme activity in the deeper soil horizons.

314 **1.12 Locality-specific enzyme activity**

315 While hydrolytic enzyme activities differed strongly between horizons, oxidative enzyme activities varied
316 significantly between both localities (**Table 1**). A stronger effect of locality on the activities of oxidative enzymes
317 could be explained by the different abundances of microorganisms between these two localities. All hydrolytic
318 enzyme activities were significantly correlated with bacterial and fungal gene abundances from both localities (**Fig.**
319 **S2**). On the contrary, the activity of the oxidative enzymes was less correlated with the abundance of microbial
320 genes in both localities (except for the topsoil of HI and the cryoOM and subsoil from DI; **Fig. S2**). In support, we
321 found that bacterial and fungal genera that could possess genes for enzymes involved in lignin degradation (e.g.,
322 laccases) were more enriched at HI locality than at DI locality (data not shown). Our previous studies showed that
323 the abundances of bacteria and fungi were strongly influenced by different localities, likely resulting from change in
324 vegetation (Varsadiya et al. 2021b, a). We assumed that the significant difference in the abundance and potential
325 composition of microbial genes could lead to varying hydrolytic and oxidative enzymes in both localities. Another
326 potential reason for the significantly different enzyme activity at both localities could be the different soil processes
327 by which SOC is translocated down the soil profile. These different processes may have fostered different microbial
328 communities. We assume that cryoturbation from HI locality lifts up material from the subsoil and the subsoil
329 conditions are likely water saturated, so cryoturbation also brings anaerobic microbes in the upper parts of the soil
330 which are adjusted to anoxic conditions. Whereas solifluction from DI locality translocate material from upper parts
331 of a slope, which is the better aerated topsoil material (compared to subsoil material) and in addition, upper slopes
332 are typically better drained since the water is percolating down the slope towards the valley, so water saturation is
333 less likely. Consequently, microbes which are translocated by solifluction are rather adjusted to oxic conditions.
334 Hence different microbial community could have major implication in these processes and therefore the enzyme
335 production. However, this still warrants proof.

336 **1.13 Sink or source of greenhouse gases emission depending on individual localities.**

337 Arctic soils are generally considered N-limited soils compared to other ecosystems (Sistla et al. 2012). The ratios
338 between the C, N, and P enzymes showed that the topsoils of both localities were co-limited by C and P, while the
339 subsoils showed a locality effect and were limited by N at HI locality and co-limited by C, P at DI locality (**Fig. 3**).
340 Co-limitation of microbial nutrients was previously observed in an incubation study of permafrost horizons (soil
341 similar to HI locality) in the Siberian Arctic (Wild et al. 2014). Therein, the authors found that the organic topsoils

342 were energy (i.e., C) limited, while the mineral subsoils were C plus N co-limited, and the cryoOM was N limited.
343 Our study also evidenced similar limitations in microbial resources, however, specific to each locality. This can be
344 explained by the fact that both localities were dominated by different soil processes (i.e., cryoturbation, solifluction)
345 and therefore exhibit different soil physico chemical and microbial characteristics (**Table S1**). N acquisition from
346 organic matter is more complex as N is distributed among several classes of different polymers and humic
347 substances, therefore N acquisition depend on the relative availability of C substrate too (Manzoni et al. 2008). We
348 found lower N acquisition than the C in deeper soil of HI locality, this result was corresponded to decrease of
349 WEOC/WETN ratio with soil depth (**Table S1**). We found significant positive correlation between WEOC/WETN
350 ratio, N enzymes, and vector A from HI locality (**Table S6**) but not from DI locality (**Table S7**). Lower ratio of
351 energy (C) to nutrient (N) in deep soil could point towards high nutrient availability but less energy. In general,
352 microbial community in the mineral subsoil is considered energy limited (Fontaine et al. 2007), as subsoil is poorly
353 rooted, and source of plant derived root exudates is also scarce. Previous study also suggested the energy limitation in
354 deeper soil of permafrost affected soil (Wild et al. 2014). Therefore, we argue that even with high amount of WETN
355 (lower WEOC/WETN ratio) in deeper soil microbes may not have enough energy to utilize those nutrients. Future
356 warming is expected to increase the primary production of plant communities in the Arctic (McGuire et al., 1997),
357 and therefore increase the plant-derived C input into the lower horizon, which may eliminate the energy limitation in
358 the mineral subsoil and cryoOM at HI locality. Conclusively, microbial communities likely will enhance SOM
359 decomposition once the energy limitation is alleviated, resulting in an increase in greenhouse gas emissions at the HI
360 locality. It is already shown in a recent study from permafrost affected soil that the increased plant-derived C
361 allocation to the deeper soil enhanced the C loss (Keuper et al. 2020).

362 In contrast to HI, the microbial community in the lower horizons at DI invested more enzyme resources to acquire C
363 and P than those of N. In general, P from the soil is mostly unavailable to microbes because of its high affinity for
364 binding to iron minerals and can become part of stabilized organic matter. However, higher soil pH can liberate
365 complexed P and can make it available to microbes (Schaller et al. 2019). Although the pH value did not differ
366 significantly between horizons and localities, we found a significant correlation between pH, En:Ep, and vector A at
367 HI locality but not at DI. Hence, we assume that other factors (dissolved nutrients, C_{tot}, N_{tot}, and microbial gene
368 abundances), but not pH, had a stronger influence on P availability for microbes at DI. For instance, we found a
369 significantly higher relative proportion of fungal genus *Hebeloma* in subsoils of DI (not published data), which is

370 known to produce a high amount of phosphatase in Arctic soils (Tibbett et al. 1998). This result partially supports
371 our finding of high phosphatase activities in deeper horizons, which indicated a higher P limitation at DI. A study
372 from Siberian Arctic also found increasing P limitation with increasing depth (Čapek et al. 2016). Similar to HI
373 locality, increasing allocation of plant derived C into deeper soil of DI locality could remove the energy limitation
374 and potentially increase SOM decomposition. However microbial community are still limited by soil P which is
375 important nutrient for microbial growth, hence, we assume that with future warming and high plant derived C
376 allocation may be stored in DI locality.

377 6. Conclusions

378 The present study provides new information about the microbial nutrient demands from soil profiles of permafrost
379 soils affected by cryoturbation and solifluction, which can help to understand C and nutrient cycles under future
380 conditions. We found that the activity of hydrolytic enzymes decreased with depth but increased when normalized
381 with the C_{tot} and microbial biomass (DNA) of the soil, while the activity of oxidative enzymes was higher in the
382 deeper soil, regardless of analysis. The microbial resource limitation in the permafrost soil horizons derived from
383 enzyme ratios and vector analysis was remarkably correlated with most of the soil biotic and abiotic parameters in
384 all horizons. This suggests that the imbalance of enzyme stoichiometry is an important factor that affects microbial
385 metabolism in permafrost soil horizons. We also found a stronger correlation between the enzyme activities and
386 stoichiometries with bacterial gene abundance than with fungal gene abundance, indicating that bacteria could be
387 key organisms involved in SOM decomposition by producing extracellular enzymes.

388 Moreover, enzyme ratio scatter plot, and vector analysis consistently suggested that microbes from the topsoil of
389 both localities were P and C limited, while mineral subsoil and cryoOM were N limited at HI locality and N, and P
390 and C co-limited at DI. Based on these findings we conclude that, although the Arctic soils are generally N limited,
391 specific co-limitation with C and P can occur in distinct horizons and localities. Collectively, we infer that warming
392 and associated higher plant-derived C input in mineral subsoil horizons can have different implications based on the
393 localities. The enzyme data suggest that DI may act as a C sink, while at HI SOM decomposition may get
394 accelerated and potentially act as a source of greenhouse gases. In conclusion, the general perception that
395 permafrost soils are future C sources during global warming may need to be modified, as soils in Arctic regions
396 react individually to changing environmental conditions.

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526 **8. Funding**

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528 **9. Author Contributions**

529 Soil samples from Herschel Island, Canada were collected by GH, samples from Disko Island, Greenland were
530 collected by MV, JB, TU, PL, and SP. MV and JB analyzed and evaluated soil physicochemical parameters and
531 quantified microbe gene. MV, JB wrote the manuscript, and PL, SP, TU, GH, GG contributed to and have approved
532 the final manuscript.

533 **10. Data availability**

534 Additional data for present study were given in online supplementary materials.

535

Under Revision

Fig. 1

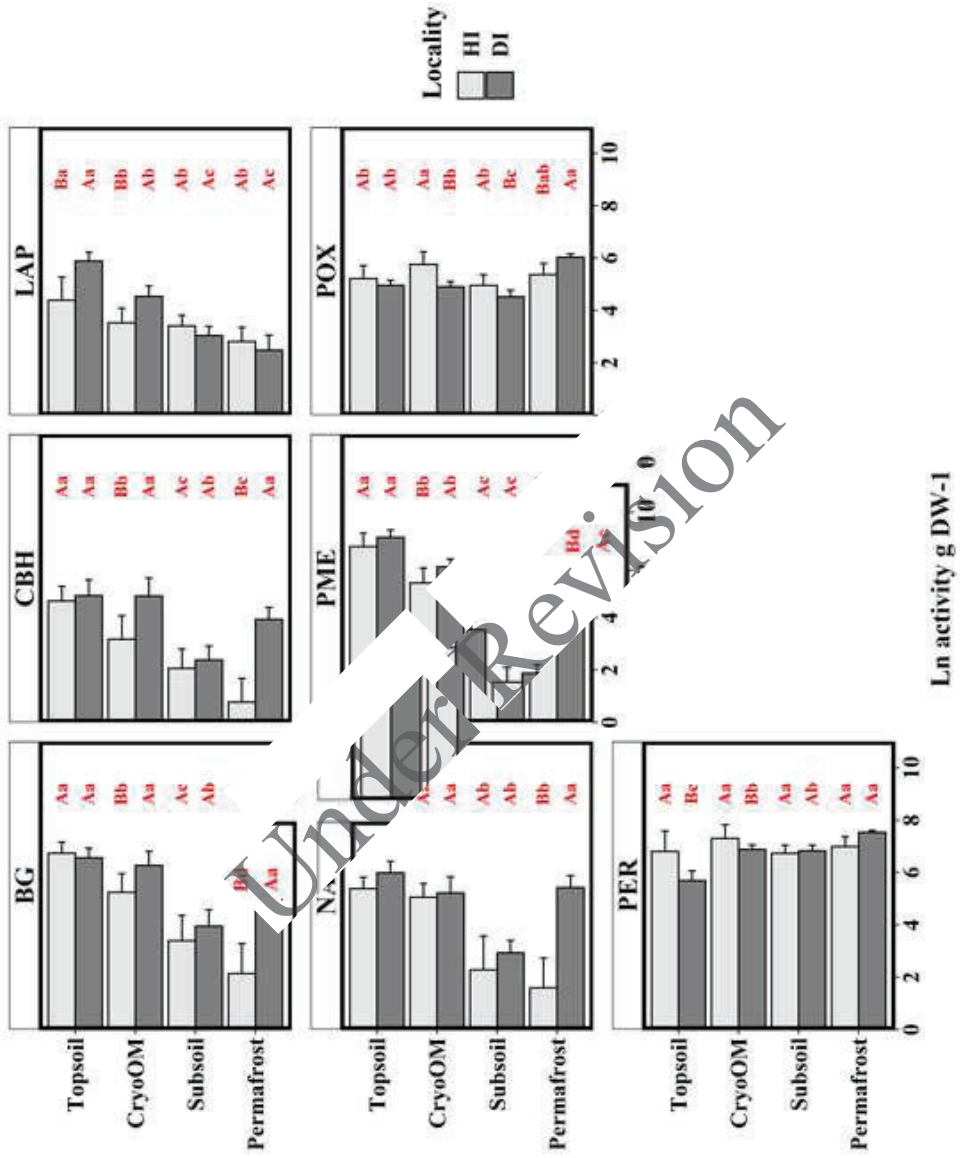


Fig. 2

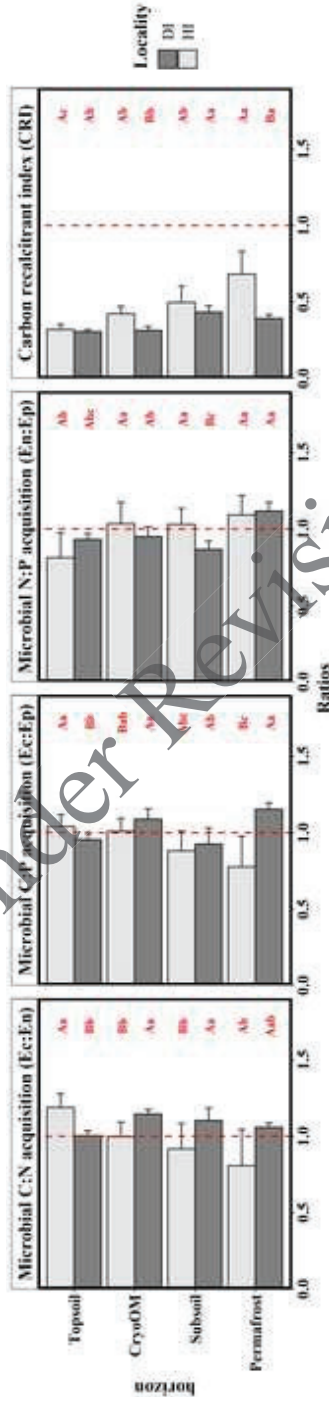


Fig. 3

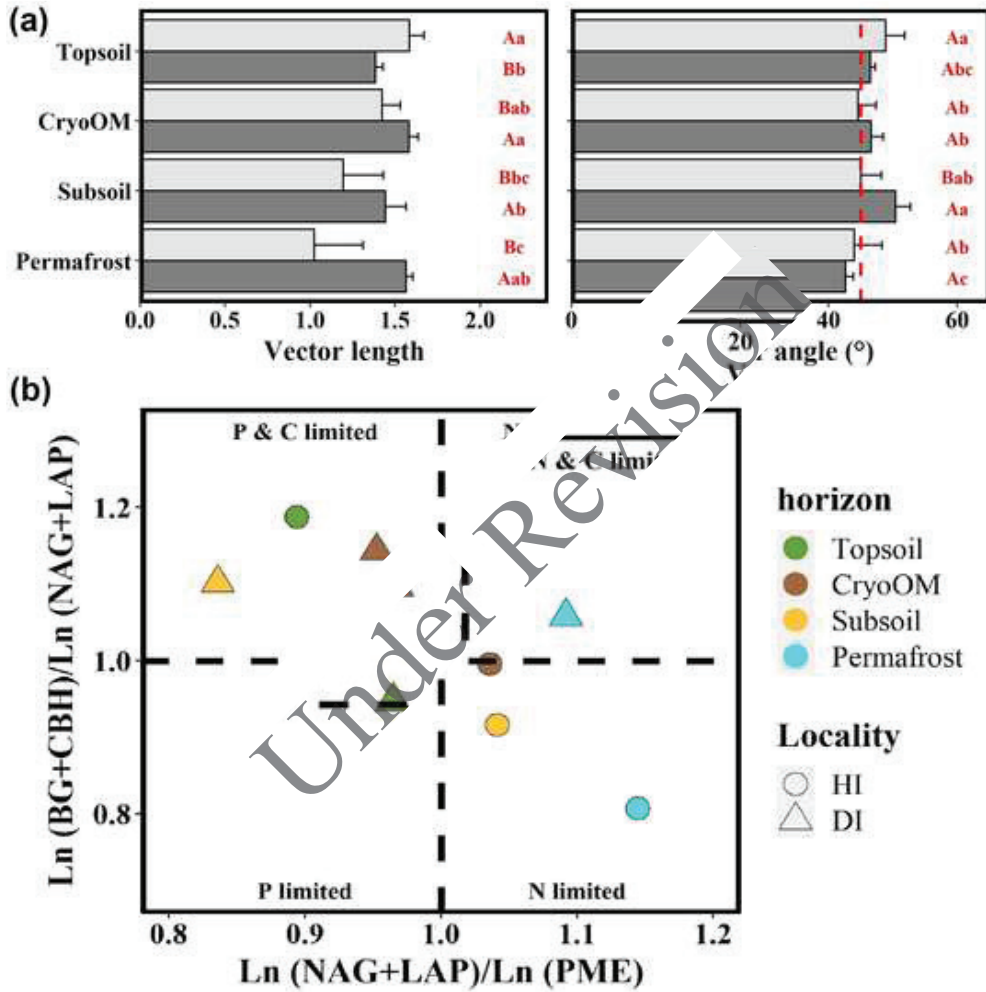


Fig. 4

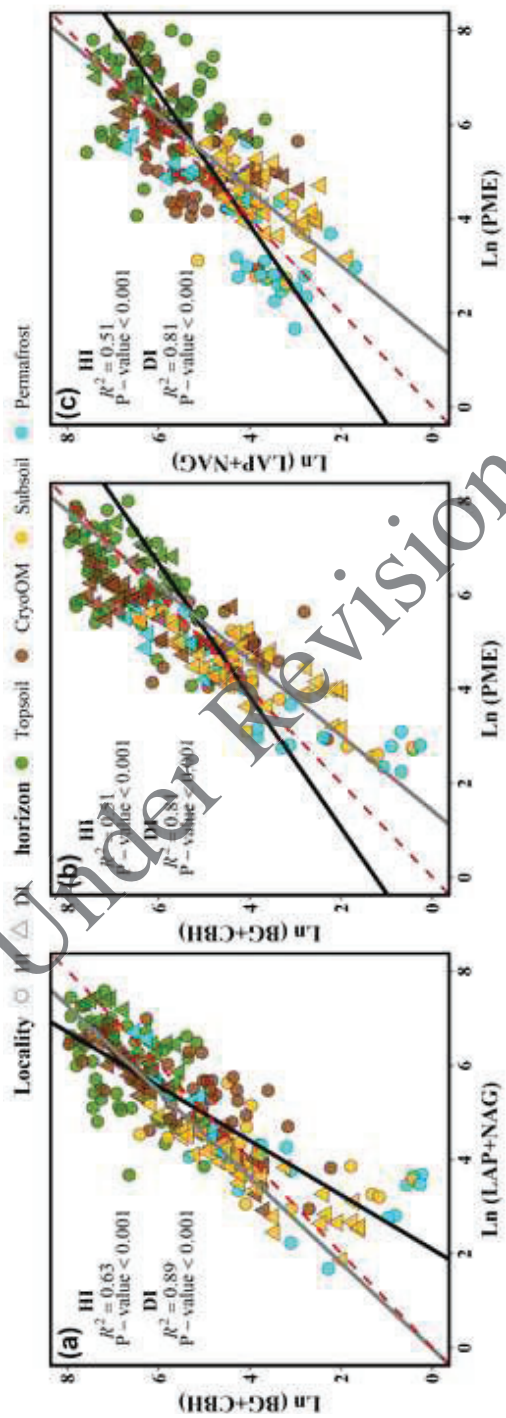


Fig. 5

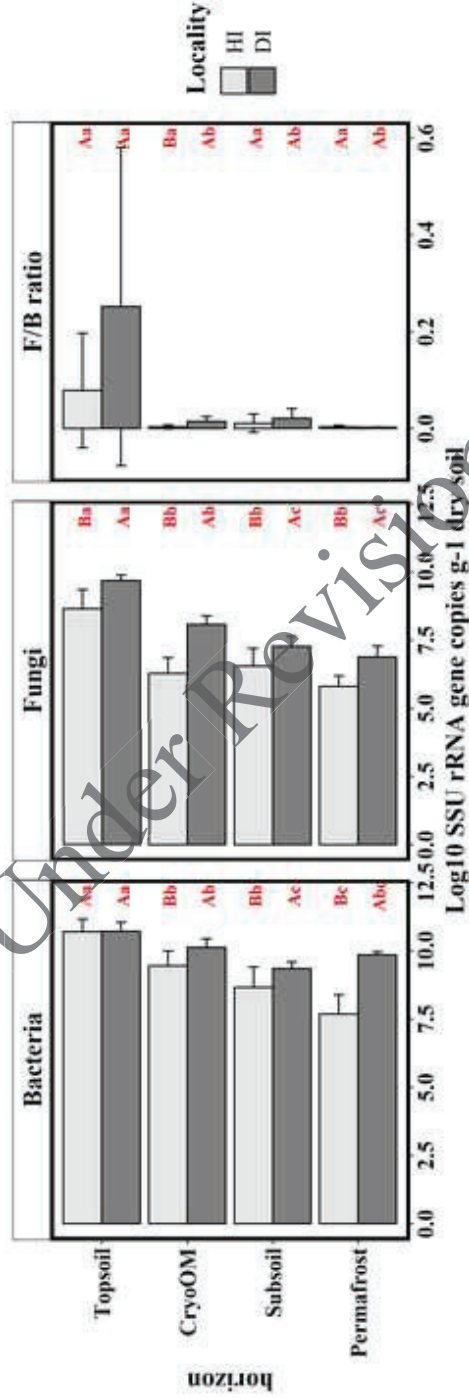


Fig. 6

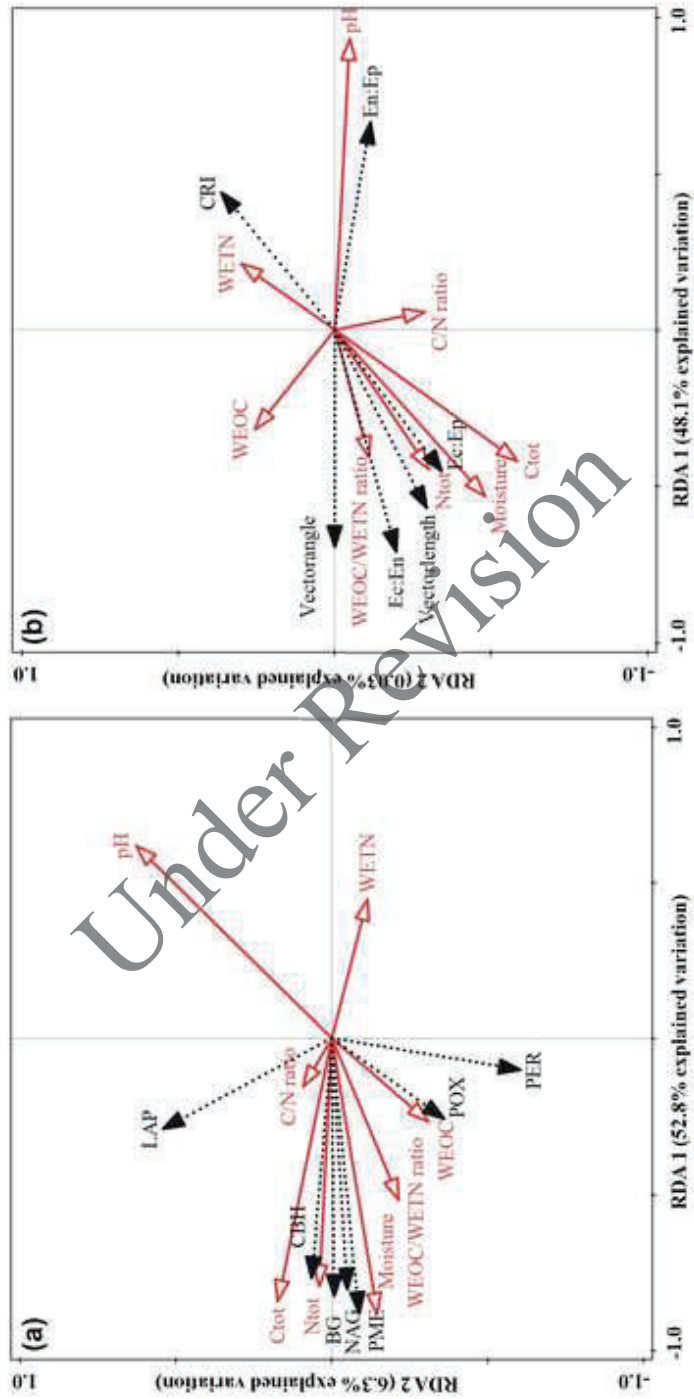


Fig. 1 Variation in natural log (Ln) transformed activity of C (BG and CBH), N (LAP and NAG), P, oxidative (POX and PER) enzyme in different horizons from HI and DI localities. The significant difference of enzyme activity between and among horizons and localities was determined by one-way ANOVA and followed by Tukey's HSD test, the difference was considered significant at $p < 0.05$. All values were presented as mean + standard error. Capital letters showed the significant difference between the two localities at the same soil horizon, and the different lower cases indicated the significant difference between soil horizons within one locality.

Fig. 2 Variation in natural log (Ln) transformed enzyme stoichiometry ratio and carbon quality index (CQI) in different horizons from HI and DI localities. The significant difference of enzyme stoichiometry ratios and CRI between and among horizons and locality were determined by one-way ANOVA and followed by Tukey's HSD test, the difference was considered significant at $p < 0.05$. All values were presented as mean + standard error. Capital letters showed the significant difference between the two localities at the same soil horizon, and the different lower cases reflect the significant difference between soil horizons within one locality.

Fig. 3 The general pattern of microbial resource limitation was analyzed by a) vector length (vector L) and vector angle (vector A) b) a scatter plot of soil enzymatic stoichiometry ratios. The significant differences of vector L and vector A between and among horizons and locality were determined by one-way ANOVA and followed by Tukey's HSD test, the difference was considered significant at $p < 0.05$. All values were presented as mean + standard error. Different colors and shapes of the points referred to different horizons and locality, respectively.

Fig. 4 Standard major axis (SMA) regressions between the natural logarithm transformed a) BG + CBH and LAP + NAG b) BG + CBH and PME and c) LAP + NAG and PME. Different colors and shapes of the points referred to different horizons and locality, respectively. All regression slopes were significant at $p < 0.05$. Reference lines with a slope of 1.0 were shown on the graphs.

Fig. 5 Log₁₀ transformed SSU rRNA gene copies of bacteria, fungi, and their ratio (F/B ratio). The significant difference between and among horizons and locality were determined by one-way ANOVA and followed by Tukey's HSD test, the difference was considered significant at $p < 0.05$. All values were presented as mean + standard error.

Fig. 6 Redundancy analysis (RDA) of a) natural log-transformed enzyme activities and b) microbial nutrient acquisition ratios and vector analysis. For the full form of abbreviations refer to the materials and methods section.

Table



Table 1. The results of two-way ANOVA examining effect of soil horizon, locality, and their interaction on enzyme

Enzymes	Horizon			Locality			Horizon*Local	
	DF	F	<i>p</i>	DF	F	P	DF	F
BG	3	87.69	<0.001	1	21.83	<0.001	3	9.85
CBH	3	55.89	<0.001	1	31.66	<0.001	3	8.07
LAP	3	35.07	<0.001	1	13.12	<0.001	3	8.09
NAG	3	74.77	<0.001	1	18.14	<0.001	3	10.77
PME	3	129.21	<0.001	1	22.09	<0.001	3	3.76
POX	3	9.06	<0.001	1	15.98	<0.001	3	6.29
PER	3	5.77	<0.001	1	6.19	<0.05	3	5.56

For abbreviation refer to materials and methods section.

Under Revision

activity.

ity
<i>p</i>
<0.001
<0.001
<0.001
<0.001
<0.05
<0.001
<0.01

Under Revision

Table 2. Pearson's correlation between enzyme activity, stoichiometry ratios, vector analysis, soil biotic and abiotic

Biotic and abiotic factors	pH	Moisture	WEOC	WETN	WEOC/WETN ratio
pH					
Moisture	-0.51 ***				
WEOC	-0.38 ***	0.66 ***			
WETN			0.39 ***		
WEOC/WETN ratio	-0.44 ***	0.65 ***	0.62 ***	-0.47 ***	
Ctot	-0.44 ***	0.9 ***	0.63 ***		0.65 ***
Ntot	-0.44 ***	0.87 ***	0.56 ***		0.56 ***
C/N ratio		0.26 ***	0.33 ***		0.36 ***
DNA	-0.34 ***	0.41 ***		-0.35 ***	0.32 ***
Bacteria	-0.36 ***	0.52 ***	0.18 **	-0.28 ***	0.41 ***
Fungi		0.4 ***		-0.28 ***	0.33 ***
F/B ratio		0.15 *	0.13 *		
BG	-0.52 ***	0.73 ***	0.33 ***	-0.27 ***	0.55 ***
CBH	-0.36 ***	0.65 ***	0.22 ***	-0.23 ***	0.42 ***
LAP	0.23 **	0.37 ***			0.16 *
NAG	-0.51 ***	0.73 ***	0.32 ***	-0.18 **	0.47 ***
PME	-0.6 ***	0.81 ***	0.46 ***	-0.21 **	0.62 ***
POX	-0.26 ***	0.41 ***	0.43 ***	0.25 ***	0.2 **
PER	-0.33 ***	0.14 *	0.26 ***		0.16 *
Ec:En	-0.59 ***	0.33 ***	0.14 *	-0.28 ***	0.38 ***
Ec:Ep		0.26 ***		-0.22 ***	0.19 **
En:Ep	0.65 ***	-0.29 ***	-0.26 ***	0.16 *	-0.39 ***
CRI	0.51 ***	-0.51 ***	-0.14 *	0.34 ***	-0.42 ***
Vector L	-0.52 ***	0.4 ***		-0.31 ***	0.37 ***
Vector A	-0.6 ***			-0.18 **	0.26 ***

Only significant correlation were shown. The values followed by “*”, “**”, and “***” were statistically significant: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. For full form of

: factors.

Ctot	Ntot	C/N ratio	DNA	Bacteria	Fungi	F/B ratio	BG	CBH
0.94 ***								
0.36 ***								
0.46 ***	0.49 ***							
0.56 ***	0.54 ***	0.18 **	0.9 ***					
0.47 ***	0.38 ***	0.4 ***	0.78 ***	0.79 ***				
0.15 *					0.2 **			
0.73 ***	0.73 ***	0.16 *	0.76 ***	0.8 ***	0.63 ***			
0.66 ***	0.63 ***	0.26 ***	0.67 ***	0.7 ***	0.63 ***		0.95 ***	
0.49 ***	0.46 ***	0.16 *	0.56 ***	0.57 ***	0.63 ***		0.53 ***	0.58 ***
0.7 ***	0.74 ***		0.68 ***	0.73 ***	0.47 ***		0.91 ***	0.84 ***
0.79 ***	0.79 ***	0.17 **	0.7 ***	0.71 ***	0.66 ***	0.21 **	0.87 ***	0.81 ***
0.27 ***	0.34 ***	-0.16 *			-0.24 ***		0.21 **	
		-0.26 ***	-0.15 *		-0.34 ***			
0.28 ***	0.27 ***		0.41 ***	0.4 ***	0.24 ***		0.64 ***	0.57 ***
0.31 ***	0.32 ***		0.46 ***	0.51 ***	0.17 *		0.71 ***	0.62 ***
-0.19 **	-0.18 **		-0.16 *			-0.15 *	-0.22 ***	
-0.51 ***	-0.55 ***		-0.75 ***	-0.73 ***	0.66 ***		-0.88 ***	-0.86 ***
0.36 ***	0.4 ***		0.6 ***	0.61 ***	0.25 ***		0.79 ***	0.68 ***

Abbreviations refer to materials and methods section.

Supplementary Materials

Table S1. Soil biotic and abiotic parameters in different horizons from HI and DI localities. Significant difference were determined by one-way ANOVA and followed by Tukey's HSD test, difference was considered significant at $p > 0.05$. All values were presented as mean \pm standard error. Capital letters showed the significant difference between the two localities at the same soil horizon, and the different lower cases reflect the significant difference between soil horizons within one locality.

Locality	Horizon	N	pH	Moisture (%)	WEOC ($\mu\text{g g}^{-1}\text{dw}$)	WETN ($\mu\text{g g}^{-1}\text{dw}$)	WEOC/WETN ratio	Clot (%)	Ntot (%)	C/N ratio	DNA (ng ul^{-1})
HI	Topsoil	53	6.14 \pm 0.5 (Ab)	72.9 \pm 6.76 (Aa)	3303.95 \pm 2571.43 (Aa)	8.93 \pm 5.18 (Ab)	392.83 \pm 140.58 (Aa)	33.5 \pm 5.18 (Aa)	1.39 \pm 0.28 (Aa)	29.41 \pm 10.42 (Aa)	56.29 \pm 23.99 (Aa)
	CryoOM	37	6.15 \pm 0.38 (Ab)	57.89 \pm 8.34 (Ab)	1147.13 \pm 749.55 (Ab)	16.64 \pm 14.89 (Ab)	122.33 \pm 68.63 (Ab)	17.1 \pm 4.28 (Ab)	1.01 \pm 0.25 (Ab)	17.18 \pm 2.23 (Ab)	13.3 \pm 11.84 (Bb)
	Subsoil	23	6.56 \pm 0.59 (Ab)	28.43 \pm 4.86 (Ac)	345.49 \pm 110.38 (Ab)	25.56 \pm 9.66 (Ab)	18.28 \pm 6.5 (Bb)	4.42 \pm 0.93 (Ac)	0.25 \pm 0.06 (Ac)	18.26 \pm 2.29 (Ab)	11.2 \pm 7 (Ab)
DI	Permafrost	23	7.47 \pm 0.56 (Aa)	37.13 \pm 8.66 (Bc)	488.57 \pm 298.44 (Ab)	49.83 \pm 24.8 (Aa)	22.78 \pm 14.64 (Ab)	4.41 \pm 1.5 (Bc)	0.26 \pm 0.13 (Bc)	21.17 \pm 3.87 (Aab)	0.5 \pm 0.29 (Bb)
	Topsoil	16	6.15 \pm 0.19 (Ab)	69.4 \pm 4.79 (Aa)	331.17 \pm 50.33 (Ba)	11.54 \pm 4.65 (Aa)	37.32 \pm 7.2 (Ba)	27.39 \pm 4.68 (Ba)	1.29 \pm 0.18 (Aa)	20.8 \pm 1.64 (Aa)	61 \pm 15.45 (Aa)
	CryoOM	38	6.39 \pm 0.2 (Ab)	54.39 \pm 4.82 (Ab)	166.24 \pm 26.94 (Bc)	6.33 \pm 2.65 (Bb)	36.47 \pm 8.15 (Ba)	14 \pm 2.88 (Ab)	0.88 \pm 0.16 (Ab)	15.81 \pm 0.67 (Ab)	32.16 \pm 9.56 (Ab)
DI	Subsoil	40	6.73 \pm 0.2 (Aa)	27.45 \pm 3.96 (Ac)	87 \pm 16.34 (Bd)	2.73 \pm 1.12 (Bc)	40.08 \pm 8.98 (Aa)	2.71 \pm 0.86 (Bc)	0.19 \pm 0.06 (Bc)	15.18 \pm 0.99 (Bb)	13.33 \pm 6.5 (Ac)
	Permafrost	8	6.21 \pm 0.05 (Ab)	52.62 \pm 5.53 (Ab)	225.73 \pm 34.79 (Ab)	8.57 \pm 2.1 (Bab)	30.34 \pm 5.3 (Aa)	9.67 \pm 2.17 (Ab)	0.63 \pm 0.15 (Ab)	15.64 \pm 0.57 (Bb)	15.28 \pm 5.42 (Abc)

Table S2. Studied enzymes, their abbreviation, commission number (EC), functions, and specific substrate used for enzyme assay.

Enzymes	Abbreviation	EC	Function	Substrates
β -Glucosidase	BG	3.2.1.21	Hydrolytic, Releases glucose from cellulose	4-MUB- β -D-glucoside
Cellobiohydrolase	CHB	3.2.1.91	Cellulose degradation: hydrolyses cellobiose dimers from non-reducing ends of cellulose molecules	4-MUB- β -D-cellobioside
			Hydrolytic, Releases disaccharides from cellulose	
N-acetyl-glucosaminidase	NAG	3.2.1.14	Hydrolytic, Degrades chitin	4-MUB-N-acetyl- β -D-glucosaminide
Leucine-amino-peptidase	LAP	3.4.11.1	Hydrolytic, Degrades protein into amino acids	L-Leucine-7-amino-4-methylcoumarin
Phosphatase	PME	3.1.3.1	Protolysis: hydrolyses leucine and other hydrophobic amino acids from the N terminus of polypeptides	4-MUB-phosphate
Peroxidase	PER	1.11.1.7	Oxidative, Releases phosphate ions from phosphate group	L-DOPA
Phenol Oxidase	POX	1.10.3.2	Oxidative, Oxidize aromatic and aliphatic hydrocarbons using peroxide	L-DOPA

Table S3. Results of two-way ANOVA examining effect of soil horizon, locality and their interaction on enzyme stoichiometric ratios.

Enzymes	Horizon			Locality			Horizon*Locality		
	DF	F	p	DF	F	p	DF	F	p
Ec:En	3	10.83	<0.001	1	8.53	<0.01	3	9.07	<0.001
Ec:Ep	3	8.74	<0.001	1	5.01	<0.05	3	6.82	<0.001
En:Ep	3	11.03	<0.001	1	2.07	0.15	3	3.84	<0.05
CRI	3	48.54	<0.001	1	28.47	<0.001	3	6.71	<0.001

Table S4. Estimated fixed effect of soil horizon, locality and their interaction on vector analysis based on two-way ANOVA analysis.

Vector	Horizon			Locality			Horizon*Locality		
	DF	F	p	DF	F	p	DF	F	p
Vectorlength	3	17.08	<0.001	1	13.14	<0.001	3	10.75	<0.001
Vectorangle	3	8.11	<0.001	1	3.92	<0.05	3	8.11	<0.001

Table S5. Standard major axis (SMA) regressions between the natural logarithm transformed a) BG + CBH and LAP + NAG, b) BG + CBH and PME and c) LAP + NAG and PME in all samples together, from individual locality for different horizons.

Variables	Locality	Horizon	Formula	R ²
Ln(BG+CBH) - Ln(LAP+NAG)	All	All (n= 238)	y= 1.33 LN(LAP+NAG)-1.33	0.66 ***
		Topsoil (n= 53)	y= 0.9 LN(LAP+NAG) -1.59	0.3 ***
		Cryosol (n= 37)	y= 1.85 LN(LAP+NAG)-4.62	0.51 ***
	HI	Subsoil (n= 23)	y= 2.71 LN(LAP+NAG)-7.68	0.44 ***
		Permafrost (n= 23)	y= 3.89 LN(LAP+NAG)-12.03	0.28 **
		Topsoil (n= 16)	y= 1.34 LN(LAP+NAG)-2.28	0.74 ***
	DI	Cryosol (n= 38)	y= 1.07 LN(LAP+NAG)0.42	0.93 ***
		Subsoil (n= 40)	y= 1.65 LN(LAP+NAG)-2	0.85 ***
		Permafrost (n= 8)	y= 0.84 LN(LAP+NAG)1.16	0.92 ***
Ln (BG+CBH) - Ln (PME)	All	All (n= 238)	y= 1.33 LN(PME)-1.77	0.75 ***
		Topsoil (n= 53)	y= 0.87 LN(PME) 1.04	0.22 ***
		Cryosol (n= 37)	y= 1.25 LN(PME)-1.28	0.68 ***
	HI	Subsoil (n= 23)	y= 1.73 LN(PME)+3.63	0.79 ***
		Permafrost (n= 23)	y= 2.31 LN(PME)-5.53	0.66 ***
		Topsoil (n= 16)	y= 1.47 LN(PME)-3.62	0.56 ***
	DI	Cryosol (n= 38)	y= 1.54 LN(PME)-2.58	0.61 ***
		Subsoil (n= 40)	y= 2.09 LN(PME)+5.11	0.5 ***
		Permafrost (n= 8)	y= 1.49 LN(PME)-1.67	0.71 **
Ln (LAP+NAG) - Ln (PME)	All	All (n= 238)	y= 0.9 LN(PME)0.21	0.57 ***
		Topsoil (n= 53)	y= 0.95 LN(PME)-0.54	0.02
		Cryosol (n= 37)	y= 0.65 LN(PME) 1.97	0.27 ***
	HI	Subsoil (n= 23)	y= 0.68 LN(PME) 1.29	0.31 **
		Permafrost (n= 23)	y= 0.63 LN(PME) 1.53	0.32 **
		Topsoil (n= 16)	y= 1.15 LN(PME)-1.41	0.63 ***
	DI	Cryosol (n= 38)	y= 1.46 LN(PME)-2.92	0.64 ***
		Subsoil (n= 40)	y= 1.3 LN(PME)-2.03	0.46 ***
		Permafrost (n= 8)	y= 1.77 LN(PME)-3.37	0.85 ***

Table S6. Pearson's correlation between enzyme activity, stoichiometry ratios, vector analysis, soil biotic and abiotic factors from HI locality only.

Biotic and abiotic	Moisture	WDOC	WFEN	WDOC/WFEN ratio	C:ar	N:ar	C:N ratio	DNA	Bacteria	Fungi	F:B ratio	BG	CBH	LAP	NAG	PME	POX	PER	Ec:En	Ec:Ep	En:Ep	CR1	Vector L	Vector A	
pH																									
Moisture	0.53 ***																								
WDOC	0.44 ***	0.71 ***																							
WFEN	0.21 *	0.51 ***	0.78 ***																						
WDOC/WFEN ratio	0.47 ***	0.39 ***	0.7 ***	0.68 ***																					
C:ar	0.46 ***	0.37 ***	0.59 ***	0.68 ***	0.93 ***																				
N:ar	0.45 ***	0.51 ***	0.56 ***	0.58 ***	0.83 ***	0.91 ***																			
C:N ratio	0.41 ***	0.41 ***	0.51 ***	0.52 ***	0.82 ***	0.85 ***	0.92 ***																		
DNA	0.37 ***	0.25 ***	0.25 ***	0.45 ***	0.51 ***	0.64 ***	0.64 ***	0.82 ***																	
Bacteria	0.36 ***	0.54 ***	0.29 ***	0.43 ***	0.52 ***	0.63 ***	0.6 ***	0.21 *	0.92 ***																
Fungi	0.14 ***	0.21 ***	0.17 ***	0.47 ***	0.51 ***	0.58 ***	0.41 ***	0.49 ***	0.79 ***	0.52 ***															
F:B ratio	0.2 *	0.28 **									0.24 **														
BG	0.55 ***	0.72 ***	0.47 ***	0.55 ***	0.74 ***	0.81 ***	0.79 ***	0.8 ***	0.81 ***	0.72 ***															
CBH	0.4 ***	0.68 ***	0.36 ***	0.55 ***	0.69 ***	0.75 ***	0.67 ***	0.72 ***	0.73 ***	0.7 ***	0.97 ***														
LAP	0.31 ***	0.19 *	0.15 ***	0.28 **	0.38 ***	0.38 ***	0.14 ***	0.55 ***	0.53 ***	0.57 ***	0.4 ***	0.45 ***													
NAG	0.53 ***	0.75 ***	0.42 ***	0.49 ***	0.63 ***	0.74 ***	0.68 ***	0.7 ***	0.72 ***	0.47 ***	0.89 ***	0.8 ***													
PME	0.62 ***	0.83 ***	0.6 ***	0.51 ***	0.81 ***	0.84 ***	0.82 ***	0.73 ***	0.71 ***	0.65 ***	0.19 *	0.38 ***	0.83 ***	0.55 ***	0.76 ***	0.76 ***	0.76 ***	0.7 ***							
POX	0.28 ***	0.35 ***	0.3 ***	0.19 *	0.26 **	0.26 **	0.35 ***	0.29 ***	0.29 ***	0.29 ***															
PER	0.37 ***	0.22 **	0.18 ***																						
Ec:En	0.65 ***	0.55 ***	0.77 ***	0.28 **	0.48 ***	0.51 ***	0.47 ***	0.19 *	0.46 ***	0.34 ***	0.76 ***	0.7 ***	0.56 ***	0.65 ***	0.2 *	0.21 ***	0.21 ***	0.21 ***							
Ec:Ep	0.29 ***			0.34 ***	0.29 ***	0.39 ***	0.24 ***		0.47 ***	0.52 ***	0.21 *	0.69 ***	0.85 ***	0.61 ***	0.65 ***	0.3 ***	0.3 ***	0.3 ***	0.62 ***						
En:Ep	0.7 ***	0.81 ***	0.89 ***	0.46 ***	0.69 ***	0.41 ***	0.37 ***		0.23 ***	0.23 ***	0.26 **	0.38 ***	0.75 ***	0.66 ***	0.71 **	0.64 ***	0.48 **	0.18 *	0.37 ***	0.62 ***					
CR1	0.54 ***	0.63 ***	0.34 ***	0.43 ***	0.46 ***	0.42 ***	0.67 ***		0.75 ***	0.73 ***	0.68 ***	0.91 ***	0.87 ***	0.28 **	0.28 **	0.28 **	0.28 **	0.28 **	0.79 ***	0.8 ***	0.31 ***				
Vector L	0.36 ***	0.37 ***	0.33 ***	0.34 ***	0.46 ***	0.57 ***	0.57 ***		0.65 ***	0.65 ***	0.38 ***	0.84 ***	0.74 ***	0.62 ***	0.62 ***	0.62 ***	0.62 ***	0.62 ***	0.91 ***	0.87 ***	0.87 ***	0.87 ***	0.87 ***		
Vector A	0.7 ***	0.46 ***	0.43 ***	0.37 ***	0.57 ***	0.25 ***	0.34 ***		0.19 *	0.21 *	0.36 ***	0.82 ***	0.82 ***	0.15 *	0.66 ***	0.18 *	0.18 *	0.18 *	0.36 ***	0.71 ***	0.71 ***	0.71 ***	0.71 ***		

Only significant correlation were shown. The values followed by *^{0.05}, **^{0.01}, and ***^{0.001} were statistically significant: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. For full form of abbreviations refer to materials and methods section.

Table S7. Pearson's correlation between enzyme activity, stoichiometry ratios, vector analysis, soil biotic and abiotic factors from DI locality only.

Biotic and abiotic	Moisture	WDOC	WFEN	WDOC/WFEN ratio	C:ar	N:ar	C:N ratio	DNA	Bacteria	Fungi	F:B ratio	BG	CBH	LAP	NAG	PME	POX	PER	Ec:En	Ec:Ep	En:Ep	CR1	Vector L	Vector A	
pH																									
Moisture	0.54 ***																								
WDOC	0.49 ***	0.89 ***																							
WFEN	0.52 ***	0.75 ***	0.81 ***																						
WDOC/WFEN ratio	0.4 *	0.57 **	0.78 ***	0.74 ***																					
C:ar	0.46 ***	0.34 ***	0.36 ***	0.71 ***	0.21 *																				
N:ar	0.36 ***	0.34 ***	0.81 ***	0.72 ***	0.24 *	0.99 ***																			
C:N ratio	0.37 ***	0.37 ***	0.51 ***	0.54 ***	0.24 **	0.91 ***	0.28 **																		
DNA	0.32 *	0.56 ***	0.53 ***	0.45 ***		0.59 ***	0.56 ***	0.47 ***																	
Bacteria	0.48 ***	0.77 ***	0.65 ***	0.66 ***		0.71 ***	0.68 ***	0.64 ***	0.82 ***																
Fungi	0.33 ***	0.59 ***	0.58 ***	0.51 ***	0.25 *	0.63 ***	0.58 ***	0.63 ***	0.68 ***	0.77 ***															
F:B ratio	0.32 *	0.76 ***	0.22 *	0.51 ***	0.25 *	0.78 ***	0.77 ***	0.29 ***	0.69 ***	0.77 ***	0.57 ***														
BG	0.3 *	0.76 ***	0.64 ***	0.57 ***	0.22 *	0.75 ***	0.74 ***	0.35 ***	0.64 ***	0.74 ***	0.57 ***	0.96 ***													
CBH	0.3 *	0.79 ***	0.61 ***	0.48 ***		0.74 ***	0.67 ***	0.64 ***	0.65 ***	0.73 ***	0.77 ***	0.96 ***	0.78 ***												
LAP	0.38 ***	0.81 ***	0.76 ***	0.65 ***	0.22 *	0.82 ***	0.79 ***	0.68 ***	0.63 ***	0.76 ***	0.58 ***	0.95 ***	0.93 ***	0.71 ***											
NAG	0.48 ***	0.87 ***	0.8 ***	0.69 ***	0.23 *	0.89 ***	0.87 ***	0.47 ***	0.69 ***	0.74 ***	0.73 ***	0.28 ***	0.84 ***	0.81 ***	0.85 ***	0.85 ***									
PME	0.48 ***	0.87 ***	0.8 ***	0.69 ***	0.23 *	0.89 ***	0.87 ***	0.47 ***	0.69 ***	0.74 ***	0.73 ***	0.28 ***	0.84 ***	0.81 ***	0.85 ***	0.85 ***									
POX	0.18 ***	0.53 ***	0.53 ***	0.29 ***	0.40 ***	0.43 ***	0.67 **	0.32 ***	0.42 ***	0.42 ***	0.59 ***	0.53 ***	0.24 *	0.63 ***	0.37 ***										
PER	0.21 **					0.28 **	0.22 **	0.51 ***		0.46 ***	0.31 **														
Ec:En	0.29 ***	0.23 *			0.36 **	0.31 **	0.24 *	0.4 ***	0.45 ***		0.32 **	0.24 *	0.28 **	0.65 ***	0.28 **	0.61 ***	0.43 ***	0.56 ***							
Ec:Ep	0.32 **	0.31 ***	0.22 **		0.36 **	0.27 **	0.28 **	0.33 ***	0.46 ***		0.59 ***	0.59 ***	0.35 ***	0.67 ***	0.21 *	0.64 ***	0.29 ***								
En:Ep	0.3 *	0.65 ***	0.55 ***	0.6 ***		0.71 **	0.71 **	0.34 ***	0.66 ***	0.73 ***	0.61 ***	0.86 ***	0.85 ***	0.79 ***	0.54 ***	0.79 ***	0.25 ***								
CR1	0.3 *	0.65 ***	0.55 ***	0.6 ***		0.71 **	0.71 **	0.34 ***	0.66 ***	0.73 ***	0.61 ***	0.86 ***	0.85 ***	0.79 ***	0.54 ***	0.79 ***	0.25 ***								
Vector L	0.36 ***	0.37 ***	0.33 ***	0.34 ***	0.46 ***	0.57 ***	0.57 ***		0.65 ***	0.65 ***	0.38 ***	0.84 ***	0.74 ***	0.62 ***	0.62 ***	0.62 ***	0.62 ***	0.91 ***	0.87 ***	0.87 ***	0.87 ***	0.87 ***	0.87 ***		
Vector A	0.42 ***	0.44 ***	0.32 **		0.49 ***	0.44 ***	0.35 ***	0.47 ***	0.58 ***	0.28 **	0.72 ***	0.68 ***	0.51 ***	0.76 ***	0.58 ***	0.66 ***	0.22 *								

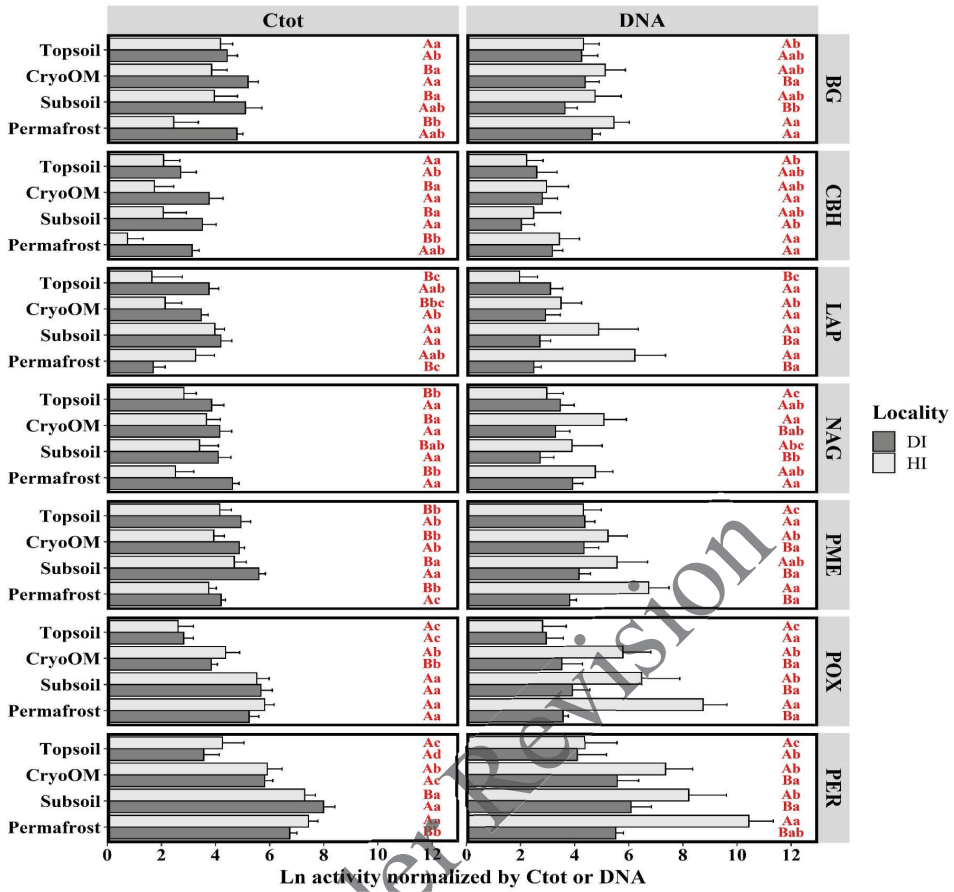


Figure S1. Variation in enzyme activity normalized to Ctot and microbial biomass (DNA).

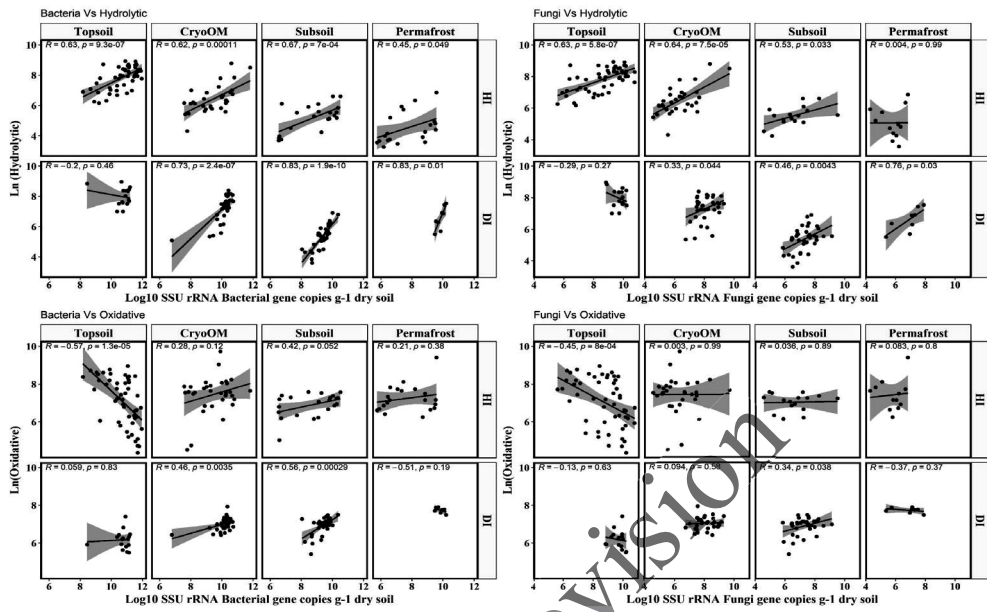


Figure S2. Pearson's correlation between enzyme activity and microbial gene abundance.

Paper IV

**Patterns of belowground active biota associated
with subducted carbon pockets in permafrost soils
of Greenland**

Petter*, S., Varsadiya*, M., Liebmann, P., Schnecker, J., Guggenberger,
G., Bárta, J., Urich, T.

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(* Equal contribution in writing manuscript)

Patterns of belowground active biota associated with subducted carbon pockets in permafrost soils of Greenland

Sebastian Petters*¹, Milan Varsadiya*², Patrick Liebmann³, Jörg Schneckner⁴, Georg Guggenberger³, Jiří Bárta^{2,5}, Tim Urich¹

¹Institute of Microbiology, Center for Functional Genomics of Microbes, University of Greifswald, Greifswald, Germany; ²Department of Ecosystem Biology, University of South Bohemia, České Budějovice, Czech Republic; ³Institute of Soil Science, Leibniz Universität Hannover, Hannover, Germany; ⁴Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria; ⁵Centre for Polar Ecology, Faculty of Science, University of South Bohemia, 37005 České Budějovice Czech Republic

*Equal contribution in writing manuscript

Abstract

Large proportions of terrestrial carbon (C) are stored in Arctic permafrost affected soil. These soils can feature buried organic matter (OM) due to cryogenic processes (cryosols) and can harbor undecomposed organic C. A lack of active predation, a driving force in the nutrient loop of food webs, can lead to a slowed-down degradation of available C. To understand the prey predation relation in the Arctic soil, we employed a metatranscriptomics approach. We collected soil samples from different horizons of two distinct vegetation sites from Disko Island, Greenland. In metatranscriptomics, a three-domain community profiling approach of different Arctic soil horizons was investigated. They were compared regarding their microbial community composition including all domains of life. Furthermore, abundances of different pro- and eukaryotic micro predator were examined, and identified taxa were assigned to functional groups involved in the C and the nitrogen (N) cycle. We found that RNA yields positively correlate with the C content and dissolved organic C of the horizon and that the composition of the microbial community in buried organic layers rather matches that of mineral subsoils instead of organic top layers. The metatranscriptomics data showed major differences in prey and predator abundance of top layers and buried horizons. The abundance of micropredators, the drivers of the nutrient loop, decreased in buried OM horizons, while myxobacteria remained remarkably constant and comprised high proportions of the total communities in all horizons. Correlations between functional guilds and biotic and abiotic parameters revealed fundamental disconnections between C and N cycles in buried layers and suggest a major impact of myxobacteria on the N cycle. The

study promotes quantitative metatranscriptomics as a promising tool for analyzing complex soil microbiomes.

Introduction

Arctic permafrost soils are important components of the global C cycle (McGuire et al. 2009). Having acted as C sinks since the beginning of the Holocene, a vast proportion of terrestrially stored organic C (OC) can be found in Arctic permafrost soils (McGuire et al. 2009; Tarnocai et al. 2009). Permafrost-affected soils (cryosols) store more than twice as much C as is currently contained in the atmosphere (Tarnocai et al. 2009). These permafrost soils can contain pockets or layers in the subsoil, that have buried OC. Two processes account for the transfer of high-organic topsoil material to these deeper layers, cryoturbation, and solifluction (Tarnocai et al. 2009). Cryoturbated mineral soils contain more than one-third of the soil OC (SOC) in Arctic permafrost soils (Kaiser et al. 2007; Tarnocai et al. 2009). Remarkably, the OC in these buried layers remains largely undecomposed as compared to OC in the topsoils. Besides physicochemical parameters like temperature, moisture, and oxygen availability, the microbial and fungal community structure and the accessibility of soil organic matter (SOM) to the decomposer community are thought to be crucial factors in the process of SOM accumulation and storage in these cryoturbated soils (Schmidt et al. 2011; Dungait et al. 2012; Schädel et al. 2014). The underlying processes behind this phenomenon remain largely unknown (Tarnocai et al. 2009), although considerable progress has been made in recent years (Wild et al. 2013, 2014; Schnecker et al. 2014; Gittel et al. 2014a, b; Varsadiya et al. 2021a, b). For instance, previous DNA-based studies revealed that the microbiome compositions of the buried organic layers rather resemble those of the neighboring mineral horizon than those of the organic top layers (Gittel et al. 2014a, b; Varsadiya et al. 2021a, b). It was also found that bacterial gene abundances in the buried organic matter were surprisingly high, as in top layers. In contrast, fungal gene abundances decreased with depth and were significantly lower in buried organic matter than in top layers, resulting in remarkably low fungal to bacterial ratios in buried organic matter. However, the main gap in our current understanding of soil organic matter stabilization in buried soil horizons and its vulnerability to decomposition is the structure and the SOM degradation capacities of the decomposer community. For instance, next to nothing is known about other members of the belowground biota, such as various

protist groups and the micro- and mesofauna. However, all members of the belowground biota interact in the soil food web.

One major function of trophic interactions is the release of nutrients, such as N (Clarholm 1985; Bonkowski 2004). It was shown that organic and inorganic N transformation rates in Siberian soils were significantly lower in cryoturbated than in organic topsoil horizons, which indicated a deceleration of the entire N cycle in these buried horizons (Wild et al. 2013). This might indicate an altered trophic structure of the microbial food web therein. The traditional view is that energy from the decomposition of OM will flow through either a “fast bacterial channel” or a “slow fungal channel” (Moore et al. 1988). The bacterial channel is characterized by rapid turnover of C and fast cycling of nutrients while the fungal channel is characterized by the slow decomposition of biopolymers (e.g., cellulose, lignin), the long generation time of fungal biomass and therefore slower turnover of C. Energy flows through bacterial and fungal channels also influence the higher trophic levels of bacterial and fungal grazers. While the major bacterial grazers are protozoa and nematoda, grazers of fungi are typically fungus-feeding microarthropods (Beare et al. 1995; Scheu and Setälä 2002). In addition to the traditionally known predators of bacteria, such as different protozoa and nematodes (Clarholm 1985; Bonkowski 2004), several bacterial groups can prey on bacteria as well (Clarholm 1985). These include, for instance, the *Bdellovibrio* and like organisms (BALO) from the order *Bdellovibrionales* (Jurkevitch 2007). Another group of predatory bacteria is the long-known group of myxobacteria, which comprise the order of *Myxococcales* (Reichenbach 1999; Keane and Berleman 2016; Petters et al. 2021). However, a broad assessment of both prokaryotic and eukaryotic players in the belowground food web has been impossible until recently (Geisen et al. 2015). The application of metatranscriptomics, a primer- and PCR-independent RNAseq approach now enables such analyses (Urich et al. 2008; Petters et al. 2021).

Using this metatranscriptomics, three-domain profiling approach we aimed at obtaining a holistic census of the biota in the active layer of cryoturbated soils in Greenland, from the upper topsoil down to the frozen permafrost. Next to the identification and quantification of bacteria, archaea, fungi, protists, and animals, we aimed to explore if altered trophic structure is present in the subsoil's horizons. Lastly, we linked the identified biota to patterns of N cycling in topsoil and buried OM pockets.

Methods

Sampling sites

Cryoturbated soils were sampled in August 2017 in Blaesedalen, Disko Island, Greenland (Figure 1). Two sites were selected based on different slope orientations and parent materials. Site 1 was located on a south-oriented slope of a former end moraine, consisting of silt-rich glacial

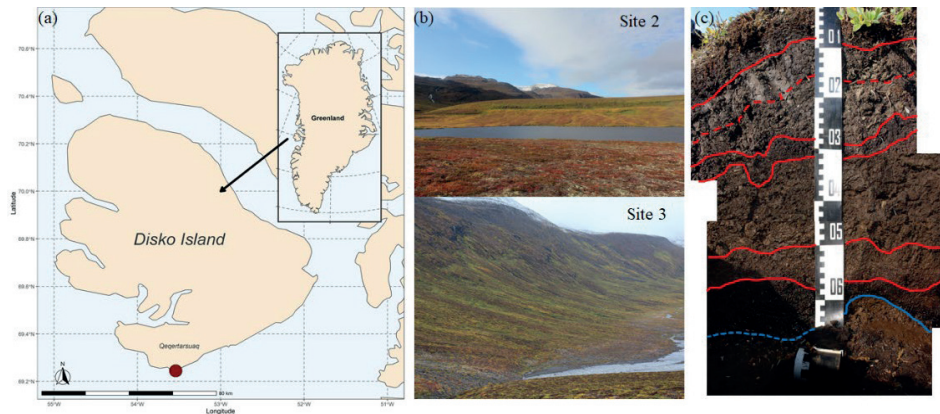


Figure 1. (a) Sampling location Disko Island, Greenland, (b) Sampling sites, and (c) Cryoturbated soil profile.

sediments and aeolian deposits as the parent material. Site 2 was located on a slope with an east orientation with basaltic bedrock as the parent material. Sites were approximately 1 km apart from each other along a northeast-southwest transect. At each site, four soil pits were excavated by preparing an up to 200 cm wide soil profile in the active layer down to the bedrock until the permafrost table (50-100 cm soil depth). The soil profile was cleaned, and soils were described and classified according to *Keys to Soil Taxonomy* (Schoeneberger, P.J., D.A. Wysocki, E.C. Benham 2012). Soil samples were taken with a sterilized soil knife into sterile plastic bags before sieving (2 mm mesh size) and homogenization. For RNA preservation, up to approximately 3 g of soil were immediately immersed with 2 volumes of LifeGuard Soil Preservation Solution (Qiagen, Hilden, Germany) and stored at 4 °C for about 1 month.

Soil physicochemical parameters

Soil water content (moisture) was measured by drying soil at 60 °C, reweighing the sample, and expressed in wet percentage. Water dissolved pH was measured from soil suspension with water at a solid to solution

ratio of 1:2.5 (w/v) using pH 3151i (Xylem incorporation GmbH, Hessen, Germany). The total C (C_{tot}) and N (N_{tot}) contents were determined from 60 °C dried and milled soil sample using an Elementar Vario Micro cube (Elementar, Langenselbold, Germany). The dissolved organic carbon (DOC) and dissolved nitrogen (DN) were quantified by mixing 1:5 (w/v) part of water for an hour and a filtered soil solution was used for LiquiTOC II (Elementar, Germany). C_{tot} and N_{tot} were expressed as percentages, whereas DOC and DN were expressed by ug/g dry soil.

RNA extraction and purification

In total 20 samples were chosen for RNA extraction. These included 18 active layer samples, comprising four samples from organic top layers (O horizon), two from mineral topsoils (A horizon), five from mineral subsoils (B horizon), and seven from buried organic layers. Two samples were from permafrost.

The lifeguard solution was removed via centrifugation. RNA was extracted from approximately 1 gram of soil with the RNeasy PowerSoil total RNA kit (Qiagen, Hilden, Germany). Additionally, DNA was coextracted using the RNeasy PowerSoil DNA elution kit (Qiagen, Hilden, Germany). RNA quality was assessed via 1% agarose gel and RNA quantity via Qubit RNA HS kit (ThermoFisher Scientific Schwerte, Germany). Residual DNA has been removed via RQ1 DNase 1 (Promega, Mannheim, Germany) digestion and cleaned using the MEGAClear kit (Life technology, Darmstadt Germany). MessageAmp-II Bacteria kit (Life technology, Darmstadt Germany) was used to amplify RNA and enrich rRNA. The amplified RNA was aliquoted and stored at -80°C or in an RNASTable LD matrix (Biomatrix, Taufkirchen, Germany) for shipment. Samples were sequenced via Illumina HiSeq PE sequencing by SEQme. Two sequencing runs were performed.

Bioinformatic analyses

Forward and reverse reads were then overlapped via FLASH (Magoc and Salzberg 2011). Quality filtering was done via prince-lite (Schmieder and Edwards 2011). SortMeRNA (Kopylova et al. 2012) was used to sort sequences into SSU rRNA, LSU rRNA, and non-rRNA. SSU rRNA sequences were length trimmed to 240 – 260 bp and subsampled to 50 000 sequences per sample via USEARCH (Edgar 2010). Sequences were mapped against the modified silvmod128 database (Lanzén et al. 2012)

via BLAST (Altschul et al. 1990) and analyzed via MEGAN5 (Huson et al. 2007).

3-Domain community profiles were exported from MEGAN and further analyzed via R (Ihaka and Gentleman 1996) using the packages *reshape2*, *skip a lot*, *RColorBrewer*, *vegan*, *riverplot*, *heatmap*. Bacterivorous bacteria, protozoa, and metazoan were identified according to (Petters et al. 2021). These included the bacterial orders Myxococcales and Bdellovibrionales, as well as the genera *Lysobacter*, *Daptobacter*, and *Vampirococcus*. Eukaryotic bacterivores screened for were nematodes (according to (Yeates et al. 1993)), as well as the Amoebozoa, Cercozoa, Ciliophora, Diplonemea, Euglenophyceae, Heterolobosea, Kinetoplastea, and Retaria (Geisen et al. 2015; Petters et al. 2021). Bacteria groups not belonging to one of the aforementioned predatory bacteria groups were defined as non-predatory prey bacteria. We are aware that this categorization is a simplification to the reality found in situ, and that several micro predator groups are facultative predatory or have a wider range of food sources. RNA per gram soil, Ctot, DOC, and PLFA data were tested for correlation.

Furthermore, SSU rRNA abundances per gram of dry soil were calculated according to (Söllinger et al. 2018), resulting in taxon-specific SSU rRNA abundances. These taxa were then functionally classified with FAPROTAX (Louca et al. 2016) to obtain an abundance of SSU rRNAs of functional groups per gram of dry soil. Specific functional groups belonging to the carbon and nitrogen cycle were correlated with abiotic parameters DOC and DN on a per gram dry soil basis. Respective investigated functional groups were aerobic chemoheterotrophy, aromatic compound degradation, cellulolysis, fermentation, hydrocarbon degradation, and methylotrophy from the C cycle and aerobic nitrite oxidation, aerobic ammonia oxidation, nitrate reduction, nitrate respiration, nitrification, nitrite denitrification, nitrous oxide denitrification, nitrogen fixation, and ureolysis from the N cycle. Furthermore, correlation analyses were done with pH, moisture, C/N ratio, Ctot, and Ntot. Correlation analyses of FAPROTAX functional groups were also done with the abundance of fungi, predatory protists, predatory bacteria, myxobacteria, and Bdellovibrionales on a per gram dry soil basis.

Results

Depth heterogeneity in Turbic cryosols and associated microbiomes

We analyzed the microbiomes in cryoturbated soils in Blaesedalen valley, Disko Island (Greenland). Overall, we investigated six pits of cryoturbated soils in two different locations. At each pit, we aimed to sample topsoil/layers (O or A horizons), cryoturbated layers (JJ), and the surrounding subsoils (Table 1). The average pH value was around 6.4 for topsoil, subsoil, and cryoturbated soil. The pH was lower in permafrost layers (around 6.2) and the organic top layer (around 6.3). The low values here may well be a result of plant exudates found in these layers. Moisture varied strongly among the different horizons, with the highest moisture content found in O and jj layers (68.7% and 57%, respectively) and the lowest in B. As expected, C_{tot} content was highest in the top O layer with 24.3% and the C_{tot} content in jj layer was the second highest (mean 15.3%), and much higher than the total C content in the surrounding mineral horizons (Table 1). Similar patterns were detected for DOC, DN, and total N. These data show the widespread presence of pockets with a high OC content in these subsoils.

We extracted RNA from the soils for metatranscriptomics analysis and quantified the RNA content per gram of soil. To explore the value of soil RNA content as a marker for microbial biomass and thus the suitability of quantitative metatranscriptomics, we compared soil RNA content with several parameters, such as C_{tot} and DOC content (Figure 2). The O horizon samples with high C_{tot} content had high RNA content, while mineral soils had a much lower RNA content. From the buried horizons an intermediate amount of RNA was extracted. Generally, a linear positive correlation was found (0.71) between RNA content and C_{tot} (Figure 2a). Similar results were obtained when comparing RNA content and DOC (0.73) (Figure 2b). This hints at a direct connection between RNA content and available organic carbon in the soils and shows how buried carbon does not appear as an exception from that pattern. Furthermore, we compared RNA content with phospholipid fatty acid (PLFA) derived C content, a widely used proxy for microbial biomass in soils (Schnecker et al. 2014). We found a linear positive correlation between RNA concentration per gram of soil and the PLFA data (Supplementary Figure S1), pointing towards the validity of RNA-based quantitative analyses, such as quantitative metatranscriptomics for soil microbiome analyses.

ID	Horizon	depth (cm)	pH	DOC (µg/g)	DN (µg/g)	DOC/DN ratio	Ctot (%)	Ntot (%)	CN ratio	Moist. (%)	OC (bulk soil)	total N (bulk soil)	δ 13C bulk	δ 15N bulk
Site 2														
Pit F Latitude: 69,27124; Longitude: -53,47444; Altitude: 92,37														
F1	O	0-6	6,3	324,8	5,3	61,1	25,3	1,0	24,3	67,9	313,6	11,8	-26,8	-1,6
F11	j	41-47	6,2	309,0	18,4	16,8	29,8	1,7	17,5	71,0	93,6	6,3	-25,2	-0,3
F13	j	47-51	6,2	257,5	19,6	13,2	27,9	1,7	16,8	66,8	284,2	16,1	-25,9	-0,5
F15	ff	51-54	6,1	180,9	5,2	34,9	7,2	0,4	17,6	44,6	334,7	17,5	-26,1	-1,9
Pit G Latitude: 69,271632; Longitude: -53,475903; Altitude: 108,88														
G1	O	0-12	6,2	393,5	15,3	25,7	30,5	1,5	20,5	75,2	310,4	14,8	-26,7	-1,3
G5	B	33-42	6,4	69,6	3,4	20,6	2,0	0,2	13,2	30,0	18,0	1,3	-24,9	0,8
G9	j	44-60	6,2	149,9	3,8	39,5	10,6	0,7	15,9	55,5	82,2	5,4	-25,5	-1,4
Pit H Latitude: Longitude: Altitude:														
H3	A	0-25	6,4	113,2	2,9	39,2	3,9	0,2	16,9	37,2	29,7	2,1	-25,4	0,6
H5	j	25-28	6,5	192,6	4,2	45,5	7,8	0,5	15,9	63,3	96,7	6,8	-25,6	0,6
H7	ff	28-48	6,3	228,7	5,8	39,8	10,9	0,8	13,9	59,7	65,6	3,9	-25,8	-0,9
H11	ff	28-48		266,5	8,7	30,8	17,3	1,2	15,0	65,7	320,6	14,1	-27,6	-2,8
Site 3														
Pit J Latitude: 69,277936; Longitude: -53,492568; Altitude: 104,46														
J1	O	0-15	6,4	216,3	8,0	27,1	34,4	1,5	22,4	70,8	96,0	6,6	-25,5	-0,4
J9	j	41-46	6,3	134,1	2,3	58,5	11,4	0,8	15,0	52,0	27,0	2,1	-25,3	1,3
J11	B	46-49	6,5	99,5	13,4	7,4	3,0	0,2	14,3	35,0	152,6	9,6	-25,8	-0,4
J13	j	49-54	6,2	152,2	3,5	43,2	16,8	1,1	15,6	54,4	33,7	2,6	-25,1	1,7
Pit K Latitude: 69,277544; Longitude: -53,49209; Altitude: 118,46														
K1	A	0-22	6,9	78,3	1,4	54,3	2,2	0,2	14,5	22,3	81,6	5,6	-25,6	0,8
K5	j	29-32	6,3	145,9	2,7	53,2	17,2	1,1	15,6	55,1	22,0	1,7	-25,4	1,2
K7	B	32-65	6,9	67,4	1,3	52,9	1,8	0,1	14,6	20,4				
Pit L Latitude: 69,277438; Longitude: -53,491029; Altitude: 115,86														
L3	A	0-41	6,7	169,1	3,6	47,6	7,1	0,5	15,0	60,6	142,7	8,2	-26,2	-1,3
L5	j	50-67	6,2	162,0	6,6	26,3	15,7	0,9	17,5	52,6	22,6	1,7	-25,1	0,8
L7	B	110-120	6,4	89,1	2,8	32,3	2,5	0,2	15,3	23,2	142,7	8,2	-26,2	-1,3

Table 1: Soil physicochemical parameters. For abbreviation refer to Materials and Methods.

Differences in the microbiome composition between samples were analyzed using non-metric multidimensional scaling (Figure 2c). Three clusters were found. One cluster contained all three microbiomes from the top O horizon and one from the mineral A horizon. Another cluster had microbiomes from the remaining two topsoil horizons, all four subsoil horizons, and five buried organic pockets grouped. And in a third one, microbiomes from the three remaining cryoturbated pockets and the two permafrost layers clustered together. With the two NMDS axes combined, a depth gradient could be identified. Microbiomes from the buried organic pockets are mostly clustered together with those from mineral subsoils. The RNA-based results go in line with DNA-based findings from permafrost soil (Gittel et al. 2014a, b; Varsadiya et al. 2021a, b). Additionally, a site effect between the 10 samples of each sampling site could be detected (Supplementary Table S1).

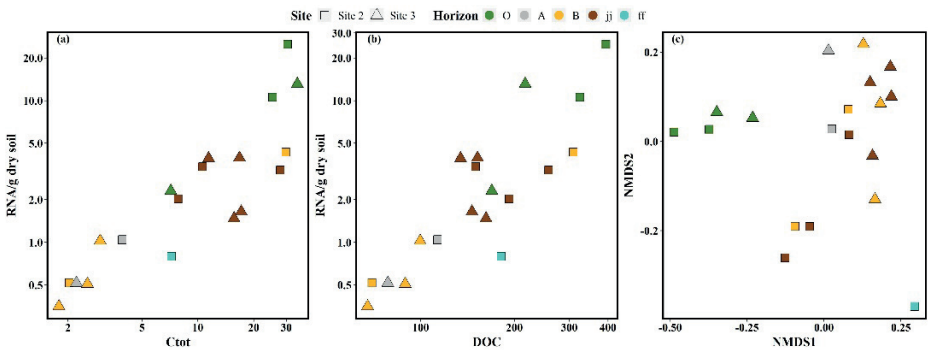


Figure 2. RNA yield and phylogenetic dissimilarity. (a) RNA yield per gram soil against total C, (b) RNA yield per gram soil against DOC, and (c) the phylogenetic dissimilarity between soil samples by NMDS plot. Different colors and shapes represented distinct horizons and sites, respectively. Green, top organic layer (O); Gray, mineral topsoil (A); Gold; mineral subsoil (B); Brown, buried OM (jj); Blue, permafrost (ff); Square, Site 2; Triangle, Site 3.

Census of (micro-)biomes in cryoturbated permafrost soils

We used SSU rRNA-based metatranscriptomics three-domain community profiling (Urich et al., 2008) to reveal for the first time the composition of Bacteria, Archaea, and Eukaryotic (micro-)organisms in permafrost-affected soil (Figure 3a). A strength of the PCR- and primer-independent

SSU rRNA approach is the direct comparability of Archaea, Bacteria, and Eukaryota within one analysis. As expected, the Bacteria were the dominant group, comprising more than 82.3% of all SSU rRNAs. Eukaryotes were less abundant, with approximately 17.5% of SSU rRNA. The Archaea domain was by far least abundant. The Archaea were dominated by the phylum Thaumarchaeota, followed by Euryarchaeota (Figure 3b). The Bacteria were composed of taxa typically found in soils and permafrost soils (Figure 3c). The most abundant classes were Actinobacteria, Gammaproteobacteria, and Thermoleophilia. Other abundant classes were Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria.

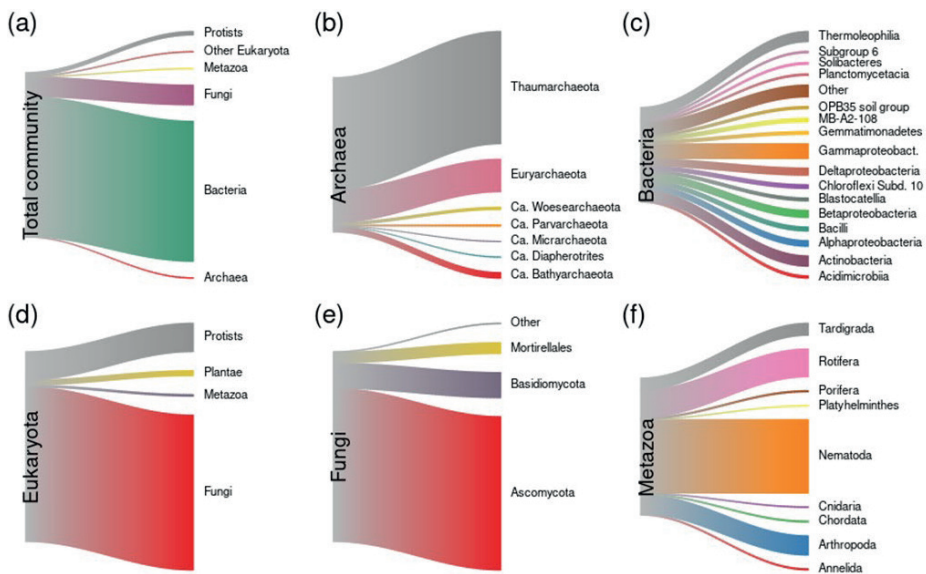


Figure 3. SSU rRNA abundance of the Arctic soil community. The size of the edges displays the number of SSU rRNA read counts. (a) Total community, (b) Archaea, (c) Bacteria, (d) Eukaryota, (e) Fungi, (f) Metazoa.

Within the same dataset alongside the two prokaryotic domains, also eukaryotic groups like Fungi, Metazoa, and different Protists were detected (Figure 3d). The most abundant eukaryotic group was the Fungi, while animals (Metazoa) were the least abundant, and various Protists groups in between. The Fungi were dominated by the Ascomycota (Figure 3e) and Basidiomycota, whereas the Mortirellales were less abundant. The Metazoa were mainly comprised of Nematoda, followed by Rotifera, Arthropoda, and Tardigrada. The three dominant Protist groups were

Alveolata, Amoebozoa, and Rhizaria (Fig. 3f). Other abundant groups were Excavata and Stramenopiles, while Hacrobia, Apusozoa, and Breviatea were less abundant.

Horizon resolved profiling reveals major differences in (micro-)biomes of topsoils and buried horizons

To assess differences in (micro-)biomes along the active layer horizons, we analyzed horizon specific SSU rRNA data (Fig. 4). There was no clear difference between horizons on the domain level (Fig. 4a), however, within each domain clear depth trends were visible. Within the Archaea, Thaumarchaeota were abundant in all active layer horizons, while Euryarchaeota dominated in the permafrost samples (Fig. 4b). The O horizons were populated by distinct bacterial communities, dominated by Gammaproteobacteria and Bacilli. In contrast, Actinobacteria and Thermoleophilia were prominent in the mineral topsoil, subsoil horizons, and the permafrost soil (Fig. 4c). The Eukaryotes were dominated by Fungi in all horizons (Fig. 4d). Among the Fungi, a clear depth trend was observed with the Mortirellales (Fig. 4e). Ascomycota was generally dominant in all horizons. Very few Metazoa were found, mainly in the O and A topsoils. In contrast, rather high abundances of protist groups were found, again, especially in the topsoils (Fig. 4d). The Metazoa showed some trends with depth as well (Figure 4f). Animals in the organic top layer were mainly comprised of Arthropoda, Nematoda, Rotifera, and Tardigrada, with Nematodes showing the most pronounced abundance, more than 40% of SSU rRNA reads. This dominance remained constant in the mineral topsoil, as did the abundance of Arthropoda, around 20%. The Tardigrada decreased from around 20%, while the Rotifera increased to more than 20%. The mineral subsoil, and the buried organic layer alike, were almost completely dominated by Nematoda and Rotifera. Especially larger-bodied animals, such as Annelida, Arthropoda, and Tardigrada decreased with depth. In the permafrost samples, only Rotifera SSU rRNA was found.

The abundance of bacterivores decreased in buried OM horizons.

We analyzed the abundance of players in the microbial food web, with a focus on bacterivorous micropredators, such as protozoa, nematodes, and predatory bacteria like myxobacteria and Bdellovibrionales (Figure 5).

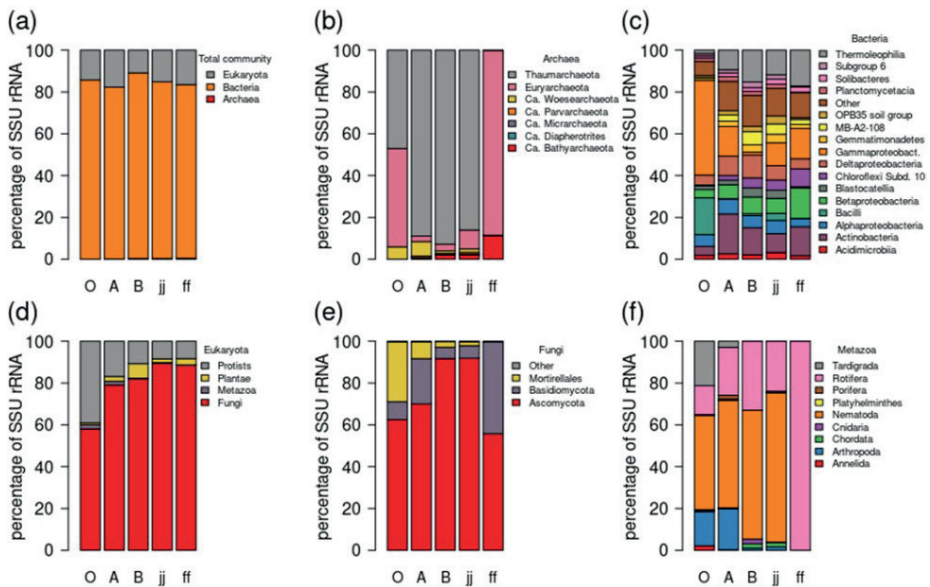


Figure 4. Horizon resolved community composition. Read counts normalized to 100%. O: organic top layer. A: mineral topsoil. B: mineral subsoil. jj: buried organic layer. ff: permafrost. (a) Total community. (b) Archaea. (c) Bacteria. (d) Eukaryota. (e) Fungi. (f) Metazoa.

The most abundant protozoa in the horizons were the Alveolata, accounting for more than 40% of all protists (Fig. 5a). Amoebozoa were also abundant, with the highest proportion in mineral topsoils, more than 40% of all protozoa groups. The third most prominent group was the Rhizaria, showing high abundances in the organic top layer and the permafrost. Overall, bacterivorous protozoa had their highest abundance in the topsoils (O and A horizons). Their relative abundance in the microbiomes significantly decreased with depth and was significantly lower in the buried organic layer, as compared to the topsoil ($p = 0.04$). Compared to other micropredators, they were the second most abundant group after the myxobacteria, generally below 1% of the total SSU rRNA in all horizons.

The Nematoda showed remarkable compositional differences between the horizons. The organic top layer was dominated by the bacterivorous Dorylaimida and Rhabditida (Fig. 5b). The mineral topsoil was dominated by Dorylaimida and the plant-parasitic Tylenchida, accounting for more

than 93.1% together. The latter heavily dominated mineral subsoil and buried carbon layers (approx. 90% of all nematodes). When compared to other micropredators across all horizons, the Nematoda were the least abundant group. In the two top layers (O and A horizons) their abundance was much higher than in the deeper horizons, including the buried layers. The difference between the organic top layer and buried organic layers was significant ($p = 0.03$).

A closer look was taken into the myxobacteria composition. They were dominated by the family *Haliangiaceae*, which comprised 40 - 60% in all horizons (Fig. 5c), similar to what has been observed in other soils (Petters et al., 2021). When compared to other bacterivorous groups, their abundance was higher (average of 4.8% of the total SSU rRNA; Fig. 5d) and remained rather constant overall horizons. It did not significantly decrease with depth. The Bdellovibrionales were less abundant, below 0.5% of the total community, and slightly decreased with depth. However, the difference between the organic top layer and the buried organic carbon layer was not significant ($p = 0.18$).

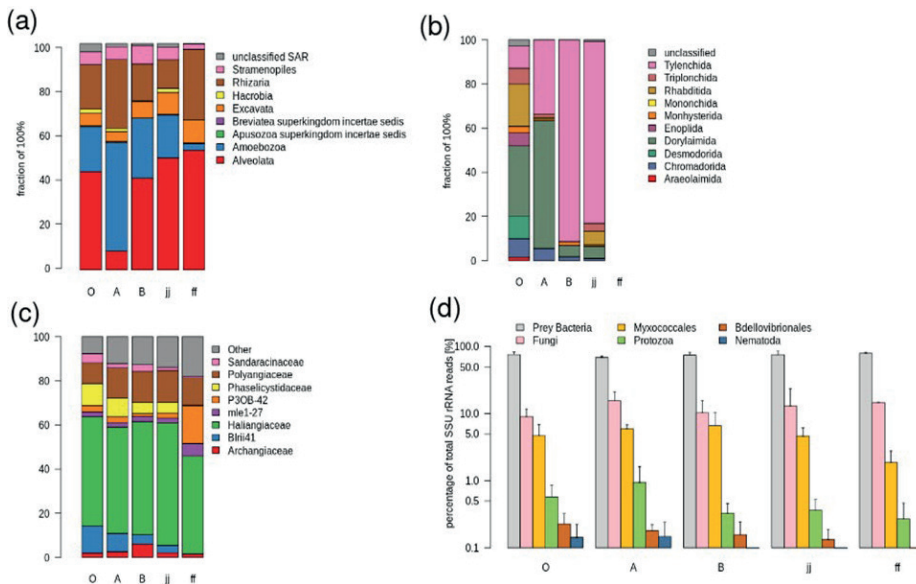


Figure 5. The abundance of prey and predatory organisms in different soil layers. O: organic top layer. A: mineral topsoil. B: mineral subsoil. jj: buried organic layer. ff: permafrost. (a) Prey and predatory organisms. (b) Myxococcales. (c) Nematoda. (d) Protists.

Links between predatory myxobacteria and soil nitrogen cycling.

Using quantitative metatranscriptomics, we aimed to resolve linkages between the abundance of micropredators, microbial functional groups of the C and N cycles, and soil abiotic parameters. For this purpose, SSU rRNA read counts were calculated per gram of soil according to (Söllinger et al. 2018). Functional groups of the soil C and N cycle were identified with FAPROTAX, and correlation matrices were calculated to identify statistically significant links between the functional groups and micro predator abundance. Analyses were done for organic top layers and buried organic pockets (Figure 6) to identify differences between microbiomes in them that might be linked to C storage. In the organic top layers, the abundance of microbial functional groups belonging to both the C cycle and N cycle were positively correlated with both biotic factors (i.e., fungi, protists, and predatory bacteria) and abiotic parameters (i.e., DOC, DN, Ctot). The only exceptions were soil pH and moisture. In contrast to that, buried organic layers showed a different pattern. Here, the functional groups associated with the C cycle positively correlated only with abiotic parameters. Contrary, the functional groups belonging to the N cycle mainly showed positive correlations with micro predator abundance. In particular, ammonia oxidizers showed a strong positive correlation with myxobacteria and predatory bacteria.

Discussion

Quantitative metatranscriptomics as a tool for soil biology

Microbiomes in cryoturbated permafrost soils are comparably poorly studied to date. In this study, we used a holistic metatranscriptomics community profiling approach (Urich et al. 2008) to obtain a broad view into all three domains of life and across all horizons of cryoturbated permafrost soils. By integrating total RNA amounts per gram soil and relative transcript abundance, we employed quantitative metatranscriptomics. Using this method, we were able to quantify amounts of SSU rRNA reads of both pro- and eukaryotic taxa per gram of soil. Ground truthing with a comparison of RNA and PLFA, an established marker of microbial biomass (Schnecker et al. 2014) showed positive correlations between RNA and PFLA data.

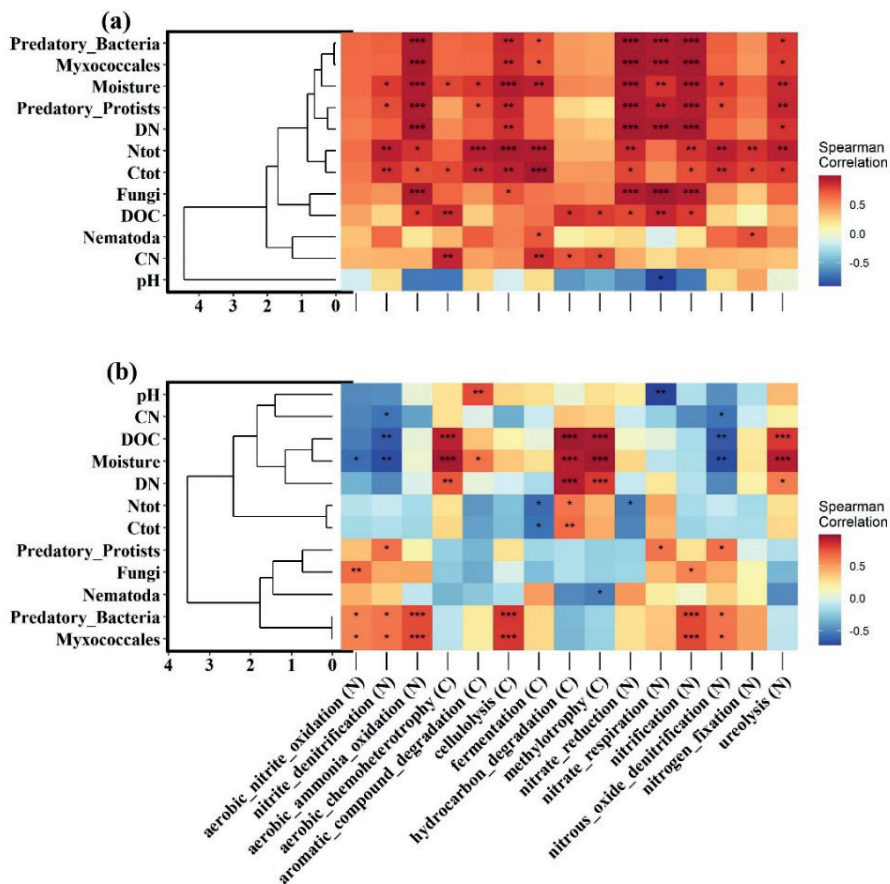


Figure 6. Correlation matrix of functional groups and different biotic (taxonomic groups) and abiotic factors. Functional groups were assigned by FAPROTAX pipeline using SSU rRNA abundances. Abundances of both functional and taxonomic groups were based on quantified SSU rRNA abundances. (a) Organic top layer. (b) Buried organic layer.

Broad and at the same time quantitative metatranscriptomics are promising tools for the analysis of complex soil communities and are not restricted to prokaryotes. In particular, it enables the simultaneous identification and comparison of eukaryotic and prokaryotic micropredators and thus studies of the soil microbial food web.

Our RNA-based analyses of the microbiomes of cryoturbated soils in Blaesedalen on Disko Island showed distinct patterns of organism distribution and abundance in all three domains of life. Generally, high microbial biomass/RNA content was found in organic topsoils and the

cryoturbated pockets as compared to the mineral top and subsoils. This is congruent with earlier studies of such soils in Siberia, Greenland, and Canada (Schnecker et al. 2014; Gittel et al. 2014a, b; Varsadiya et al. 2021a, b). Another similarity was the observation that the microbial community in the buried pockets was more similar to the surrounding mineral subsoils than to the organic topsoils (Gittel et al. 2014a, b; Varsadiya et al. 2021a, b). These two findings point to a potentially global pattern in cryoturbated permafrost soil microbiomes. This deserves further attention since the underlying structuring mechanisms might hold the key to the retarded SOM decomposition in the subsoil.

Significance of micropredators for OC decomposition in buried organic pockets

As mentioned before, micropredators of bacteria are key players in the soil microbial food web. By preying on other organisms, specific enzymes can set free the nutrients that are then again available for other organisms. This process drives the activity of the microbial food web and its structure (Clarholm 1985; Christensen et al. 1992; Griffiths and Caul 1993; Griffiths et al. 1993; Bonkowski et al. 2000). Thus, a lack of active micropredators might lead to a hampered degradation of OC and a slow-down of this microbial loop entire. When comparing the top layers of the horizons to buried carbon, the abundance of prey bacteria remained constant with slight changes in their composition. Micropredators showed a decreased abundance in deeper layers, including buried organic pockets. Remarkably, the abundance of myxobacteria was found to be rather constant over the soil horizons. The aforementioned hypothesis, where one major role in a slowed-down degradation of C in buried organic matter was a decrease of active micropredators, was partially confirmed. On the one hand, bacterivorous protozoa and nematodes as well as Bdellovibrionales were found in reduced abundances. The decreases in eukaryotic and prokaryotic (Bdellovibrionales) predators' abundance with increasing depth have been observed in previous study (Petters et al. 2021). With increasing depth, the bacterial biomass decreases due to lower availability of SOC which could diminish the growth of predators. Not only the prey microbial biomass, but also the soil pore size could restrict the eukaryotic predators to access the bacterial prey. Deeper soil are more compact and pore size decreases with increasing depth which could result in compartmentalization of prey and predators organisms, as was observed in previous studies (Griffiths 1990; Rønn et al. 2001; Erktan et al. 2020). However, small sized prokaryotic predators such as myxobacteria could be

able to assess prey bacteria via small sized pores. In support of this idea, we also found constant abundance of myxobacteria across the soil profile, which was already evidenced recently (Petters et al. 2021). Thus, microbes living in non-continuous capillary pores are protected from bigger cell sized eukaryotic predators in subsoil but not from similar sized prokaryotic predators such as myxobacteria.

Furthermore, we found significant positive correlations between ammonia oxidizers and predatory bacteria, predatory protists, and fungi. It is known that micropredation provides ammonia oxidizers with ammonia (Treuner-Lange et al. 2017). There are two types of hydrolytic enzymes, the aminotransferase, and the glutamate dehydrogenase, that cleave amino acids and liberate ammonium. We found critical differences between top organic matter and buried organic layers for the correlations. On top of that, the activity and number of microbial functional guilds involved in the C and N cycle were controlled by both the number of available forms (DOC and DN) and the activity of predators in the organic layer. In contrast, in buried OM layer, predators regulate the activity and amount of N guilds (i.e., nitrifiers and denitrifiers), but the activity of C guilds (i.e., methanotrophic, methanogenic, lignin degradation, and hydrocarbon decomposition) was controlled by the amount of available C. These findings showed that C and N cycle in buried organic layers were controlled by different mechanisms: C cycle was controlled bottom-up, but N cycle top-down. In particular, ammonia oxidizers were mainly positively associated with myxobacteria. These findings suggest a closed cycle in these horizons and render myxobacteria an important part of this potential loop. This fundamental disconnection of the C and N cycles may be one of the reasons for the very slow decomposition of SOM in buried organic layers. These findings confirm previous studies (Schnecker et al. 2014). While in the organic top layer ammonia oxidizers seemed to correlate with a variety of taxonomic groups and micropredators, they only showed positive correlations with myxobacteria in buried organic layers. However, the predatory behavior of myxobacteria is not entirely clear, since the majority of myxobacteria are facultative predatory and can have a saprotrophic lifestyle as well (Rosenberg et al. 2014). Thus, it remains unclear whether and to what extent different groups of myxobacteria exhibit prey on bacteria *in situ*. Nevertheless, the effects of these altered food web structures in deeper organic layers could harbor interesting insights into C storage and nutrient cycles of Arctic permafrost soils and remain a subject of deeper analyses.

Conclusion

The present study provides comprehensive information about active three domain of life in different horizons of Arctic permafrost through the metatranscriptomics approach and to best of our knowledge this the first study evaluating and quantifying active biota of different horizons of the Arctic permafrost parallel from same soil sample. This approach allowed to study abundance and composition of active prey-predator throughout the soil profile which suggested that predatory protist and nematodes abundance decreased with depth, whereas micropredator, myxobacteria, remained constant across the soil profile. We found strong positive correlation between N functional guilds and prokaryotic predators (myxobacteria) in buried OM, whereas C functional guilds with soil physicochemical parameters which suggested different biotic and abiotic control on N and C cycles in buried OM. This finding elucidates the important role of myxobacteria in microbial loop in this peculiar soil. Furthermore, the correlation between biogeochemical cycling guilds, abundance of active prokaryotic and eukaryotic predators, soil physicochemical parameter can hint the role of different trophic levels in biogeochemical cycles and could help us to better predict the vulnerability of buried OM in the Arctic permafrost.

Acknowledgments

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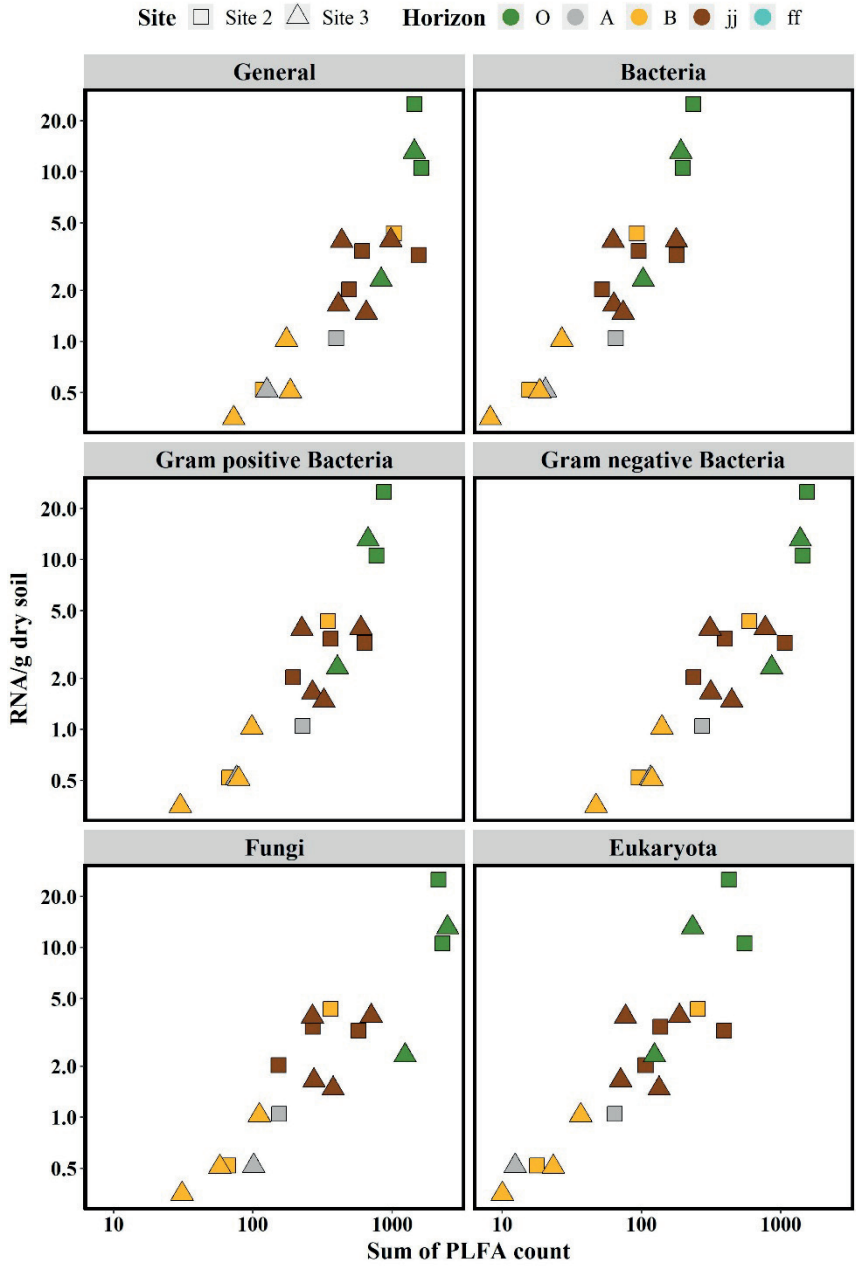
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Supplementary Materials

Supplementary Table S1: PERMANOVA analysis

Domain	Category	F-Model	R ²	P-value
All	Horizon	9.2	0.44	0.0001
	Site	9.9	0.12	0.0001
	Horizon*Site	2.7	0.1	0.0009
Bacteria	Horizon	10.3	0.47	0.0001
	Site	10.8	0.12	0.0001
	Horizon*Site	2.6	0.09	0.003
Fungi	Horizon	5	0.3	0.0001
	Site	9.3	0.14	0.0001
	Horizon*Site	3	0.13	0.0008
Eukaryota	Horizon	5.2	0.3	0.0001
	Site	8.9	0.13	0.0001
	Horizon*Site	3.4	0.15	0.0001

Supplementary Figure S1: Correlation between RNA content per gram of soil and sum of PLFA count at different domain levels.



10 CURRICULUM VITAE

Personal information

First name /Surname: Milan Varsadiya
Permanent address: Flat-D/1003, Shikshapatri Heights, Sayan Road, Amroli, Surat City, 394107, Gujarat, India
Telephone (office): +420 387 772 347
E-mail: milanvarsadiya@gmail.com
Date and place of birth: 15th January 1990, Chital Amreli, India
University address: Department of Ecosystem Biology, Faculty of Science, University of South Bohemia Branišovská 1760, 370 05 České Budějovice Czech Republic
Webpage: <http://kbe.prf.jcu.cz/en/milankumar-varsadiya>
<https://www.researchgate.net/profile/Milan-Varsadiya>
ORCHID ID: 0000-0001-6311-6439

Education

October 2016- University of South Bohemia in České Budějovice, České Budějovice, Czech Republic- PhD study (supervisor: Assoc. Prof. Ing. Jiří Bárta, Ph.D.)
April 2013 - September 2015 Technical University of Munich, Munich, Germany- Master in Horticulture Sciences (supervisor: Prof. Dr. agr. Dr. agr. habil. Dieter Treutter)
February 2014 – June 2014 Corvinus University, Budapest, Hungary- Erasmus semester as part of Master of Horticulture Sciences
April 2010 – March 2012 Veer Narmad South Gujarat University, Surat, India- MSc in Biotechnology (supervisor: Prof. Dr. R. Krishnamurthy)

April 2007 – March 2010 Sardar Patel University, Anand, India- BSc in Biotechnology

Scientific focus

Ecology of soil microorganisms involved in soil organic matter transformation in the Arctic Permafrost soil.

Work and Sciences experience

Member of fieldwork and sampling campaign in Disko Island, Greenland 2017 (Soil samples collection, *in-situ* DNA extraction)

Team member on the international project- MiCryoFun (Microorganisms in Arctic cryoturbated soils- drivers of organic matter transformations in a changing climate)

Team member on the international project- CryoVulcan (Vulnerability of carbon in Cryosols- substrate-microorganisms-aggregate interactions)

International courses

Soil-Water-Plants Interactions, University of Life Sciences, Tartu, Estonia, 2017

Stable Isotopic Course, Freising, Germany, 2017

Personal Skills and Competencies

Mother tongue: Gujarati

Other languages: Hindi
English, B2
German, A1
Czech, A1

Driving license: B

Publication in peer-reviewed scientific journals

Varsadiya, M., Urich, T., Hugelius, G., and Bárta, J. (2021a). Fungi in Permafrost-Affected Soils of the Canadian Arctic: Horizon- and Site-Specific Keystone Taxa Revealed by Co-Occurrence Network. *Microorganisms* 9, 1943. doi:10.3390/microorganisms9091943.

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Gałązka, A., Marzec-Grządziel, A., **Varsadiya, M.**, Niedźwiecki, J., Gawryjolek, K., Furtak, K., Przybyś, M., Grządziel, J. (2022). Biodiversity and Metabolic Potential of Bacteria in Bulk Soil from the Peri-Root Zone of Black Alder (*Alnus glutinosa*), Silver Birch (*Betula pendula*) and Scots Pine (*Pinus sylvestris*). *International Journal of Molecular Sciences* 23, 2633. doi:10.3390/ijms23052633

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Gałązka, A., Marzec-Grządziel, A., Grządziel, J., **Varsadiya, M.**, Pawlik, L. (2022). Fungal diversity and activity under trees as an indicator of potential biological weathering and soil formation – towards a better understanding of the Earth system dynamics (Under review in *Environment of Total Sciences*)

International conferences and workshop

Varsadiya, M., Bárta, J. Effect of root exudates on organic matter decomposition and microbial functioning in Arctic permafrost soils. ESM workshop 2016, Prague, Czech Republic. (talk)

Varsadiya, M., Šantrůčková, H., Urich, T., Hugelius, G., Bárta, J. Functional potential of microbial communities in cryoturbated organic

matter from Herschel Island. Ecology of Soil Microorganism (ESM) 2018, Helsinki, Finland. (poster)

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Varsadiya, M., Walter, D., Liebman, P., Petters, S., Šantrůčková, H., Urich, T., Guggenberger, G., Bárta, J. Potential enzymatic activity and bacterial community composition of permafrost affected (PAS) soil from Greenland. Pedologické day 2019, Srní, Czech Republic. (poster).

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milanvarsadiya@gmail.com

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University of South Bohemia in České Budějovice

Faculty of Science

Branišovská 1760

CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201

www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.c

