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

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

Ph.D. Thesis

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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

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❖ Acknowledgments

"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!)

I owe my deepest gratitude to Katka Diáková for her help in text correction and invaluable opinions.

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

❖ Table of Contents

❖ 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
N2O	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 (CO_2) and methane (CH_4) (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.

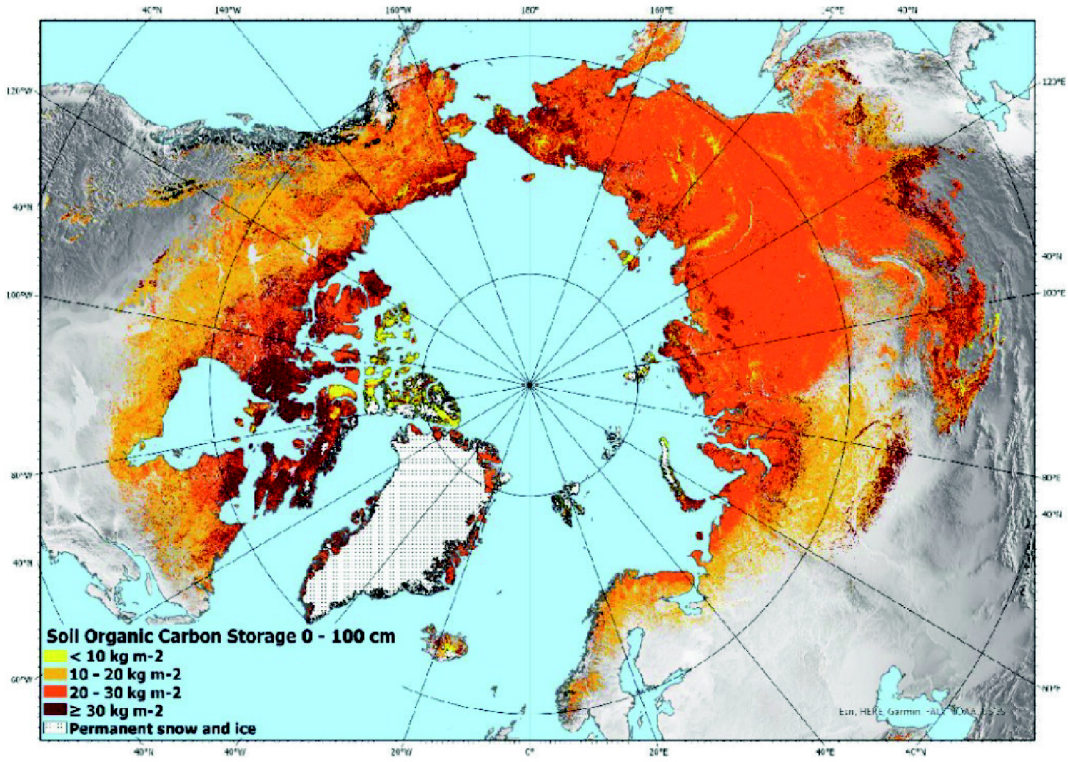

Figure 1: Estimated total storage of SOC (kg C m^2) to a depth of 0-100 cm in the northern circumpolar (Palmtag et al. 2022).

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).

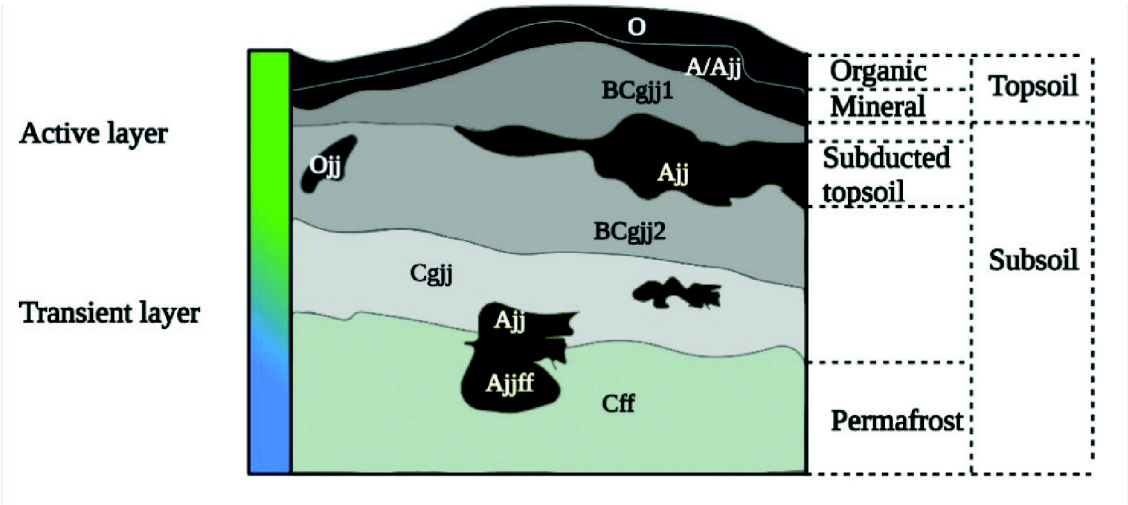

Figure 2: Schematic diagram of typical cryosols (modified from Gentsch et al. 2015a). O, organic layer; A, mineral topsoil; Ojj/Ajj, buried topsoil; B/C, mineral B/C horizons; Cff, permafrost.

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 CO_2, CH_4, and nitrous oxide (N_2O), 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).

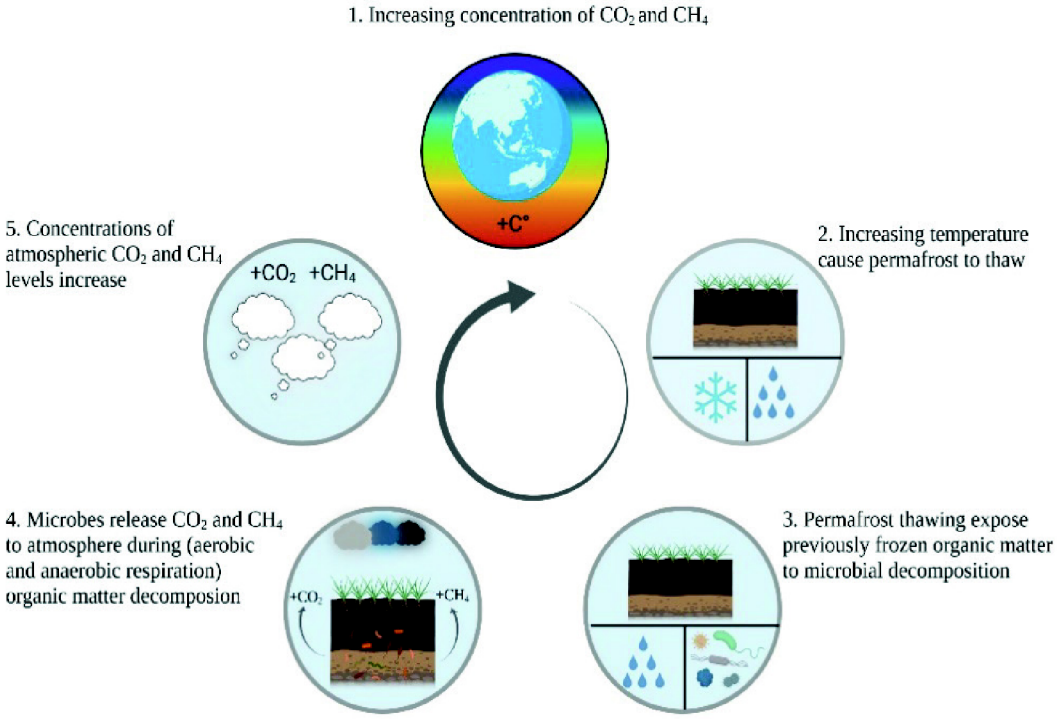

Figure 3: Positive feedback between global warming and permafrost Thaw (Original Varsadiya M).

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 CO_2 (Stackhouse et al. 2015; Drake et al. 2015) but a significant fraction (0 - 1 Tg CH_4 yr^{-1}) can be emitted as CH_4 (Saunois et al. 2020). The main producers of CH_4 are methanogenic archaea in anoxic environments that either reduce CO_2 by H_2 (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 the in 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 CH_4 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 CH_4, therefore, they dominate mostly in places where CH_4 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 CH_4 production rates; therefore, the diversity and ratios between type I vs. type II

methanotrophs will have an effect on the mitigation of CH_4 flux from the Arctic cryosols.

In contrast to aerobic CH_4 consumption, anaerobic consumption of CH_4 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 CH_4 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 CH_4 consists of altering the last step of methanogenesis that produces CH_4. 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 CH_4 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 CH_4 through anaerobic CH_4 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 CH_4 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 (Schirrmeister 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 (N_2). Among them, N_2-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, N_2-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 N_2-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 N_2 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 N_2-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 N_2-fixation, nitrification, and denitrification (Mengel 1996). Nitrifying bacteria are stimulated by the release of ammonium (NH_4^+) from protozoan grazers, presumably by preying on fast-growing bacterial competitors (Griffiths 1989; Alphei 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).

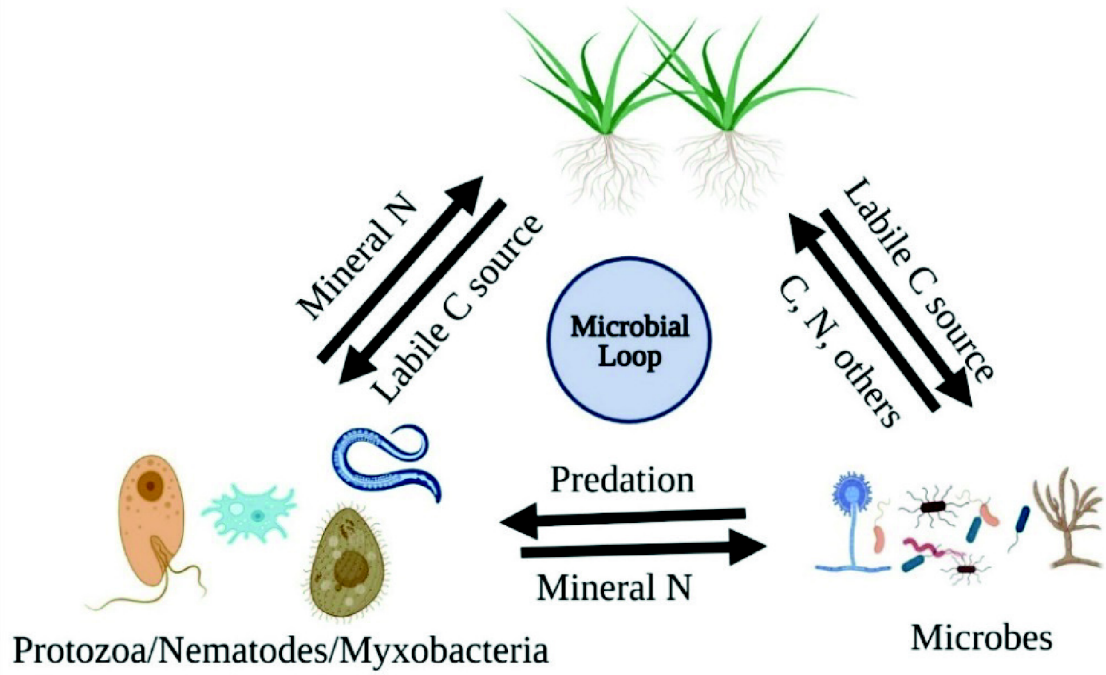

Figure 4: Schematic overview of the simplified illustration of microbial loop concept as applied to soil community (created by Varsadiya M according to Clarholm 1985).

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 CO_2 and CH_4. 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 inducive. 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 CH_4 production and N_2 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.

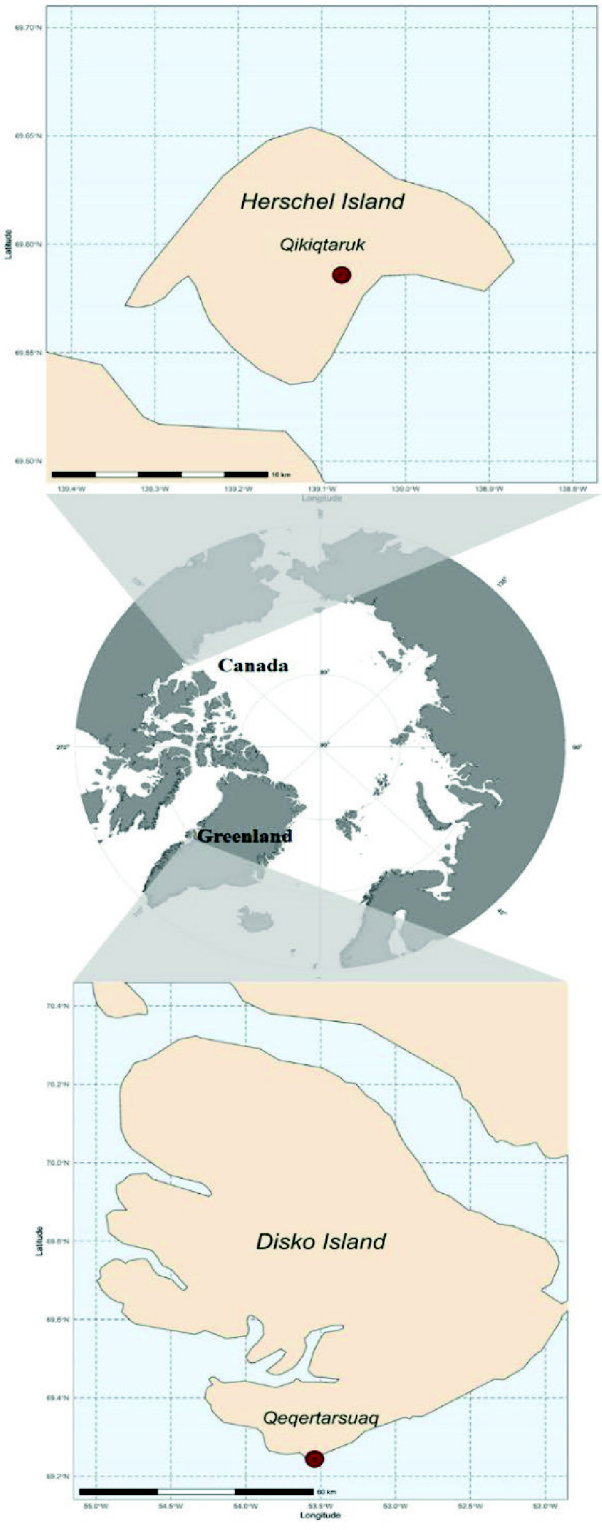

Figure 5: Sampling locations.

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 CH_4 emission (by methanogens- *mcrA* gene) and N_2 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 (Põlme 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).

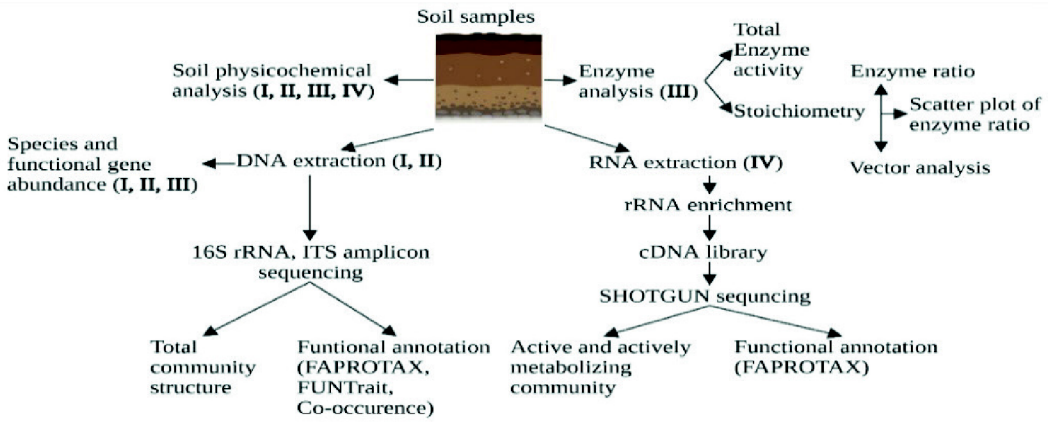

Figure 6: Analysis carried out in the present study.

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 N_2-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 (Upson 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* (Põlme 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 *Baglietoa* 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 CH_4 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 *Caldisericaceae*). *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 *Caldisericaceae* 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 (CO_2 and H_2) 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 CH_4 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 CH_4 production in cryoOM

Arctic cryosols can act as a source or sinks of CH_4 which can be decided by the balance between the upward diffusion of CH_4 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 CH_4 emission potential. However, we found hydrogenotrophic methanogens (using only H_2 and CO_2 as substrate) more proportional than the acetoclastic (broad range of substrate, for example, acetate, methanol, and H_2) 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 CH_4 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 CH_4 as a C source produced by methanogens (Kalyuzhnaya et al. 2006), and cryoOM may be a potential hotspot for CH_4 release as observed in previous studies (Ganzert et al. 2007; Jiang et al. 2010; Olefeldt et al. 2013; Lau et al. 2015b).

5.5 N_2 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 CO_2 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 CO_2 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 CO_2 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 alike 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, N_2-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; Alphei 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 $\delta^{15}N$) 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 CH_4 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 CH_4 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 CH_4 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, N_2-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.

8 REFERENCES

Achtnich C, Bak F, Conrad R (1995) Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. Biol Fertil Soils 19:65–72. https://doi.org/10.1007/BF00336349

Agerer R (2001) Exploration types of ectomycorrhizae. Mycorrhiza 11:107–114. https://doi.org/10.1007/s005720100108

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycological Progress 5:67–107. https://doi.org/10.1007/s11557-006-0505-x

Allison SD, Gartner TB, Holland K, et al (2007) Soil enzymes:linking proteomics and ecological process. In: Manual of environmental microbiology. ASM Press, Washington, DC, pp 704–711

Allison SD, Weintraub MN, Gartner TB, Waldrop MP (2010) Evolutionary-Economic Principles as Regulators of Soil Enzyme Production and Ecosystem Function. In: Soil Enzymology. pp 229–243

Alphei J, Bonkowski M, Scheu S (1996) Protozoa, Nematoda and Lumbricidae in the rhizosphere of Hordelymus europeaus (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. Oecologia 106:111–126. https://doi.org/10.1007/BF00334413

Anisimov OA, Shiklomanov NI, Nelson FE (1997) Global warming and active-layer thickness: results from transient general circulation models. Global and Planetary Change 15:61–77. https://doi.org/10.1016/S0921-8181(97)00009-X

Atkin OK (1996) Reassessing the nitrogen relations of Arctic plants: a mini-review. Plant Cell Environ 19:695–704. https://doi.org/10.1111/j.1365-3040.1996.tb00404.x

Ausec L, van Elsas JD, Mandic-Mulec I (2011a) Two- and three-domain bacterial laccase-like genes are present in drained peat soils. Soil Biology and Biochemistry 43:975–983. https://doi.org/10.1016/j.soilbio.2011.01.013

Ausec L, Zakrzewski M, Goesmann A, et al (2011b) Bioinformatic analysis reveals high diversity of bacterial genes for laccase-like enzymes. PLoS ONE 6:. https://doi.org/10.1371/journal.pone.0025724

Bai R, Wang J-T, Deng Y, et al (2017) Microbial Community and Functional Structure Significantly Varied among Distinct Types of Paddy Soils But Responded Differently along Gradients of Soil Depth Layers. Frontiers in Microbiology 8:. https://doi.org/10.3389/fmicb.2017.00945

Baldwin SA, Khoshnoodi M, Rezadehbashi M, et al (2015) The Microbial Community of a Passive Biochemical Reactor Treating Arsenic, Zinc, and Sulfate-Rich Seepage. Frontiers in Bioengineering and Biotechnology 3:. https://doi.org/10.3389/fbioe.2015.00027

Balk M, Weijma J, Friedrich MW, Stams AJM (2003) Methanol utilization by a novel thermophilic homoacetogenic bacterium, Moorella mulderi sp. nov., isolated from a bioreactor. Archives of Microbiology 179:315–320. https://doi.org/10.1007/s00203-003-0523-x

Balk M, Weijma J, Stams AJM (2002) Thermotoga lettingae sp. nov., a novel thermophilic, methanol-degrading bacterium isolated from a thermophilic anaerobic reactor. International Journal of Systematic and Evolutionary Microbiology 52:1361–1368. https://doi.org/10.1099/00207713-52-4-1361

Banerjee S, Schlaeppi K, van der Heijden MGA (2018) Keystone taxa as drivers of microbiome structure and functioning. Nature Reviews Microbiology 16:567–576. https://doi.org/10.1038/s41579-018-0024-1

Barberán A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME Journal 6:343–351. https://doi.org/10.1038/ismej.2011.119

Barbier BA, Dziduch I, Liebner S, et al (2012) Methane-cycling communities in a permafrost-affected soil on Herschel Island, Western Canadian Arctic: active layer profiling of mcrA and pmoA genes. FEMS Microbiology Ecology 82:287–302. https://doi.org/10.1111/j.1574-6941.2012.01332.x

Bar-Or I, Elvert M, Eckert W, et al (2017) Iron-Coupled Anaerobic Oxidation of Methane Performed by a Mixed Bacterial-Archaeal Community Based on Poorly Reactive Minerals. Environ Sci Technol 51:12293–12301. https://doi.org/10.1021/acs.est.7b03126

Barsdate RJ, Alexander V (1975) The Nitrogen Balance of Arctic Tundra: Pathways, Rates, and Environmental Implications. Journal of Environmental Quality 4:111–117. https://doi.org/10.2134/jeq1975.00472425000400010025x

Bell TH, Klironomos JN, Henry HAL (2010) Seasonal Responses of Extracellular Enzyme Activity and Microbial Biomass to Warming and Nitrogen Addition. Soil Science Society of America Journal 74:820–828. https://doi.org/10.2136/sssaj2009.0036

Bender M, Conrad R (1995) Effect of CH4 concentrations and soil conditions on the induction of CH4 oxidation activity. Soil Biology and Biochemistry 27:1517–1527. https://doi.org/10.1016/0038-0717(95)00104-M

Biasi C, Wanek W, Rusalimova O, et al (2005) Microtopography and plant-cover controls on nitrogen dynamics in hummock tundra ecosystems in Siberia. Arctic, Antarctic, and Alpine Research 37:435–443. https://doi.org/10.1657/1523-0430(2005)037[0435:MAPCON]2.0.CO;2

Bjorbækmo MFM, Carlsen T, Brysting A, et al (2010) High diversity of root associated fungi in both alpine and arctic Dryas octopetala. BMC Plant Biology 10:244–244. https://doi.org/10.1186/1471-2229-10-244

Björk RG, Klemedtsson L, Molau U, et al (2007) Linkages between N turnover and plant community structure in a tundra landscape. Plant and Soil 294:247–261. https://doi.org/10.1007/s11104-007-9250-4

Blagodatskaya E, Blagodatsky S, Khomyakov N, et al (2016) Temperature sensitivity and enzymatic mechanisms of soil organic matter decomposition along an altitudinal gradient on Mount Kilimanjaro. Scientific Reports 6:22240–22240. https://doi.org/10.1038/srep22240

Blazewicz SJ, Petersen DG, Waldrop MP, Firestone MK (2012) Anaerobic oxidation of methane in tropical and boreal soils: Ecological significance in terrestrial methane cycling: ANAEROBIC OXIDATION OF METHANE IN SOILS. J Geophys Res 117:n/a-n/a. https://doi.org/10.1029/2011JG001864

Bockheim JG (2007) Importance of Cryoturbation in Redistributing Organic Carbon in Permafrost-Affected Soils. Soil Science Society of America Journal 71:1335–1342. https://doi.org/10.2136/sssaj2006.0414N

Bodegom PM, Scholten JCM, Stams AJM (2004) Direct inhibition of methanogenesis by ferric iron. FEMS Microbiology Ecology 49:261–268. https://doi.org/10.1016/j.femsec.2004.03.017

Bödeker ITM, Lindahl BD, Olson Å, Clemmensen KE (2016) Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. Functional Ecology 30:1967–1978. https://doi.org/10.1111/1365-2435.12677

Boetius A, Ravenschlag K, Schubert CJ, et al (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626. https://doi.org/10.1038/35036572

Bonkowski M, Griffiths B, Scrimgeour C (2000) Substrate heterogeneity and microfauna in soil organic ‘hotspots’ as determinants of nitrogen capture and growth of ryegrass. Applied Soil Ecology 14:37–53. https://doi.org/10.1016/S0929-1393(99)00047-5

Boylen CW, Ensign JC (1970) Intracellular Substrates for Endogenous Metabolism During Long-Term Starvation of Rod and Spherical Cells of Arthrobacter crystallopoietes. Journal of Bacteriology 103:578–587. https://doi.org/10.1128/jb.103.3.578-587.1970

Breidenbach B, Conrad R (2015) Seasonal dynamics of bacterial and archaeal methanogenic communities in flooded rice fields and effect of drainage. Frontiers in Microbiology 5:. https://doi.org/10.3389/fmicb.2014.00752

Breidenbach B, Pump J, Dumont MG (2016) Microbial Community Structure in the Rhizosphere of Rice Plants. Frontiers in Microbiology 6:. https://doi.org/10.3389/fmicb.2015.01537

Buckley Daniel H., Graber Joseph R., Schmidt Thomas M. (1998) Phylogenetic Analysis of Nonthermophilic Members of the Kingdom Crenarchaeota and Their Diversity and Abundance in Soils. Applied and Environmental Microbiology 64:4333–4339. https://doi.org/10.1128/AEM.64.11.4333-4339.1998

Bugg TDH, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. Current Opinion in Biotechnology 22:394–400. https://doi.org/10.1016/j.copbio.2010.10.009

Caldwell BA, Jumpponen A, Trappe JM (2000) Utilization of Major Detrital Substrates by Dark-Septate, Root Endophytes. Mycologia 92:230. https://doi.org/10.2307/3761555

Caldwell SL, Laidler JR, Brewer EA, et al (2008) Anaerobic Oxidation of Methane: Mechanisms, Bioenergetics, and the Ecology of Associated Microorganisms. Environ Sci Technol 42:6791–6799. https://doi.org/10.1021/es800120b

Čapek P, Diáková K, Dickopp J-E, et al (2015) The effect of warming on the vulnerability of subducted organic carbon in arctic soils. Soil Biology and Biochemistry 90:19–29. https://doi.org/10.1016/j.soilbio.2015.07.013

Carteron A, Beigas M, Joly S, et al (2021) Temperate Forests Dominated by Arbuscular or Ectomycorrhizal Fungi Are Characterized by Strong Shifts from Saprotrophic to Mycorrhizal Fungi with Increasing Soil Depth. Microbial Ecology 82:377–390. https://doi.org/10.1007/s00248-020-01540-7

Chambers SM, Burke RM, Brooks PR, Cairney JWG (1999) Molecular and biochemical evidence for manganese-dependent peroxidase activity in Tylospora fibrillosa. Mycological Research 103:1098–1102. https://doi.org/10.1017/S095375629900831X

Chan OC, Claus P, Casper P, et al (2005) Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. Environmental Microbiology 7:1139–1149. https://doi.org/10.1111/j.1462-2920.2005.00790.x

Chan OC, Yang X, Fu Y, et al (2006) 16S rRNA gene analyses of bacterial community structures in the soils of evergreen broad-leaved forests in south-west China. FEMS Microbiology Ecology 58:247–259. https://doi.org/10.1111/j.1574-6941.2006.00156.x

Chapin DM (1996) Nitrogen Mineralization, Nitrification, and Denitrification in a High Arctic Lowland Ecosystem, Devon Island, N.W.T., Canada. Arctic and Alpine Research 28:85–85. https://doi.org/10.2307/1552089

Chaya A, Kurosawa N, Kawamata A, et al (2019) Community Structures of Bacteria, Archaea, and Eukaryotic Microbes in the Freshwater Glacier Lake Yukidori-Ike in Langhovde, East Antarctica. Diversity 11:105–105. https://doi.org/10.3390/d11070105

Clarholm M (1985) Possible roles for roots, bacteria, protozoa and fungi in supplying nitrogen to plants. Ecological Interact in soil 4:355–365

Conant RT, Ryan MG, Ågren GI, et al (2011) Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology 17:3392–3404. https://doi.org/10.1111/j.1365-2486.2011.02496.x

Conrad R (2005) Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. Organic Geochemistry 36:739–752. https://doi.org/10.1016/j.orggeochem.2004.09.006

Coyte KZ, Schluter J, Foster KR (2015) The ecology of the microbiome: Networks, competition, and stability. Science 350:663–666. https://doi.org/10.1126/science.aad2602

Crevecoeur S, Vincent WF, Comte J, et al (2017) Diversity and potential activity of methanotrophs in high methane-emitting permafrost thaw ponds. PLOS ONE 12:e0188223–e0188223. https://doi.org/10.1371/journal.pone.0188223

Crevecoeur S, Vincent WF, Lovejoy C (2016) Environmental selection of planktonic methanogens in permafrost thaw ponds. Scientific Reports 6:31312–31312. https://doi.org/10.1038/srep31312

Dao TT, Gentsch N, Mikutta R, et al (2018) Fate of carbohydrates and lignin in north-east Siberian permafrost soils. Soil Biology and Biochemistry 116:311–322. https://doi.org/10.1016/j.soilbio.2017.10.032

Dao TT, Mikutta R, Sauheitl L, et al (2022) Lignin preservation and microbial carbohydrate metabolism in permafrost soils. Journal of Geophysical Research: Biogeosciences. https://doi.org/10.1029/2020JG006181

de Jong AEE, in 't Zandt MH, Meisel OH, et al (2018) Increases in temperature and nutrient availability positively affect methane-cycling microorganisms in Arctic thermokarst lake sediments. Environmental Microbiology 20:4314–4327. https://doi.org/10.1111/1462-2920.14345

Della Monica IF, Saparrat MCN, Godeas AM, Scervino JM (2015) The co-existence between DSE and AMF symbionts affects plant P pools through P mineralization and solubilization processes. Fungal Ecology 17:10–17. https://doi.org/10.1016/j.funeco.2015.04.004

Deng Y, Jiang Y-H, Yang Y, et al (2012) Molecular ecological network analyses. BMC Bioinformatics 13:113. https://doi.org/10.1186/1471-2105-13-113

Dennis PG, Rushton SP, Newsham KK, et al (2012) Soil fungal community composition does not alter along a latitudinal gradient through the maritime and sub-Antarctic. Fungal Ecology 5:403–408. https://doi.org/10.1016/j.funeco.2011.12.002

Deslippe JR, Hartmann M, Grayston SJ, et al (2016) Stable isotope probing implicates a species of C ortinarius in carbon transfer through ectomycorrhizal fungal mycelial networks in A rctic tundra. New Phytologist 210:383–390. https://doi.org/10.1111/nph.13797

Deslippe JR, Hartmann M, Simard SW, Mohn WW (2012) Long-term warming alters the composition of Arctic soil microbial communities. FEMS Microbiology Ecology 82:303–315. https://doi.org/10.1111/j.1574-6941.2012.01350.x

Deslippe JR, Simard SW (2011) Below-ground carbon transfer among Betula nana may increase with warming in Arctic tundra. New Phytologist 192:689–698. https://doi.org/10.1111/j.1469-8137.2011.03835.x

Desyatkin A, Fedorov P, Filippov N, Desyatkin R (2020) Climate Change and Its Influence on the Active Layer Depth in Central Yakutia. Land 10:3–3. https://doi.org/10.3390/land10010003

Dobiński W (2020) Permafrost active layer. Earth-Science Reviews 208:103301. https://doi.org/10.1016/j.earscirev.2020.103301

Doronina NV (2004) Methylobacillus pratensis sp. nov., a novel non-pigmented, aerobic, obligately methylotrophic bacterium isolated from meadow grass. INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY 54:1453–1457. https://doi.org/10.1099/ijs.0.02956-0

Drake TW, Wickland KP, Spencer RGM, et al (2015) Ancient low–molecular-weight organic acids in permafrost fuel rapid carbon dioxide production upon thaw. Proceedings of the National Academy of Sciences 112:13946–13951. https://doi.org/10.1073/pnas.1511705112

Dukunde A, Schneider D, Schmidt M, et al (2019) Tree Species Shape Soil Bacterial Community Structure and Function in Temperate Deciduous Forests. Front Microbiol 10:1519. https://doi.org/10.3389/fmicb.2019.01519

Dunfield PF, Yuryev A, Senin P, et al (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450:879–882. https://doi.org/10.1038/nature06411

Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18:1781–1796. https://doi.org/10.1111/j.1365-2486.2012.02665.x

Dupont YL, Olesen JM (2009) Ecological modules and roles of species in heathland plant-insect flower visitor networks. Journal of Animal Ecology 78:346–353. https://doi.org/10.1111/j.1365-2656.2008.01501.x

Eilers KG, Debenport S, Anderson S, Fierer N (2012) Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry 50:58–65. https://doi.org/10.1016/j.soilbio.2012.03.011

Eilers KG, Lauber CL, Knight R, Fierer N (2010) Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. Soil Biology and Biochemistry 42:896–903. https://doi.org/10.1016/j.soilbio.2010.02.003

Ekelund F, Rønn R (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. FEMS Microbiology Reviews 15:321–353. https://doi.org/10.1111/j.1574-6976.1994.tb00144.x

Elmendorf SC, Henry GHR, Hollister RD, et al (2012) Global assessment of experimental climate warming on tundra vegetation: Heterogeneity over space and time. Ecology Letters 15:164–175. https://doi.org/10.1111/j.1461-0248.2011.01716.x

Erktan A, Rillig MC, Carminati A, et al (2020) Protists and collembolans alter microbial community composition, C dynamics and soil aggregation in simplified consumer–prey systems. Biogeosciences 17:4961–4980. https://doi.org/10.5194/bg-17-4961-2020

Faust K, Sathirapongsasuti JF, Izard J, et al (2012) Microbial Co-occurrence Relationships in the Human Microbiome. PLoS Computational Biology 8:e1002606. https://doi.org/10.1371/journal.pcbi.1002606

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364. https://doi.org/10.1890/05-1839

Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35:167–176. https://doi.org/10.1016/S0038-0717(02)00251-1

Filley TR, Cody GD, Goodell B, et al (2002) Lignin demethylation and polysaccharide decomposition in spruce sapwood degraded by brown rot fungi. Organic Geochemistry 33:111–124. https://doi.org/10.1016/S0146-6380(01)00144-9

Fontaine S, Barot S, Barré P, et al (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450:277–280. https://doi.org/10.1038/nature06275

Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry 35:837–843. https://doi.org/10.1016/S0038-0717(03)00123-8

Frank-Fahle BA, Yergeau É, Greer CW, et al (2014) Microbial functional potential and community composition in permafrost-affected soils of the NW Canadian Arctic. PLoS ONE 9:. https://doi.org/10.1371/journal.pone.0084761

Freedman Z, Zak DR (2014) Atmospheric N Deposition Increases Bacterial Laccase-Like Multicopper Oxidases: Implications for Organic Matter Decay. Applied and Environmental Microbiology 80:4460–4468. https://doi.org/10.1128/AEM.01224-14

Frey B, Walthert L, Perez-Mon C, et al (2021) Deep Soil Layers of Drought-Exposed Forests Harbor Poorly Known Bacterial and Fungal Communities. Frontiers in Microbiology 12:. https://doi.org/10.3389/fmicb.2021.674160

Fujimura KE, Egger KN (2012) Host plant and environment influence community assembly of High Arctic root-associated fungal communities. Fungal Ecology 5:409–418. https://doi.org/10.1016/j.funeco.2011.12.010

Fujimura KE, Egger KN, Henry GHR (2008) The effect of experimental warming on the root-associated fungal community of Salix arctica. The ISME Journal 2:105–114. https://doi.org/10.1038/ismej.2007.89

Ganzert L, Jurgens G, Münster U, Wagner D (2007) Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. pp 476–488

Gao Z, Karlsson I, Geisen S, et al (2019) Protists: Puppet Masters of the Rhizosphere Microbiome. Trends in Plant Science 24:165–176. https://doi.org/10.1016/j.tplants.2018.10.011

Gatheru Waigi M, Sun K, Gao Y (2017) Sphingomonads in Microbe-Assisted Phytoremediation: Tackling Soil Pollution. Trends in Biotechnology 35:883–899. https://doi.org/10.1016/j.tibtech.2017.06.014

Gentsch N, Mikutta R, Alves RJE, et al (2015a) Storage and transformation of organic matter fractions in cryoturbated permafrost soils across the Siberian Arctic. Biogeosciences 12:4525–4542. https://doi.org/10.5194/bg-12-4525-2015

Gentsch N, Mikutta R, Shibistova O, et al (2015b) Properties and bioavailability of particulate and mineral-associated organic matter in Arctic permafrost soils, Lower Kolyma Region, Russia. European Journal of Soil Science. https://doi.org/10.1111/ejss.12269

Giblin AE, Nadelhoffer KJ, Shaver GR, et al (1991) Biogeochemical Diversity Along a Riverside Toposequence in Arctic Alaska. Ecological Monographs 61:415–435. https://doi.org/10.2307/2937049

Gittel A, Bárta J, Kohoutová I, et al (2014a) Distinct microbial communities associated with buried soils in the Siberian tundra. The ISME Journal 8:841–853. https://doi.org/10.1038/ismej.2013.219

Gittel A, Bárta J, Kohoutová I, et al (2014b) Site- and horizon-specific patterns of microbial community structure and enzyme activities in permafrost-affected soils of Greenland. Frontiers in Microbiology 5:541–541. https://doi.org/10.3389/fmicb.2014.00541

Godden B, Ball AS, Helvenstein P, et al (1992) Towards Elucidation of the Lignin Degradation Pathway in Actinomycetes. Journal of General Microbiology 138:2441–2448. https://doi.org/10.1099/00221287-138-11-2441

Goldfarb KC, Karaoz U, Hanson CA, et al (2011) Differential Growth Responses of Soil Bacterial Taxa to Carbon Substrates of Varying Chemical Recalcitrance. Frontiers in Microbiology 2:. https://doi.org/10.3389/fmicb.2011.00094

Griffiths BS (1990) A comparison of microbial-feeding nematodes and protozoa in the rhizosphere of different plants. Biology and Fertility of Soils 9:83–88. https://doi.org/10.1007/BF00335867

Griffiths BS (1989) Enhanced nitrification in the presence of bacteriophagous protozoa. Soil Biology and Biochemistry 21:1045–1051. https://doi.org/10.1016/0038-0717(89)90042-4

Guo J, Wu Y, Wu X, et al (2021) Soil bacterial community composition and diversity response to land conversion is depth-dependent. Global Ecology and Conservation 32:e01923. https://doi.org/10.1016/j.gecco.2021.e01923

Harms TK, Jones JB (2012) Thaw depth determines reaction and transport of inorganic nitrogen in valley bottom permafrost soils. Global Change Biology 18:2958–2968. https://doi.org/10.1111/j.1365-2486.2012.02731.x

Hartley AE, Schlesinger WH (2002) Potential environmental controls on nitrogenase activity in biological crusts of the northern Chihuahuan Desert. Journal of Arid Environments 52:293–304. https://doi.org/10.1006/jare.2002.1007

He R, Wooller MJ, Pohlman JW, et al (2012) Diversity of active aerobic methanotrophs along depth profiles of arctic and subarctic lake water column and sediments. The ISME Journal 6:1937–1948. https://doi.org/10.1038/ismej.2012.34

Henry HAL (2013) Reprint of "Soil extracellular enzyme dynamics in a changing climate." Soil Biology and Biochemistry 56:53–59. https://doi.org/10.1016/j.soilbio.2012.10.022

Heyer J, Berger U, Hardt M, Dunfield PF (2005) Methylohalobius crimeensis gen. nov., sp. nov., a moderately halophilic, methanotrophic bacterium isolated from hypersaline lakes of Crimea. International Journal of Systematic and Evolutionary Microbiology 55:1817–1826. https://doi.org/10.1099/ijs.0.63213-0

Hobara S, McCalley C, Koba K, et al (2006) Nitrogen Fixation in Surface Soils and Vegetation in an Arctic Tundra Watershed: A Key Source of Atmospheric Nitrogen. Arctic, Antarctic, and Alpine Research 38:363–372. https://doi.org/10.1657/1523-0430(2006)38[363:NFISSA]2.0.CO;2

Hobbie JE, Hobbie EA (2006) 15N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. Ecology 87:816–822. https://doi.org/10.1890/0012-9658(2006)87[816:NISFAP]2.0.CO;2

Høj L, Olsen RA, Torsvik VL (2005) Archaeal communities in High Arctic wetlands at Spitsbergen, Norway (78°N) as characterized by 16S rRNA gene fingerprinting. FEMS Microbiology Ecology 53:89–101. https://doi.org/10.1016/j.femsec.2005.01.004

Hsu S-F, Buckley DH (2009) Evidence for the functional significance of diazotroph community structure in soil. The ISME Journal 3:124–136. https://doi.org/10.1038/ismej.2008.82

Huber KJ, Pascual J, Foesel BU, Overmann J (2017) Blastocatellaceae. In: Whitman WB, Rainey F, Kämpfer P, et al. (eds) Bergey's Manual of Systematics of Archaea and Bacteria, 1st edn. Wiley, pp 1–4

Hugelius G, Kuhry P, Tarnocai C, Virtanen T (2010) Soil organic carbon pools in a periglacial landscape: A case study from the central Canadian Arctic. Permafrost and Periglacial Processes 21:16–29. https://doi.org/10.1002/ppp.677

Intergovernmental Panel on Climate Change (ed) (2014) Climate Change 2013 - The Physical Science Basis. Cambridge University Press, Cambridge

Jackson RB, Lajtha K, Crow SE, et al (2017) The Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls. Annual Review of Ecology, Evolution, and Systematics 48:419–445. https://doi.org/10.1146/annurev-ecolsys-112414-054234

Jenkins O, Jones D (1987) Taxonomic Studies on Some Gram-negative Methylotrophic Bacteria. Microbiology 133:453–473. https://doi.org/10.1099/00221287-133-2-453

Ji Y, Liu P, Conrad R (2018) Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to progressing rice straw degradation. Soil Biology and Biochemistry 124:70–80. https://doi.org/10.1016/j.soilbio.2018.05.029

Jiang N, Wang Y, Dong X (2010) Methanol as the Primary Methanogenic and Acetogenic Precursor in the Cold Zoige Wetland at Tibetan Plateau. Microbial Ecology 60:206–213. https://doi.org/10.1007/s00248-009-9602-0

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications. https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

Jobbágy EG, Jackson RB (2001) The distribution of soil nutrients with depth: Global patterns and the imprint of plants. Biogeochemistry 53:51–77. https://doi.org/10.1023/A:1010760720215

Jurgens G, GlÃ¶ckner F-O, Amann R, et al (2000) Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescent in situ hybridization1. FEMS Microbiology Ecology 34:45–56. https://doi.org/10.1111/j.1574-6941.2000.tb00753.x

Jurgens G, Lindström K, Saano A (1997) Novel group within the kingdom Crenarchaeota from boreal forest soil. Applied and Environmental Microbiology 63:803–805. https://doi.org/10.1128/aem.63.2.803-805.1997

Kadnikov VV, Savvichev AS, Mardanov AV, et al (2019) Microbial communities involved in the methane cycle in the near-bottom water layer and sediments of the meromictic subarctic Lake Svetloe. Antonie van Leeuwenhoek 112:1801–1814. https://doi.org/10.1007/s10482-019-01308-1

Kaiser C, Meyer H, Biasi C, et al (2007) Conservation of soil organic matter through cryoturbation in arctic soils in Siberia. Journal of Geophysical Research: Biogeosciences 112:1–8. https://doi.org/10.1029/2006JG000258

Kalyuzhnaya MG, Bowerman S, Lara JC, et al (2006) Methylotenera mobilis gen. nov., sp. nov., an obligately methylamine-utilizing bacterium within the family Methylophilaceae. INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY 56:2819–2823. https://doi.org/10.1099/ijs.0.64191-0

Kaštovská E, Straková P, Edwards K, et al (2018) Cotton-Grass and Blueberry have Opposite Effect on Peat Characteristics and Nutrient Transformation in Peatland. Ecosystems 21:443–458. https://doi.org/10.1007/s10021-017-0159-3

Kellner H, Luis P, Zimdars B, et al (2008) Diversity of bacterial laccase-like multicopper oxidase genes in forest and grassland Cambisol soil samples. Soil Biology and Biochemistry 40:638–648. https://doi.org/10.1016/j.soilbio.2007.09.013

Keough BP, Schmidt TM, Hicks RE (2003) Archaeal nucleic acids in picoplankton from great lakes on three continents. Microbial Ecology 46:238–248. https://doi.org/10.1007/s00248-003-1003-1

Keuper F, Bodegom PM, Dorrepaal E, et al (2012) A frozen feast: thawing permafrost increases plant-available nitrogen in subarctic peatlands. Global Change Biology 18:1998–2007. https://doi.org/10.1111/j.1365-2486.2012.02663.x

Kielak AM, Barreto CC, Kowalchuk GA, et al (2016) The Ecology of Acidobacteria: Moving beyond Genes and Genomes. Frontiers in Microbiology 7:. https://doi.org/10.3389/fmicb.2016.00744

Kim HM, Jung JY, Yergeau E, et al (2014) Bacterial community structure and soil properties of a subarctic tundra soil in Council, Alaska. FEMS Microbiology Ecology. https://doi.org/10.1111/1574-6941.12362

Klüber HD, Conrad R (1998) Effects of nitrate, nitrite, NO and N2O on methanogenesis and other redox processes in anoxic rice field soil. FEMS Microbiology Ecology 25:301–318. https://doi.org/10.1111/j.1574-6941.1998.tb00482.x

Knief C (2015) Diversity and Habitat Preferences of Cultivated and Uncultivated Aerobic Methanotrophic Bacteria Evaluated Based on pmoA as Molecular Marker. Frontiers in Microbiology 6:. https://doi.org/10.3389/fmicb.2015.01346

Knittel K, Boetius A (2009) Anaerobic Oxidation of Methane: Progress with an Unknown Process. Annu Rev Microbiol 63:311–334. https://doi.org/10.1146/annurev.micro.61.080706.093130

Knoblauch C, Zimmermann U, Blumenberg M, et al (2008) Methane turnover and temperature response of methane-oxidizing bacteria in permafrost-affected soils of northeast Siberia. Soil Biology and Biochemistry 40:3004–3013. https://doi.org/10.1016/j.soilbio.2008.08.020

Knorr K-H, Horn MA, Borken W (2015) Significant nonsymbiotic nitrogen fixation in Patagonian ombrotrophic bogs. Glob Change Biol 21:2357–2365. https://doi.org/10.1111/gcb.12849

Kobabe S, Wagner D, Pfeiffer EM (2004) Characterisation of microbial community composition of a Siberian tundra soil by fluorescence in situ hybridisation. FEMS Microbiology Ecology 50:13–23. https://doi.org/10.1016/j.femsec.2004.05.003

Koch O, Tscherko D, Kandeler E (2007) Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Global Biogeochemical Cycles 21:n/a-n/a. https://doi.org/10.1029/2007GB002983

Kotsyurbenko OR (2005) Trophic interactions in the methanogenic microbial community of low-temperature terrestrial ecosystems. FEMS Microbiology Ecology 53:3–13. https://doi.org/10.1016/j.femsec.2004.12.009

Koyama A, Wallenstein MD, Simpson RT, Moore JC (2014) Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. Frontiers in Microbiology 5:516. https://doi.org/10.3389/fmicb.2014.00516

Kuhry P, Dorrepaal E, Hugelius G, et al (2010) Short communication: Potential remobilization of belowground permafrost carbon under future global warming. Permafrost and Periglacial Processes 21:208–214. https://doi.org/10.1002/ppp.684

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5

Labeda DP, Liu KC, Casida LE (1976) Colonization of soil by Arthrobacter and Pseudomonas under varying conditions of water and nutrient availability as studied by plate counts and transmission electron microscopy. Applied and Environmental Microbiology 31:551–561. https://doi.org/10.1128/aem.31.4.551-561.1976

Lal R (2004) Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science 304:1623–1627. https://doi.org/10.1126/science.1097396

Larmola T, Leppänen SM, Tuittila E-S, et al (2014) Methanotrophy induces nitrogen fixation during peatland development. Proc Natl Acad Sci USA 111:734–739. https://doi.org/10.1073/pnas.1314284111

Lau MCY, Stackhouse BT, Layton AC, et al (2015a) An active atmospheric methane sink in high Arctic mineral cryosols. The ISME Journal 9:1880–1891. https://doi.org/10.1038/ismej.2015.13

Lau MCY, Stackhouse BT, Layton AC, et al (2015b) An active atmospheric methane sink in high Arctic mineral cryosols. The ISME Journal 9:1880–1891. https://doi.org/10.1038/ismej.2015.13

Lavergne C, Aguilar-Muñoz P, Calle N, et al (2021) Temperature differently affected methanogenic pathways and microbial communities in sub-Antarctic freshwater ecosystems. Environment International 154:106575–106575. https://doi.org/10.1016/j.envint.2021.106575

Lavoie M, Mack MC, Schuur EAG (2011) Effects of elevated nitrogen and temperature on carbon and nitrogen dynamics in Alaskan arctic and boreal soils. J Geophys Res 116:G03013. https://doi.org/10.1029/2010JG001629

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. European Journal of Soil Biology 37:25–50. https://doi.org/10.1016/S1164-5563(01)01067-6

Le Roes-Hill M, Khan N, Burton SG (2011) Actinobacterial peroxidases: An unexplored resource for biocatalysis. Applied Biochemistry and Biotechnology 164:681–713. https://doi.org/10.1007/s12010-011-9167-5

Lennon JT (2020) Microbial Life Deep Underfoot. mBio 11:. https://doi.org/10.1128/mBio.03201-19

Li J, Pancost RD, Naafs BDA, et al (2019) Multiple environmental and ecological controls on archaeal ether lipid distributions in saline ponds. Chemical Geology 529:119293–119293. https://doi.org/10.1016/j.chemgeo.2019.119293

Lidstrom ME (2006) Aerobic Methylotrophic Prokaryotes. In: The Prokaryotes. Springer New York, New York, NY, pp 618–634

Liebner S, Wagner D (2007) Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia. Environmental Microbiology 9:107–117. https://doi.org/10.1111/j.1462-2920.2006.01120.x

Liengen T, Olsen RA (1997) Nitrogen Fixation by Free-Living Cyanobacteria from Different Coastal Sites in a High Arctic Tundra, Spitsbergen. Arctic and Alpine Research 29:470. https://doi.org/10.2307/1551994

Lipson DA, Haggerty JM, Srinivas A, et al (2013) Metagenomic Insights into Anaerobic Metabolism along an Arctic Peat Soil Profile. PLoS ONE 8:e64659. https://doi.org/10.1371/journal.pone.0064659

Lipson DA, Monson RK (1998) Plant-microbe competition for soil amino acids in the alpine tundra: Effects of freeze-thaw and dry-rewet events. Oecologia. https://doi.org/10.1007/s004420050393

Louca S, Parfrey LW, Doebeli M (2016) Decoupling function and taxonomy in the global ocean microbiome. Science. https://doi.org/10.1126/science.aaf4507

Luo L, Meng H, Gu J-D (2017) Microbial extracellular enzymes in biogeochemical cycling of ecosystems. Journal of Environmental Management 197:539–549. https://doi.org/10.1016/j.jenvman.2017.04.023

Lupatini M, Suleiman AKA, Jacques RJS, et al (2014) Network topology reveals high connectance levels and few key microbial genera within soils. Frontiers in Environmental Science 2:. https://doi.org/10.3389/fenvs.2014.00010

Ma B, Wang H, Dsouza M, et al (2016) Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. The ISME Journal 10:1891–1901. https://doi.org/10.1038/ismej.2015.261

Macias-Fauria M, Forbes BC, Zetterberg P, Kumpula T (2012) Eurasian Arctic greening reveals teleconnections and the potential for structurally novel ecosystems. Nature Climate Change 2:613–618. https://doi.org/10.1038/nclimate1558

Mapelli F, Marasco R, Rizzi A, et al (2011) Bacterial Communities Involved in Soil Formation and Plant Establishment Triggered by Pyrite Bioweathering on Arctic Moraines. Microbial Ecology 61:438–447. https://doi.org/10.1007/s00248-010-9758-7

Martín González AM, Dalsgaard B, Olesen JM (2010a) Centrality measures and the importance of generalist species in pollination networks. Ecological Complexity 7:36–43. https://doi.org/10.1016/j.ecocom.2009.03.008

Martín González AM, Dalsgaard B, Olesen JM (2010b) Centrality measures and the importance of generalist species in pollination networks. Ecological Complexity 7:36–43. https://doi.org/10.1016/j.ecocom.2009.03.008

Martinez-Cruz K, Leewis M-C, Herriott IC, et al (2017a) Anaerobic oxidation of methane by aerobic methanotrophs in sub-Arctic lake sediments. Science of The Total Environment 607–608:23–31. https://doi.org/10.1016/j.scitotenv.2017.06.187

Martinez-Cruz K, Leewis M-C, Herriott IC, et al (2017b) Anaerobic oxidation of methane by aerobic methanotrophs in sub-Arctic lake sediments. Science of The Total Environment 607–608:23–31. https://doi.org/10.1016/j.scitotenv.2017.06.187

Masuda Y, Itoh H, Shiratori Y, Senoo K (2018) Metatranscriptomic insights into microbial consortia driving methane metabolism in paddy soils. Soil Science and Plant Nutrition 64:455–464. https://doi.org/10.1080/00380768.2018.1457409

McCarthy AJ (1987) Lignocellulose-degrading actinomycetes. FEMS Microbiology Letters 46:145–163. https://doi.org/10.1016/0378-1097(87)90061-9

McKane RB, Johnson LC, Shaver GR, et al (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415:68–71. https://doi.org/10.1038/415068a

Mekonnen ZA, Riley WJ, Berner LT, et al (2021) Arctic tundra shrubification: a review of mechanisms and impacts on ecosystem carbon balance. Environmental Research Letters 16:053001–053001. https://doi.org/10.1088/1748-9326/abf28b

Mengel K (1996) Turnover of organic nitrogen in soils and its availability to crops. Plant and Soil 181:83–93. https://doi.org/10.1007/BF00011295

Metje M, Frenzel P (2007) Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. Environmental Microbiology 9:954–964. https://doi.org/10.1111/j.1462-2920.2006.01217.x

Mikkonen A, Santalahti M, Lappi K, et al (2014) Bacterial and archaeal communities in long-term contaminated surface and subsurface soil evaluated through coextracted RNA and DNA. FEMS Microbiology Ecology 90:103–114. https://doi.org/10.1111/1574-6941.12376

Miller K, Lai C-T, Dahlgren R, Lipson D (2019) Anaerobic Methane Oxidation in High-Arctic Alaskan Peatlands as a Significant Control on Net CH4 Fluxes. Soil Syst 3:7. https://doi.org/10.3390/soilsystems3010007

Mondav R, McCalley CK, Hodgkins SB, et al (2017) Microbial network, phylogenetic diversity and community membership in the active layer across a permafrost thaw gradient. Environmental Microbiology 19:3201–3218. https://doi.org/10.1111/1462-2920.13809

Mongodin EF, Shapir N, Daugherty SC, et al (2006) Secrets of Soil Survival Revealed by the Genome Sequence of Arthrobacter aurescens TC1. PLoS Genetics 2:e214. https://doi.org/10.1371/journal.pgen.0020214

Mooshammer M, Wanek W, Schnecker J, et al (2012) Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. Ecology 93:770–782. https://doi.org/10.1890/11-0721.1

Mori K, Yamaguchi K, Sakiyama Y, et al (2009) Caldisericum exile gen. nov., sp. nov., an anaerobic, thermophilic, filamentous bacterium of a novel bacterial phylum, Caldiserica phyl. nov., originally called the candidate phylum OP5, and description of Caldisericaceae fam. nov., Caldisericales ord. no. INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY 59:2894–2898. https://doi.org/10.1099/ijs.0.010033-0

Mukherjee S, Juottonen H, Siivonen P, et al (2014) Spatial patterns of microbial diversity and activity in an aged creosote-contaminated site. The ISME Journal 8:2131–2142. https://doi.org/10.1038/ismej.2014.151

Mundra S, Halvorsen R, Kauserud H, et al (2016) Ectomycorrhizal and saprotrophic fungi respond differently to long-term experimentally increased snow depth in the High Arctic. MicrobiologyOpen 5:856–869. https://doi.org/10.1002/mbo3.375

Nakamura K, Kawabata T, Yura K, Go N (2003) Novel types of two-domain multi-copper oxidases: possible missing links in the evolution. FEBS Letters 553:239–244. https://doi.org/10.1016/S0014-5793(03)01000-7

Näsholm T, Ekblad A, Nordin A, et al (1998) Boreal forest plants take up organic nitrogen. Nature 392:914–916. https://doi.org/10.1038/31921

Nemergut DR, Cleveland CC, Wieder WR, et al (2010) Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biology and Biochemistry 42:2153–2160. https://doi.org/10.1016/j.soilbio.2010.08.011

Newsham KK, Upson R, Read DJ (2009) Mycorrhizas and dark septate root endophytes in polar regions. Fungal Ecology 2:10–20. https://doi.org/10.1016/j.funeco.2008.10.005

Nordin A, Högberg P, Näsholm T (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. Oecologia 129:125–132. https://doi.org/10.1007/s004420100698

Nordin A, Schmidt IK, Shaver GR (2004) Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. Ecology 85:955–962. https://doi.org/10.1890/03-0084

Nosko P, Bliss LC, Cook FD (1994) The Association of Free-Living Nitrogen-Fixing Bacteria with the Roots of High Arctic Graminoids. Arctic and Alpine Research 26:180. https://doi.org/10.2307/1551782

Nowinski NS, Trumbore SE, Schuur EAG, et al (2008) Nutrient Addition Prompts Rapid Destabilization of Organic Matter in an Arctic Tundra Ecosystem. Ecosystems 11:16–25. https://doi.org/10.1007/s10021-007-9104-1

Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature 405:299–304. https://doi.org/10.1038/35012500

Oh S-Y, Park MS, Lim YW (2019) The Influence of Microfungi on the Mycelial Growth of Ectomycorrhizal Fungus Tricholoma matsutake. Microorganisms 7:169. https://doi.org/10.3390/microorganisms7060169

Olefeldt D, Turetsky MR, Crill PM, McGuire AD (2013) Environmental and physical controls on northern terrestrial methane emissions across permafrost zones. Global Change Biology 19:589–603. https://doi.org/10.1111/gcb.12071

Olesen JM, Bascompte J, Dupont YL, Jordano P (2007) The modularity of pollination networks. Proceedings of the National Academy of Sciences 104:19891–19896. https://doi.org/10.1073/pnas.0706375104

Op den Camp HJM, Islam T, Stott MB, et al (2009) Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environmental Microbiology Reports 1:293–306. https://doi.org/10.1111/j.1758-2229.2009.00022.x

Palmtag J, Hugelius G, Lashchinskiy N, et al (2015) Storage, Landscape Distribution, and Burial History of Soil Organic Matter in Contrasting Areas of Continuous Permafrost. Arctic, Antarctic, and Alpine Research 47:71–88. https://doi.org/10.1657/AAAR0014-027

Palmtag J, Obu J, Kuhry P, et al (2022) A high-spatial resolution soil carbon and nitrogen dataset for the northern permafrost region, based on circumpolar land cover upscaling. Earth Syst Sci Data Discuss 2022:1–28. https://doi.org/10.5194/essd-2022-8

Palmtag J, Ramage J, Hugelius G, et al (2016) Controls on the storage of organic carbon in permafrost soil in northern Siberia. European Journal of Soil Science 67:478–491. https://doi.org/10.1111/ejss.12357

Parker TC, Thurston AM, Raundrup K, et al (2021) Shrub expansion in the Arctic may induce large-scale carbon losses due to changes in plant-soil interactions. Plant and Soil 463:643–651. https://doi.org/10.1007/s11104-021-04919-8

Patzner MS, Mueller CW, Malusova M, et al (2020) Iron mineral dissolution releases iron and associated organic carbon during permafrost thaw. Nat Commun 11:6329. https://doi.org/10.1038/s41467-020-20102-6

Paul SS, Dey A, Baro D, Punia BS (2017) Comparative community structure of archaea in rumen of buffaloes and cattle. Journal of the Science of Food and Agriculture 97:3284–3293. https://doi.org/10.1002/jsfa.8177

Pearson RG, Phillips SJ, Loranty MM, et al (2013) Shifts in Arctic vegetation and associated feedbacks under climate change. Nature Climate Change 3:673–677. https://doi.org/10.1038/nclimate1858

Pegoraro E, Mauritz M, Bracho R, et al (2019) Glucose addition increases the magnitude and decreases the age of soil respired carbon in a long-term permafrost incubation study. Soil Biology and Biochemistry 129:201–211. https://doi.org/10.1016/j.soilbio.2018.10.009

Petters S, Groß V, Söllinger A, et al (2021) The soil microbial food web revisited: Predatory myxobacteria as keystone taxa? The ISME Journal 15:2665–2675. https://doi.org/10.1038/s41396-021-00958-2

Põlme S, Abarenkov K, Henrik Nilsson R, et al (2020) FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Diversity 105:1–16. https://doi.org/10.1007/s13225-020-00466-2

Promputtha I, Hyde KD, McKenzie EHC, et al (2010) Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? Fungal Diversity 41:89–99. https://doi.org/10.1007/s13225-010-0024-6

Prosser JI, Head IM, Stein LY (2014) The Family Nitrosomonadaceae. In: Rosenberg E, DeLong EF, Lory S, et al. (eds) The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 901–918

Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends in Microbiology 20:523–531. https://doi.org/10.1016/j.tim.2012.08.001

Qian X, Chen L, Guo X, et al (2018) Shifts in community composition and co-occurrence patterns of phyllosphere fungi inhabiting Mussaenda shikokiana along an elevation gradient. PeerJ 6:e5767. https://doi.org/10.7717/peerj.5767

Rafrafi Y, Trably E, Hamelin J, et al (2013) Sub-dominant bacteria as keystone species in microbial communities producing bio-hydrogen. International Journal of Hydrogen Energy 38:4975–4985. https://doi.org/10.1016/j.ijhydene.2013.02.008

Raymond J, Siefert JL, Staples CR, Blankenship RE (2004) The Natural History of Nitrogen Fixation. Molecular Biology and Evolution 21:541–554. https://doi.org/10.1093/molbev/msh047

Reed SC, Townsend AR, Cleveland CC, Nemergut DR (2010) Microbial community shifts influence patterns in tropical forest nitrogen fixation. Oecologia 164:521–531. https://doi.org/10.1007/s00442-010-1649-6

Reiche M, Torburg G, KÃ¼sel K (2008) Competition of Fe(III) reduction and methanogenesis in an acidic fen: Fe(III) reduction and methanogenesis in a fen. FEMS Microbiology Ecology 65:88–101. https://doi.org/10.1111/j.1574-6941.2008.00523.x

Reitschuler C, Hofmann K, Illmer P (2016) Abundances, diversity and seasonality of (non-extremophilic) Archaea in Alpine freshwaters. Antonie van Leeuwenhoek 109:855–868. https://doi.org/10.1007/s10482-016-0685-6

Ricketts MP, Matamala R, Jastrow JD, et al (2020) The effects of warming and soil chemistry on bacterial community structure in Arctic tundra soils. Soil Biology and Biochemistry 148:107882. https://doi.org/10.1016/j.soilbio.2020.107882

Ridl J, Kolar M, Strejcek M, et al (2016) Plants Rather than Mineral Fertilization Shape Microbial Community Structure and Functional Potential in Legacy Contaminated Soil. Frontiers in Microbiology 7:. https://doi.org/10.3389/fmicb.2016.00995

Rinnan R, Michelsen A, Bååth E, Jonasson S (2007) Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. Global Change Biol 13:28–39. https://doi.org/10.1111/j.1365-2486.2006.01263.x

Rønn RM, Griffiths BS, Young IM (2001) Protozoa, nematodes and N-mineralization across a prescribed soil textural gradient. Pedobiologia 45:481–495. https://doi.org/10.1078/0031-4056-00101

Rosswall T (1982) Microbiological regulation of the biogeochemical nitrogen cycle. Plant Soil 67:15–34. https://doi.org/10.1007/BF02182752

Rousk K, Rousk J, Jones DL, et al (2013) Feather moss nitrogen acquisition across natural fertility gradients in boreal forests. Soil Biology and Biochemistry 61:86–95. https://doi.org/10.1016/j.soilbio.2013.02.011

Rumpel C, Kögel-Knabner I (2011) Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant and Soil 338:143–158. https://doi.org/10.1007/s11104-010-0391-5

Šantrůčková H, Kotas P, Bárta J, et al (2018) Significance of dark CO2 fixation in arctic soils. Soil Biology and Biochemistry 119:11–21. https://doi.org/10.1016/j.soilbio.2017.12.021

Saunois M, Stavert AR, Poulter B, et al (2020) The Global Methane Budget 2000–2017. Earth System Science Data 12:1561–1623. https://doi.org/10.5194/essd-12-1561-2020

Saxena J, Minaxi, Jha A (2014) Impact of a phosphate solubilizing bacterium and an arbuscular mycorrhizal fungus (Glomus etunicatum) on growth, yield and P concentration in wheat plants. Clean Soil Air Water 42:1248–1252. https://doi.org/10.1002/clen.201300492

Schädel C, Schuur EAG, Bracho R, et al (2014) Circumpolar assessment of permafrost C quality and its vulnerability over time using long-term incubation data. Glob Change Biol 20:641–652. https://doi.org/10.1111/gcb.12417

Schellenberger S, Kolb S, Drake HL (2010) Metabolic responses of novel cellulolytic and saccharolytic agricultural soil Bacteria to oxygen. Environmental Microbiology 12:845–861. https://doi.org/10.1111/j.1462-2920.2009.02128.x

Scheller S, Yu H, Chadwick GL, et al (2016) Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. Science 351:703–707. https://doi.org/10.1126/science.aad7154

Schimel J (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35:549–563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602. https://doi.org/10.1890/03-8002

Schimel JP, Chapin FS (1996) Tundra Plant Uptake of Amino Acid and NH4+ Nitrogen in Situ: Plants Complete Well for Amino Acid N. Ecology 77:2142–2147. https://doi.org/10.2307/2265708

Schirrmeister L, Grosse G, Wetterich S, et al (2011) Fossil organic matter characteristics in permafrost deposits of the northeast Siberian Arctic. J Geophys Res 116:G00M02. https://doi.org/10.1029/2011JG001647

Schlegel M, Münsterkötter M, Güldener U, et al (2016) Globally distributed root endophyte Phialocephala subalpina links pathogenic and saprophytic lifestyles. BMC Genomics 17:1015–1015. https://doi.org/10.1186/s12864-016-3369-8

Schleper C, Nicol GW (2010) Ammonia-Oxidising Archaea – Physiology, Ecology and Evolution. In: Advances in Microbial Physiology. Elsevier, pp 1–41

Schmidt IK, Jonasson S, Shaver GR, et al (2002) [No title found]. Plant and Soil 242:93–106. https://doi.org/10.1023/A:1019642007929

Schnecker J, Wild B, Hofhansl F, et al (2014) Effects of Soil Organic Matter Properties and Microbial Community Composition on Enzyme Activities in Cryoturbated Arctic Soils. PLoS ONE 9:e94076–e94076. https://doi.org/10.1371/journal.pone.0094076

Schnecker J, Wild B, Takriti M, et al (2015) Microbial community composition shapes enzyme patterns in topsoil and subsoil horizons along a latitudinal transect in Western Siberia. Soil Biology and Biochemistry 83:106–115. https://doi.org/10.1016/j.soilbio.2015.01.016

Schütte UME, Henning JA, Ye Y, et al (2019) Effect of permafrost thaw on plant and soil fungal community in a boreal forest: Does fungal community change mediate plant productivity response? Journal of Ecology 107:1737–1752. https://doi.org/10.1111/1365-2745.13139

Schuur EAG, Abbott B (2011) Climate change: High risk of permafrost thaw. Nature 480:32–33. https://doi.org/10.1038/480032a

Schuur EAG, Bockheim J, Canadell JG, et al (2008) Vulnerability of Permafrost Carbon to Climate Change: Implications for the Global Carbon Cycle. BioScience 58:701–714. https://doi.org/10.1641/B580807

Schuur EAG, McGuire AD, Schädel C, et al (2015) Climate change and the permafrost carbon feedback. Nature 520:171–179. https://doi.org/10.1038/nature14338

Semrau JD, DiSpirito AA, Yoon S (2010) Methanotrophs and copper. FEMS Microbiol Rev 34:496–531. https://doi.org/10.1111/j.1574-6976.2010.00212.x

Setälä H, McLean MA (2004) Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139:98–107. https://doi.org/10.1007/s00442-003-1478-y

Shen C, Liang W, Shi Y, et al (2014) Contrasting elevational diversity patterns between eukaryotic soil microbes and plants. Ecology 95:3190–3202. https://doi.org/10.1890/14-0310.1

Shi Y, Xiang X, Shen C, et al (2015) Vegetation-Associated Impacts on Arctic Tundra Bacterial and Microeukaryotic Communities. Applied and Environmental Microbiology 81:492–501. https://doi.org/10.1128/AEM.03229-14

Singleton CM, McCalley CK, Woodcroft BJ, et al (2018) Methanotrophy across a natural permafrost thaw environment. The ISME Journal 12:2544–2558. https://doi.org/10.1038/s41396-018-0065-5

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42:391–404. https://doi.org/10.1016/j.soilbio.2009.10.014

Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798. https://doi.org/10.1038/nature08632

Sinsabaugh RL, Lauber CL, Weintraub MN, et al (2008) Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11:1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26:1305–1311. https://doi.org/10.1016/0038-0717(94)90211-9

Sistla SA, Asao S, Schimel JP (2012) Detecting microbial N-limitation in tussock tundra soil: Implications for Arctic soil organic carbon cycling. Soil Biology and Biochemistry 55:78–84. https://doi.org/10.1016/j.soilbio.2012.06.010

Skennerton CT, Ward LM, Michel A, et al (2015) Genomic Reconstruction of an Uncultured Hydrothermal Vent Gammaproteobacterial Methanotroph (Family Methylothermaceae) Indicates Multiple Adaptations to Oxygen Limitation. Frontiers in Microbiology 6:. https://doi.org/10.3389/fmicb.2015.01425

Smemo KA, Yavitt JB (2011) Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems? Biogeosciences 8:779–793. https://doi.org/10.5194/bg-8-779-2011

Söllinger A, Tveit AT, Poulsen M, et al (2018) Holistic Assessment of Rumen Microbiome Dynamics through Quantitative Metatranscriptomics Reveals Multifunctional Redundancy during Key Steps of Anaerobic Feed Degradation. mSystems 3:e00038-18. https://doi.org/10.1128/mSystems.00038-18

Stackebrandt E, Schumann P (2014) The family Cellulomonadaceae. In: The Prokaryotes: Actinobacteria. pp 163–184

Stackhouse BT, Vishnivetskaya TA, Layton A, et al (2015) Effects of simulated spring thaw of permafrost from mineral cryosol on CO 2 emissions and atmospheric CH 4 uptake. Journal of Geophysical Research: Biogeosciences 120:1764–1784. https://doi.org/10.1002/2015JG003004

Stewart KJ, Brummell ME, Coxson DS, Siciliano SD (2013) How is nitrogen fixation in the high arctic linked to greenhouse gas emissions? Plant Soil 362:215–229. https://doi.org/10.1007/s11104-012-1282-8

Stewart KJ, Coxson D, Grogan P (2011) Nitrogen Inputs by Associative Cyanobacteria across a Low Arctic Tundra Landscape. Arctic, Antarctic, and Alpine Research 43:267–278. https://doi.org/10.1657/1938-4246-43.2.267

Stockem W, Klein H-P (1979) Pinocytosis and locomotion in amoebae. Protoplasma 100:33–43. https://doi.org/10.1007/BF01276299

Sun S, Li S, Avera BN, et al (2017) Soil Bacterial and Fungal Communities Show Distinct Recovery Patterns during Forest Ecosystem Restoration. Applied and Environmental Microbiology 83:. https://doi.org/10.1128/AEM.00966-17

Surono, Narisawa K (2017) The dark septate endophytic fungus Phialocephala fortinii is a potential decomposer of soil organic compounds and a promoter of Asparagus officinalis growth. Fungal Ecology 28:1–10. https://doi.org/10.1016/j.funeco.2017.04.001

Takai K (2019) The Nitrogen Cycle: A Large, Fast, and Mystifying Cycle. Microbes and Environments 34:223–225. https://doi.org/10.1264/jsme2.ME3403rh

Talbot JM, Allison SD, Treseder KK (2008) Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Functional Ecology 22:955–963. https://doi.org/10.1111/j.1365-2435.2008.01402.x

Tamas I, Smirnova AV, He Z, Dunfield PF (2014) The (d)evolution of methanotrophy in the Beijerinckiaceae—a comparative genomics analysis. The ISME Journal 8:369–382. https://doi.org/10.1038/ismej.2013.145

Tao J, Meng D, Qin C, et al (2018) Integrated network analysis reveals the importance of microbial interactions for maize growth. Applied Microbiology and Biotechnology 102:3805–3818. https://doi.org/10.1007/s00253-018-8837-4

Tape K, Sturm M, Racine C (2006) The evidence for shrub expansion in Northern Alaska and the Pan-Arctic. Global Change Biology 12:686–702. https://doi.org/10.1111/j.1365-2486.2006.01128.x

Tarnocai C, Canadell JG, Schuur EAG, et al (2009) Soil organic carbon pools in the northern circumpolar permafrost region: SOIL ORGANIC CARBON POOLS. Global Biogeochem Cycles 23:n/a-n/a. https://doi.org/10.1029/2008GB003327

Teske A, Callaghan AV, LaRowe DE (2014) Biosphere frontiers of subsurface life in the sedimented hydrothermal system of Guaymas Basin. Frontiers in Microbiology 5:. https://doi.org/10.3389/fmicb.2014.00362

Thakur MP, Geisen S (2019) Trophic Regulations of the Soil Microbiome. Trends in Microbiology 27:771–780. https://doi.org/10.1016/j.tim.2019.04.008

Thomson BC, Ostle N, McNamara N, et al (2010) Vegetation Affects the Relative Abundances of Dominant Soil Bacterial Taxa and Soil Respiration Rates in an Upland Grassland Soil. Microbial Ecology 59:335–343. https://doi.org/10.1007/s00248-009-9575-z

Timling I, Taylor DL (2012) Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi. Fungal Ecology 5:419–429. https://doi.org/10.1016/j.funeco.2012.01.009

Treuner-Lange A, Bruckskotten M, Rupp O, et al (2017) Whole-Genome Sequence of the Fruiting Myxobacterium Cystobacter fuscus DSM 52655. Genome Announcements 5:. https://doi.org/10.1128/genomeA.01196-17

Tripathi BM, Kim1 HM, Jung JY, et al (2019) Distinct Taxonomic and Functional Profiles of the Microbiome Associated With Different Soil Horizons of a Moist Tussock Tundra in Alaska. Frontiers in Microbiology 10:. https://doi.org/10.3389/fmicb.2019.01442

Trotsenko YA, Khmelenina VN (2005) Aerobic methanotrophic bacteria of cold ecosystems. FEMS Microbiology Ecology 53:15–26. https://doi.org/10.1016/j.femsec.2005.02.010

Trotsenko YA, Murrell JC (2008) Metabolic Aspects of Aerobic Obligate Methanotrophy⋆. In: Advances in Applied Microbiology. Elsevier, pp 183–229

Tveit A, Schwacke R, Svenning MM, Urich T (2013) Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. The ISME Journal 7:299–311. https://doi.org/10.1038/ismej.2012.99

Tveit AT, Urich T, Svenning MM (2014) Metatranscriptomic analysis of arctic peat soil microbiota. Applied and Environmental Microbiology 80:5761–5772. https://doi.org/10.1128/AEM.01030-14

Upson R, Read DJ, Newsham KK (2009) Nitrogen form influences the response of Deschampsia antarctica to dark septate root endophytes. Mycorrhiza 20:1–11. https://doi.org/10.1007/s00572-009-0260-3

Urbanová Z, Bárta J (2014) Microbial community composition and in silico predicted metabolic potential reflect biogeochemical gradients between distinct peatland types. FEMS Microbiology Ecology 90:633–646. https://doi.org/10.1111/1574-6941.12422

Urich T, Lanzén A, Qi J, et al (2008) Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome. PLoS ONE 3:e2527. https://doi.org/10.1371/journal.pone.0002527

Van Breemen N, Finzi AC (1998) Plant-soil interactions: ecological aspects and evolutionary implications. In: Van Breemen N (ed) Plant-induced soil changes: Processes and feedbacks. Springer Netherlands, Dordrecht, pp 1–19

van der Wal A, Geydan TD, Kuyper TW, de Boer W (2013) A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiol Rev 37:477–494. https://doi.org/10.1111/1574-6976.12001

Van Vliet-Lanoë B (1991) Differential frost heave, load casting and convection: Converging mechanisms; a discussion of the origin of cryoturbations. Permafrost and Periglacial Processes 2:123–139. https://doi.org/10.1002/ppp.3430020207

Venkatachalam S, Kannan VM, Saritha VN, et al (2021) Bacterial diversity and community structure along the glacier foreland of Midtre Lovénbreen, Svalbard, Arctic. Ecological Indicators 126:107704. https://doi.org/10.1016/j.ecolind.2021.107704

Ver Eecke HC, Akerman NH, Huber JA, et al (2013) Growth kinetics and energetics of a deep-sea hyperthermophilic methanogen under varying environmental conditions. Environmental Microbiology Reports n/a-n/a. https://doi.org/10.1111/1758-2229.12065

Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. The ISME Journal 5:1067–1071. https://doi.org/10.1038/ismej.2010.191

Wagg C, Schlaeppi K, Banerjee S, et al (2019) Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nature Communications 10:4841. https://doi.org/10.1038/s41467-019-12798-y

Wagner D (2017) Effect of varying soil water potentials on methanogenesis in aerated marshland soils. Scientific Reports 7:14706–14706. https://doi.org/10.1038/s41598-017-14980-y

Walker DA (2000) Hierarchical subdivision of Arctic tundra based on vegetation response to climate, parent material and topography. Global Change Biology 6:19–34. https://doi.org/10.1046/j.1365-2486.2000.06010.x

Walker DA, Raynolds MK, Daniëls FJA, et al (2005) The Circumpolar Arctic vegetation map. Journal of Vegetation Science 16:267–282. https://doi.org/10.1111/j.1654-1103.2005.tb02365.x

Walker JKM, Phillips LA, Jones MD (2014) Ectomycorrhizal fungal hyphae communities vary more along a pH and nitrogen gradient than between decayed wood and mineral soil microsites. Botany 92:453–463. https://doi.org/10.1139/cjb-2013-0239

Walker TN, Garnett MH, Ward SE, et al (2016) Vascular plants promote ancient peatland carbon loss with climate warming. Glob Change Biol 22:1880–1889. https://doi.org/10.1111/gcb.13213

Wallenstein MD, McMahon S, Schimel J (2007) Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. pp 428–435

Wallenstein MD, Mcmahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Global Change Biology. https://doi.org/10.1111/j.1365-2486.2008.01819.x

Wang X, Zhang Z, Yu Z, et al (2020) Composition and diversity of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau. Science of The Total Environment 747:141358. https://doi.org/10.1016/j.scitotenv.2020.141358

Wardle DA, Yeates GW (1993) The dual importance of competition and predation as regulatory forces in terrestrial ecosystems: evidence from decomposer food-webs. Oecologia 93:303–306. https://doi.org/10.1007/BF00317685

Warneke C, Karl T, Judmaier H, et al (1999) Acetone, methanol, and other partially oxidized volatile organic emissions from dead plant matter by abiological processes: Significance for atmospheric HO x chemistry. Global Biogeochemical Cycles 13:9–17. https://doi.org/10.1029/98GB02428

Washburn AL 1980. GeocryologyJW New York (1980) A survey of periglacial processes and enviroments

Wei J, Gao J, Wang N, et al (2019) Differences in soil microbial response to anthropogenic disturbances in Sanjiang and Momoge Wetlands, China. FEMS Microbiology Ecology fiz110. https://doi.org/10.1093/femsec/fiz110

Weintraub MN, Schimel JP (2005) Nitrogen Cycling and the Spread of Shrubs Control Changes in the Carbon Balance of Arctic Tundra Ecosystems. BioScience 55:408–415. https://doi.org/10.1641/0006-3568(2005)055[0408:NCATSO]2.0.CO;2

Wieczorek AS, Schmidt O, Chatzinotas A, et al (2019) Ecological Functions of Agricultural Soil Bacteria and Microeukaryotes in Chitin Degradation: A Case Study. Front Microbiol 10:1293. https://doi.org/10.3389/fmicb.2019.01293

Wild B, Schnecker J, Alves RJE, et al (2014) Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. Soil Biology and Biochemistry 75:143–151. https://doi.org/10.1016/j.soilbio.2014.04.014

Wild B, Schnecker J, Bárta J, et al (2013) Nitrogen dynamics in Turbic Cryosols from Siberia and Greenland. Soil Biology and Biochemistry 67:85–93. https://doi.org/10.1016/j.soilbio.2013.08.004

Wu B, Hussain M, Zhang W, et al (2019) Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. Mycology 10:127–140. https://doi.org/10.1080/21501203.2019.1614106

Wu D, Zhao Y, Cheng L, et al (2021a) Activity and structure of methanogenic microbial communities in sediments of cascade hydropower reservoirs, Southwest China. Science of The Total Environment 786:147515–147515. https://doi.org/10.1016/j.scitotenv.2021.147515

Wu X, Chauhan A, Layton AC, et al (2021b) Comparative Metagenomics of the Active Layer and Permafrost from Low-Carbon Soil in the Canadian High Arctic. Environ Sci Technol 55:12683–12693. https://doi.org/10.1021/acs.est.1c00802

Wu X, Xu H, Liu G, et al (2018) Effects of permafrost collapse on soil bacterial communities in a wet meadow on the northern Qinghai-Tibetan Plateau. BMC Ecology 18:27. https://doi.org/10.1186/s12898-018-0183-y

Yang G, Peng Y, Abbott BW, et al (2021) Phosphorus rather than nitrogen regulates ecosystem carbon dynamics after permafrost thaw. Glob Change Biol 27:5818–5830. https://doi.org/10.1111/gcb.15845

Yao H, Conrad R (1999) Thermodynamics of methane production in different rice paddy soils from China, the Philippines and Italy. Soil Biology and Biochemistry 31:463–473. https://doi.org/10.1016/S0038-0717(98)00152-7

Yergeau E, Hogues H, Whyte LG, Greer CW (2010) The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. The ISME Journal 4:1206–1214. https://doi.org/10.1038/ismej.2010.41

Zevenhuizen LPTM (1966) Formation and function of the glycogen-like polysaccharide ofArthrobacter. Antonie van Leeuwenhoek 32:356–372. https://doi.org/10.1007/BF02097485

Zhang T, Wang N-F, Liu H-Y, et al (2016) Soil pH is a Key Determinant of Soil Fungal Community Composition in the Ny-Ålesund Region, Svalbard (High Arctic). Frontiers in Microbiology 7:. https://doi.org/10.3389/fmicb.2016.00227

Zhang X, Xie Z, Ma Z, et al (2022) A Microbial-Explicit Soil Organic Carbon Decomposition Model (MESDM): Development and Testing at a Semiarid Grassland Site. Journal of Advances in Modeling Earth Systems 14:. https://doi.org/10.1029/2021MS002485

Zhao D, Wu S (2019) Projected Changes in Permafrost Active Layer Thickness Over the Qinghai-Tibet Plateau Under Climate Change. Water Resources Research 55:7860–7875. https://doi.org/10.1029/2019WR024969

Zhou J, He Z, Yang Y, et al (2015) High-Throughput Metagenomic Technologies for Complex Microbial Community Analysis: Open and Closed Formats. mBio 6:e02288-14. https://doi.org/10.1128/mBio.02288-14

Zielke M, Solheim B, Spjelkavik S, Olsen RA (2005) Nitrogen Fixation in the High Arctic: Role of Vegetation and Environmental Conditions. null 37:372–378. https://doi.org/10.1657/1523-0430(2005)037[0372:NFITHA]2.0.CO;2

Zimov SA (2006) CLIMATE CHANGE: Permafrost and the Global Carbon Budget. Science 312:1612–1613. https://doi.org/10.1126/science.1128908

9 ATTACHED PUBLICATIONS

Paper I

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

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RESEARCH ARTICLE

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

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

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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 CO_2 and CH_4 release.

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

INTRODUCTION

Turbic 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; Dungait *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 CH_4 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 CH_4 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. oligotrophs 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 N_2 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 N_2 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 Artic, 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 (Ojj 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 (Ctot) and total nitrogen (Ntot) 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 uL 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, Pyrococus 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 N_2 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 Doebeli 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)
	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)
UT	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)
	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)
WT	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 rarefication, respectively. In total, one sample from the topsoil of the WT was removed after rarefication. 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 *Epsilonbacteraeota*. 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

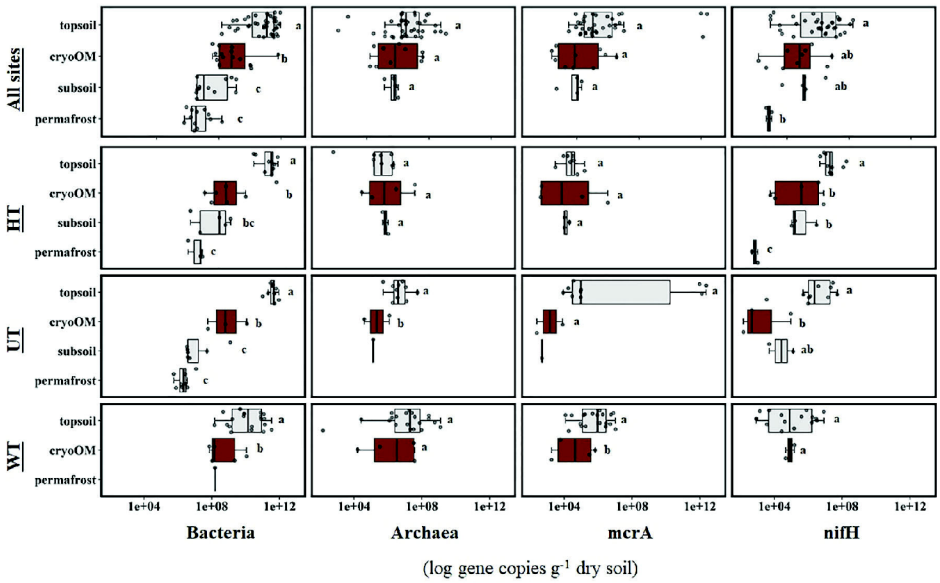

Figure 1. Box-and-whisker plot showing log-transformed gene copies per gram dry soil of individual soil horizons from each tundra types determined for bacteria, archaea, *mcrA*-methanogens and *nifH*-diazotrophs by qPCR. Different letters depicted significant differences between soil horizons ($P < 0.05$, 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-foldchanges and

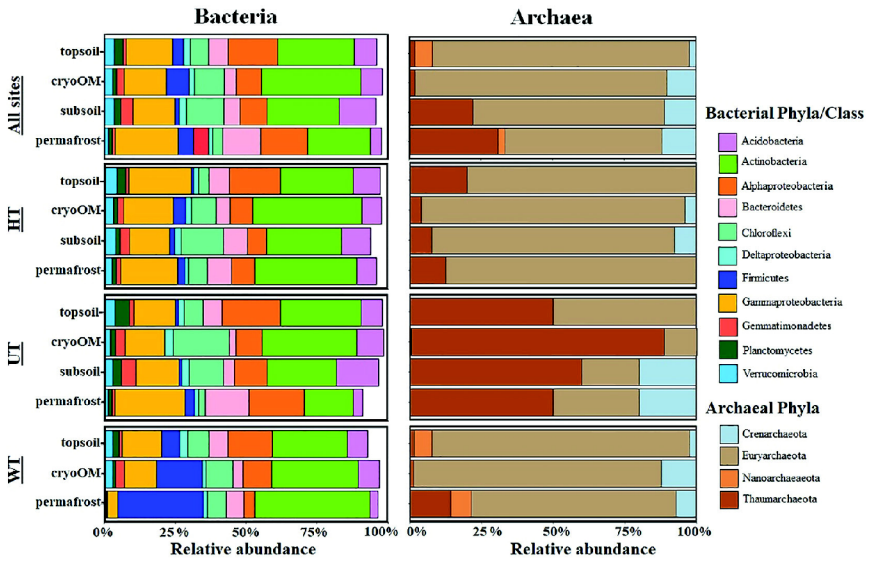

Figure 2. Relative distribution of bacterial and archaeal phyla/classes between different horizons based on targeted 16S rRNA sequencing. Left panels: Only those bacterial phyla with >1% relative abundance was shown, phylum *Proteobacteria* were shown at respective class levels. Right panels: Relative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

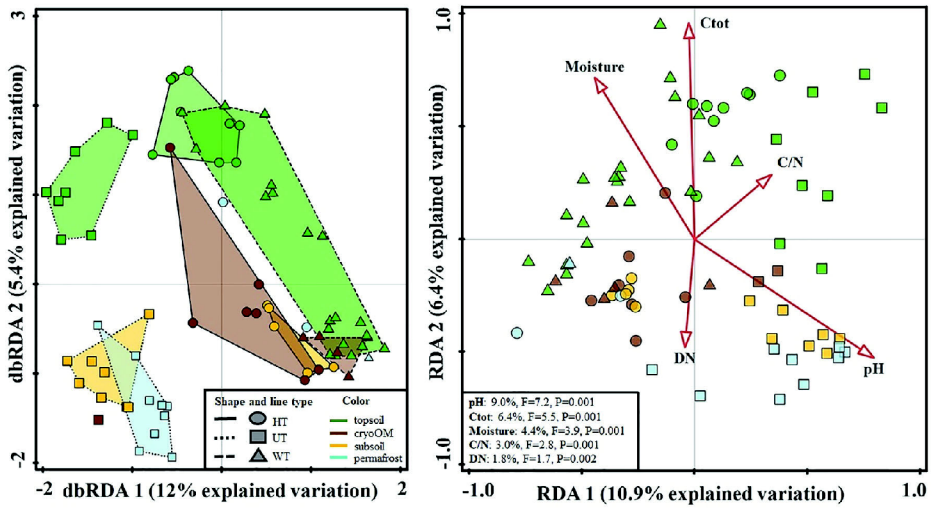

Figure 3. The phylogenetic dissimilarity between soil horizons and tundra types. **(A)** Distance-based RDA (dbRDA) of microbial communities of different horizons from distinct tundra types. Different colored envelopes represented different horizons, whereas the shape of points and line type represented 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 approximatelt 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.msu.edu/, Fish *et al.* 2013).

	Class	Genus	ARN	All sites				HT				UT				WT			
				topsoil	cryoOM	subsoil	permafrost	topsoil	cryoOM	subsoil	permafrost	topsoil	cryoOM	subsoil	permafrost	topsoil	cryoOM	permafrost
Methanogens (*mcrA*)		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	1.0
	Methanobacteria	*Methanobacterium*	2	3.5	1.3	1.2	0.6	2.0	4.7	2.3	0.3	1.0			1.0	7.4	0.6	0.5
	Methanomicrobia	*uncultured archaeon (Rice Cluster II)*	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	0.2
	Methanomicrobia	*Methanosarcina*	3	1.0	0.1				0.1							3.0	0.2	
	Methanomicrobia	*Methanosaeta*	1	0.5	0.1		0.4								1.0	1.4	0.4	0.1
		other	NA	0.2	0.1		0.4								1.0	0.5	0.5	0.2
Diazotrophs (*nifH*)		All diazotrophs	NA	12.3	4.7	6.0	4.0	9.0	7.0	5.0	2.0	8.0	2.0	9.0	7.0	20.0	5.0	1.0
	Gammaproteobacteria	*Pseudomonas*	5	2.5	0.6	0.9	0.5	3.0	0.7	0.6	0.4	1.4	0.2	1.1	1.1	3.1	0.9	
	Gammaproteobacteria	*Rhodoferax*	3	0.8	0.6	1.2	0.5	0.3	1.2	1.9	0.2	0.3	0.2	0.5	1.4	1.8	0.4	
	Actinobacteria	*Arthrobacter*	5	0.9	1.0	0.4	0.4	0.7	1.9	0.3	0.6	0.3	0.3	0.6	0.2	1.8	0.9	0.3
	Alphaproteobacteria	*Bradyrhizobium*	1	1.9	0.5	0.5	0.1	1.9	0.8	0.6	0.1	0.7	0.1	0.4	0.2	3.1	0.7	
	Alphaproteobacteria	*Sphingomonas*	2	1.9	0.4	0.5	0.4	0.6	0.4	0.2	0.1	0.9	0.3	0.9	0.9	4.2	0.6	0.1
	Actinobacteria	*Nocardioides*	3	0.6	0.1	0.3	0.1	0.4	0.1			1.2	0.3	0.6	0.3	0.3		
	Gammaproteobacteria	*Polaromonas*	1	0.1	0.2	0.1	0.4	0.1	0.3	0.1		0.1	0.2	0.1	1.3	0.1		
	Alphaproteobacteria	*Brevundimonas*	2	0.1		0.1	0.4	0.1		0.1		0.1	0.1	0.1	1.1	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

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

Bradyrhizobium, Sphingomonas (*Alphaproteobacteria*); *Pseudomonas, Rhodoferax* (*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 *Rhodoferax* 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*, *Acidobacteriia,* 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

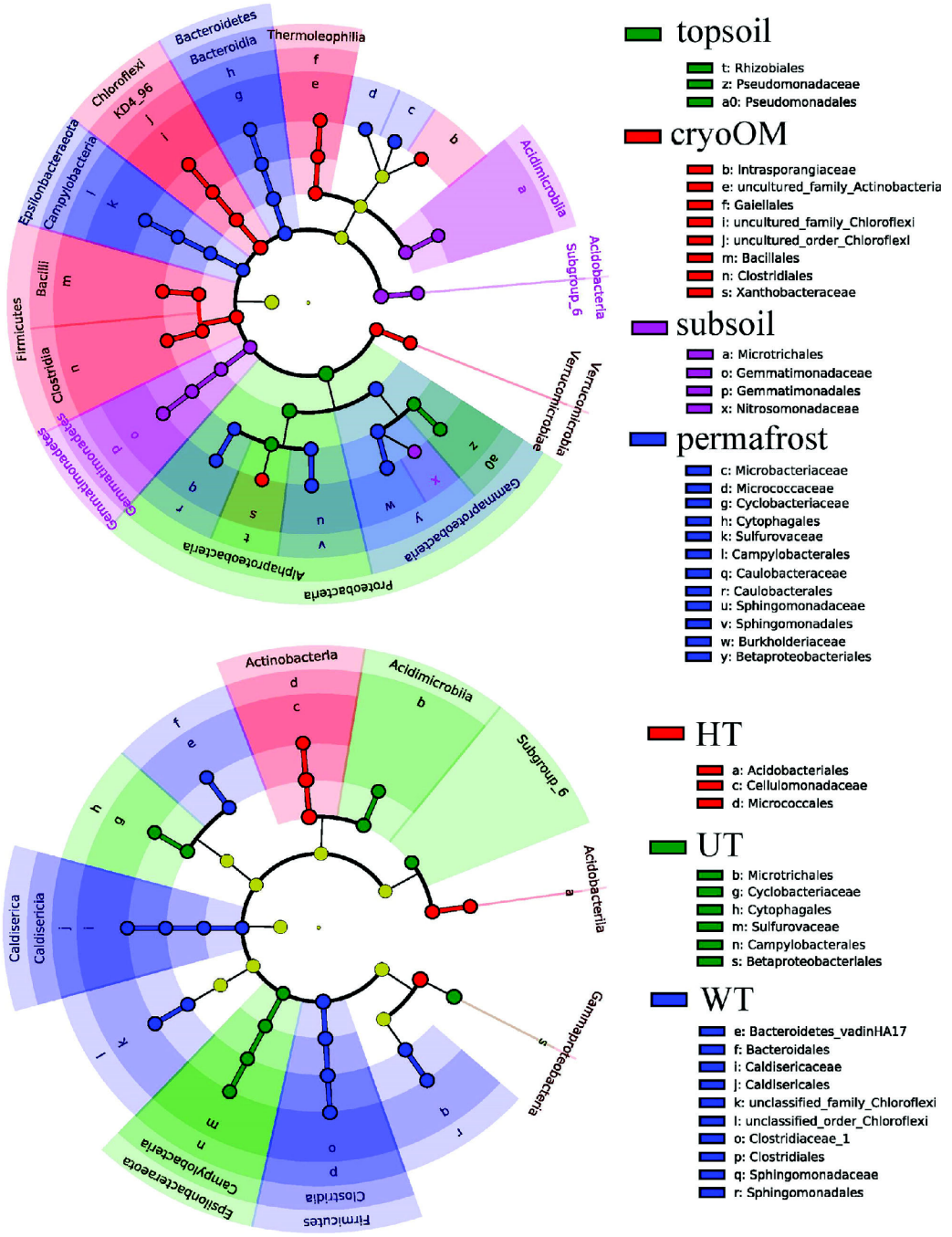

Figure 4. Cladogram reporting results from the LEfSe analysis for horizons **(A)** and tundra types **(B)** at Phylum/Class/Order/Family level. In the figure, the edges closest to the center represent the zOTUs at the phylum level, whereas the edges on the outer circle represent the zOTUs at the family level. The color of the dots and sectors indicate which phyla/classes/orders/families respective were significantly differentially more 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, Caldisericaceae, 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 anaplerotic 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 *Acidobacterila*, 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 *Caldisericaceae*. 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 *Caldisericaceae* 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 CH_4 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 CH_4 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 N_2 (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 N_2.

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 N_2 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 CH_4 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

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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.

REFERENCES

Altshuler I, Ronholm J, Layton A *et al.* Denitrifiers, nitrogen-fixing bacteria and N2O soil gas flux in high Arctic ice-wedge polygon cryosols. *FEMS Microbiol Ecol* 2019;**95**, DOI: 10.1093/femsec/fiz049.

Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 2001;**26**:32–46.

Bae H-S, Morrison E, Chanton JP *et al.* Methanogens are major contributors to nitrogen fixation in soils of the Florida Everglades. Vieille C (ed.). *Appl Environ Microbiol* 2018;**84**, DOI: 10.1128/AEM.02222-17.

Barbier BA, Dziduch I, Liebner S *et al.* Methane-cycling communities in a permafrost-affected soil on Herschel Island, Western Canadian Arctic: active layer profiling of mcrA and pmoA genes. *FEMS Microbiol Ecol* 2012;**82**:287–302.

Biasi C, Wanek W, Rusalimova O *et al.* Microtopography and plant-cover controls on nitrogen dynamics in hummock tundra ecosystems in Siberia. *Arctic, Antarct Alp Res* 2005;**37**: 435–43.

Bockheim JG, Tarnocai C. Recognition of cryoturbation for classifying permafrost-affected soils. *Geoderma* 1998;**81**:281–93.

Bolyen E, Rideout JR, Dillon MR *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;**37**:852–7.

Brabban AD, Orcutt EN, Zinder SH. Interactions between nitrogen fixation and osmoregulation in the methanogenic archaeon *Methanosarcina barkeri* 227. *Appl Environ Microbiol* 1999;**65**:1222–7.

Burn C. *Climate, in: Herschel Island Qikiqtaryuk: A Natural and Cultural History of Yukon's Arctic Island*. Whitehorse: University of Calgary Press, 2012, 48–53.

Burn CR, Zhang Y. Permafrost and climate change at Herschel Island (Qikiqtaruq), Yukon Territory, Canada. *J Geophys Res Earth Surf* 2009;**114**:1–16.

Caporaso JG, Lauber CL, Walters WA *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci* 2011;**108**:4516–22.

Conrad R. Microbial ecology of methanogens and methanotrophs. 2007, 1–63. DOI: 10.1016/S0065-2113(07)96005-8.

Čapek P, Diáková K, Dickopp JE *et al.* The effect of warming on the vulnerability of subducted organic carbon in arctic soils. *Soil Biol Biochem* 2015;**90**:19–29.

Dang H, Yang J, Li J *et al.* Environment-dependent distribution of the sediment nifH-harboring microbiota in the northern South China Sea. *Appl Environ Microbiol* 2013;**79**:121–32.

Dao TT, Gentsch N, Mikutta R *et al.* Fate of carbohydrates and lignin in north-east Siberian permafrost soils. *Soil Biol Biochem* 2018;**116**:311–22.

Davis KER, Sangwan P, Janssen PH. Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow-growing and mini-colony-forming soil bacteria. *Environ Microbiol* 2011;**13**:798–805.

de Graaff M-A, Van Kessel C, Six J. Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated CO2. *Soil Biol Biochem* 2009;**41**:1094–103.

Doronina NV. *Methylobacillus pratensis* sp. nov., a novel non-pigmented, aerobic, obligately methylotrophic bacterium isolated from meadow grass. *Int J Syst Evol Microbiol* 2004;**54**:1453–7.

Doronina NV. Phylogenetic position and emended description of the genus Methylovorus. *Int J Syst Evol Microbiol* 2005;**55**: 903–6.

Douglas GM, Maffei VJ, Zaneveld JR *et al.* PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 2020;**38**:685–8.

Dungait JAJ, Hopkins DW, Gregory AS *et al.* Soil organic matter turnover is governed by accessibility not recalcitrance. *Glob Chang Biol* 2012;**18**:1781–96.

Edgar R. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv* 2016, DOI: 10.1101/081257.

Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 2015;**31**:3476–82.

Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013, DOI: 10.1038/nmeth.2604.

Eilers KG, Lauber CL, Knight R *et al.* Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol Biochem* 2010;**42**: 896–903.

FAO. *World Reference Base for Soil Resources 2006: A Framework for International Classification, Correlation and Communication*, 2006.

Fierer N, Bradford MA, Jackson RB. Toward an ecological classification of soil bacteria. *Ecology* 2007;**88**:1354–64.

Finzi AC, Abramoff RZ, Spiller KS *et al.* Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Glob Chang Biol* 2015;**21**:2082–94.

Fish JA, Chai B, Wang Q *et al.* FunGene: the functional gene pipeline and repository. *Front Microbiol* 2013;**4**:291.

Gaby JC, Buckley DH. A comprehensive evaluation of PCR primers to amplify the nifH gene of nitrogenase. Balcazar JL (ed.). *PLoS One* 2012;**7**:1–12.

Ganzert L, Jurgens G, Münster U *et al.* Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. *FEMS Microbiol Ecol*. 2007;**59**: 476–88.

Gentsch N, Wild B, Mikutta R *et al.* Temperature response of permafrost soil carbon is attenuated by mineral protection. *Glob Chang Biol* 2018;**24**:3401–15.

Gittel A, Bárta J, Kohoutová I *et al.* Distinct microbial communities associated with buried soils in the Siberian tundra. *ISME J* 2014b;**8**:841–53.

Gittel A, Bárta J, Kohoutová I *et al.* Site- and horizon-specific patterns of microbial community structure and enzyme activities in permafrost-affected soils of Greenland. *Front Microbiol* 2014a;**5**:541.

Gupta RS, Mok A. Phylogenomics and signature proteins for the alpha Proteobacteria and its main groups. *BMC Microbiol* 2007;**7**:106.

Hales BA, Edwards C, Ritchie DA *et al.* Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* 1996. DOI: 10.1128/AEM.62.2.668-675.1996.

Harden JW, Koven CD, Ping C-L *et al.* Field information links permafrost carbon to physical vulnerabilities of thawing. *Geophys Res Lett* 2012;**39**, DOI: 10.1029/2012GL051958.

Hugelius G, Strauss J, Zubrzycki S *et al.* Estimated stocks of circumpolar permafrost carbon with quantified uncertainty ranges and identified data gaps. *Biogeosciences* 2014;**11**: 6573–93.

Izquierdo JA, Nüsslein K. Distribution of extensive nifH gene diversity Across physical Soil microenvironments. *Microb Ecol* 2006;**51**:441–52.

Jackson RB, Lajtha K, Crow SE *et al.* The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. *Annu Rev Ecol Evol Syst* 2017;**48**:419–45.

Jenkins O, Jones D. Taxonomic studies on some Gram-negative methylotrophic bacteria. *Microbiology* 1987;**133**:453–73.

Kaiser C, Meyer H, Biasi C *et al.* Conservation of soil organic matter through cryoturbation in arctic soils in Siberia. *J Geophys Res Biogeosciences* 2007;**112**:1–8.

Kalyuzhnaya MG, Bowerman S, Lara JC *et al. Methylotenera mobilis* gen. nov., sp. nov., an obligately methylamine-utilizing bacterium within the family Methylophilaceae. *Int J Syst Evol Microbiol* 2006;**56**:2819–23.

Klappenbach JA, Dunbar JM, Schmidt TM. rRNA operon copy number reflects ecological strategies of bacteria. *Appl Environ Microbiol* 2000;**66**:1328–33.

Koyama A, Wallenstein MD, Simpson RT *et al.* Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. *Front Microbiol* 2014;**5**:516.

Kruger M, Frenzel P. Effects of N-fertilisation on CH4 oxidation and production, and consequences for CH4 emissions from microcosms and rice fields. *Glob Chang Biol* 2003;**9**:773–84.

Lau MCY, Stackhouse BT, Layton AC *et al.* An active atmospheric methane sink in high Arctic mineral cryosols. *ISME J* 2015;**9**:1880–91.

Legendre P, Andersson MJ. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecol Monogr* 1999;**69**:512.

Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. *Science (80-)* 2016;**353**:1272–7.

Mizukami S, Takeda K, Akada S *et al.* Isolation and characteristics of Methanosaeta in paddy field soils. *Biosci Biotechnol Biochem* 2006;**70**:828–35.

Molles MCJ. *Ecology: Concepts and Applications*. New York, NY: McGraw-Hill, 2001.

Mori K, Yamaguchi K, Sakiyama Y *et al.* Caldisericum exile gen. nov., sp. nov., an anaerobic, thermophilic, filamentous bacterium of a novel bacterial phylum, Caldiserica phyl. nov., originally called the candidate phylum OP5, and description of Caldisericaceae fam. nov., Caldisericales ord. no. *Int J Syst Evol Microbiol* 2009;**59**:2894–8.

Oksanen J, Kindt R, Legendre P *et al. The Vegan Package Title Community Ecology Package*. Http://CranR-ProjectOrg/, Http://R-ForgeR-ProjectOrg/Projects/Vegan/ 2007.

Olefeldt D, Turetsky MR, Crill PM *et al.* Environmental and physical controls on northern terrestrial methane emissions across permafrost zones. *Glob Chang Biol* 2013;**19**:589–603.

Palmtag J, Kuhry P. Grain size controls on cryoturbation and soil organic carbon density in permafrost - affected soils. 2018:112–20. DOI: 10.1002/ppp.1975.

Parks DH, Beiko RG. Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* 2010;**26**:715–21.

Penton CR, Yang C, Wu L *et al.* NifH-harboring bacterial community composition across an Alaskan Permafrost Thaw Gradient. *Front Microbiol* 2016;**7**:1894.

Perner M, Gonnella G, Kurtz S *et al.* Handling temperature bursts reaching 464°C: different microbial strategies in the Sisters Peak hydrothermal chimney. Drake HL (ed.). *Appl Environ Microbiol* 2014;**80**:4585–98.

Ping C-L, Clark MH, Kimble JM *et al.* Sampling protocols for permafrost-affected soils. *Soil Horizons* 2013;**54**:13.

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res* 2013, DOI: 10.1093/nar/gks1219.

R Development Core Team R. *R: A Language and Environment for Statistical Computing*, 2011.

Roy R, Conrad R. Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. *FEMS Microbiol Ecol* 1999;**28**:49–61.

Schellenberger S, Kolb S, Drake HL. Metabolic responses of novel cellulolytic and saccharolytic agricultural soil Bacteria to oxygen. *Environ Microbiol* 2010;**12**:845–61.

Schimel JP, Bennett J. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 2004;**85**:591–602.

Schmidt MWI, Torn MS, Abiven S *et al.* Persistence of soil organic matter as an ecosystem property. *Nature* 2011;**478**:49–56.

Schnecker J, Wild B, Hofhansl F *et al.* Effects of soil organic matter properties and microbial community composition on enzyme activities in cryoturbated Arctic soils. *PLoS One* 2014;**9**:e94076.

Schoeneberger PJ, Wysocki DA, Benham EC *et al.* Field book for describing and sampling soils. *Nat Resour Conserv Serv Natl Soil Surv Center, Lincoln, NE* 2012, DOI: 10.1111/j.1600-0587.2009.05973.x.

Segata N, Izard J, Waldron L *et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12**:R60.

Seo J-S, Chong H, Park HS *et al.* The genome sequence of the ethanologenic bacterium *Zymomonas mobilis* ZM4. *Nat Biotechnol* 2005;**23**:63–8.

Shi Y, Xiang X, Shen C *et al.* Vegetation-associated impacts on Arctic tundra bacterial and microeukaryotic communities. Schloss PD (ed.). *Appl Environ Microbiol* 2015;**81**:492–501.

Smilauer P, Leps J. *Multivariate Analysis of Ecological Data Using Canoco* 5., 2014.

Smith CAS, Kennedy CE, Hargrave AE *et al. Soil and Vegetation of Herschel Island, Yukon Territory*. Yukon Soil Survey Report, **vol. 1**, Land Resource Research Centre, Agriculture Canada, Ottawa, 1989.

Ström L, Mastepanov M, Christensen TR. Species-specific effects of vascular plants on carbon turnover and methane emissions from wetlands. *Biogeochemistry* 2005;**75**:65–82.

Söllinger A, Tveit AT, Poulsen M *et al.* Holistic assessment of rumen microbiome dynamics through quantitative metatranscriptomics reveals multifunctional redundancy during key steps of anaerobic feed Degradation. Jansson JK (ed.). *mSystems* 2018;**3**:e00038–18.

Šantrůčková H, Kotas P, Bárta J *et al.* Significance of dark CO2 fixation in arctic soils. *Soil Biol Biochem* 2018;**119**:11–21.

Takai K. The nitrogen cycle: a large, fast, and mystifying cycle. *Microbes Environ* 2019;**34**:223–5.

Thauer RK, Kaster A-K, Seedorf H *et al.* Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat Rev Microbiol* 2008;**6**:579–91.

Thompson LR, Sanders JG, McDonald D *et al.* A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 2017;**551**:457–63.

Urbanová Z, Bárta J. Recovery of methanogenic community and its activity in long-term drained peatlands after rewetting. *Ecol Eng* 2020;**150**:105852.

van Vliet-Lanoë B. Chronostratigraphy and paleoclimatic meaning of cryogenic deformations in the Central European loess. *GeoJournal* 1991;**24**:157–63.

Wagner R, Zona D, Oechel W *et al.* Microbial community structure and soil pH correspond to methane production in Arctic Alaska soils. *Environ Microbiol* 2017;**19**:3398–410.

Wallenstein MD, McMahon S, Schimel J. Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. *FEMS Microbiol Ecol*. **Vol 59**. 2007, 428–35.

Weintraub MN, Schimel JP. Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in Arctic tundra soils. *Ecosystems* 2003;**6**:129–43.

Wild B, Gentsch N, Čapek P *et al.* Plant-derived compounds stimulate the decomposition of organic matter in arctic permafrost soils. *Sci Rep* 2016;**6**:25607.

Wild B, Schnecker J, Alves RJE *et al.* Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. *Soil Biol Biochem* 2014;**75**:143–51.

Wild B, Schnecker J, Bárta J *et al*. Nitrogen dynamics in turbic cryosols from Siberia and Greenland. *Soil Biol Biochem* 2013;**67**:85–93.

Yu Y, Lee C, Kim J *et al*. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol Bioeng* 2005;**89**:670–9.

Zhou J, Deng Y, Shen L *et al*. Temperature mediates continental-scale diversity of microbes in forest soils. *Nat Commun* 2016;**7**:12083.

Zhou J, He Z, Yang Y *et al*. High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. *MBio* 2015;**6**: e02288–14.

Paper II

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

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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

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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 °C with the mean monthly air temperature varying between −26.3 °C (February) and 8.7 °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 °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 (Ctot) and nitrogen (Ntot) content were determined from 60 °C dried soil sample (8–10 mg) using an Elementar Vario Micro cube (Elementar, Langenselbold, Germany) and expressed in percentage. The carbon to nitrogen ratio (C/N ratio) was calculated by dividing Ctot with Ntot. 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 ug 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 uM), respectively [54]. A 200 µL sample of the soil suspension and 50µL substrate (β-D-glucopyranoside, N-cellobiopyranoside, phosphate, N-acetglucosaminide, 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 ß-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 (appx. 0.25 g) using a DNeasy PowerSoilTM DNA Isolation Kit (Qiagen, Düsseldorf, Germany). Extracted DNA was stored at −20 °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_2Owater).

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 (Zi) which describes how well a node is connected with other nodes within its own module. The second parameter is connectivity between modules (Pi) which suggests how well a node is connected to different modules. The threshold values of Zi and Pi 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 Zi (<2.5) and Pi 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 Zi (<2.5) but a high Pi value (>0.62) (i.e., these nodes tend to have more connections with several modules); (3) module hubs (generalist), which have a high Zi (>2.5) but a low Pi 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 Zi (>2.5) and Pi (>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 Zi-Pi 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 deferred 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)
	CryoOM	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 (b)	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)
	Permafrost	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)
	CryoOM	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)
	Permafrost	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)
	CryoOM	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
Site 4	Topsoil	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)
	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)
	Subsoil	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)

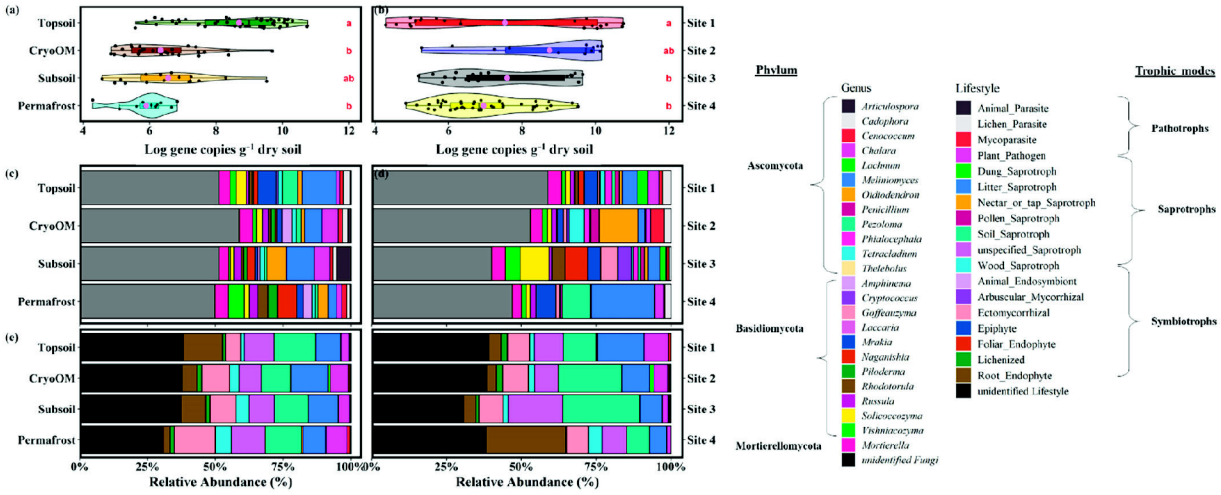

Figure 1. Fungi gene abundance and community composition. Log-transformed fungal gene copies per gram dry weight of soil are shown for (**a**) horizon and (**b**) tundra site. Based on Tukey's HSD post hoc tests, gene abundance that differed between horizons and tundra sites was represented by different 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

each other from Site 1 and Site 2, whereas two dispersed clusters were found for topsoil from Site 3. Samples from Site 4 were separated from other sites' samples. The RDA-based forward selection was used to identify the most important environmental factors affecting the fungal communities, we found pH and DN as the main contributors.

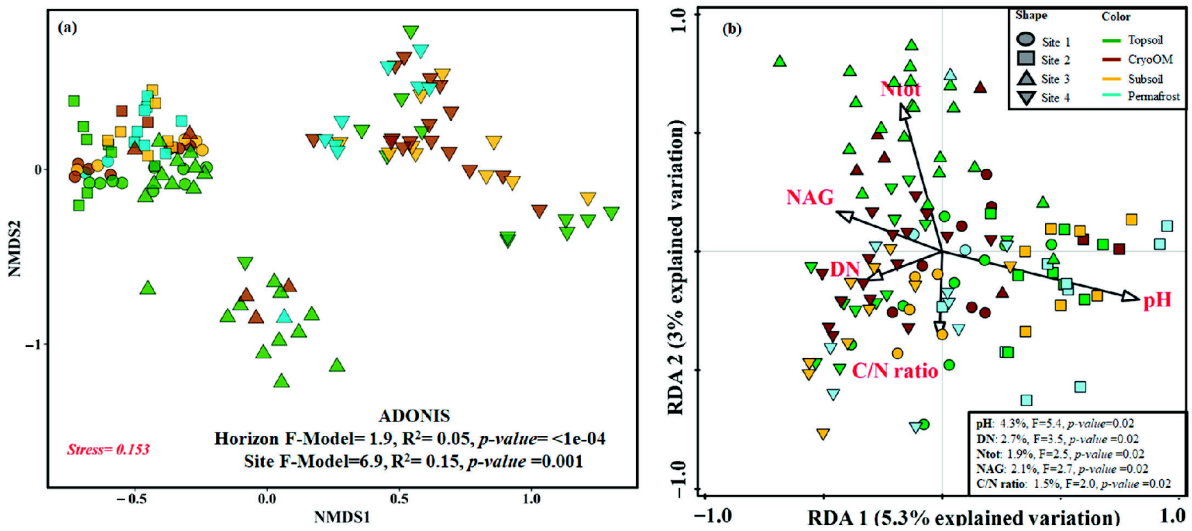

Figure 2. The phylogenetic dissimilarity between soil horizons and tundra types. (**a**) NMDS of fungal communities of different horizons from distinct tundra types; (**b**) RDA biplot of fungal diversity and environmental factors. Significant effect of soil parameters (black arrow in figure) on fungal communities were identified by forward selection. The proportion of variability explained by significant soil parameters are given in the lower right corner.

3.3. Key Topological Properties of Co-Occurrence Network

To identify the interaction of fungal taxa, we constructed a co-occurrence network from each horizon and tundra site (Figure 3). The respective global topological properties of the co-occurrence network with the corresponding random network are given in Table 2.

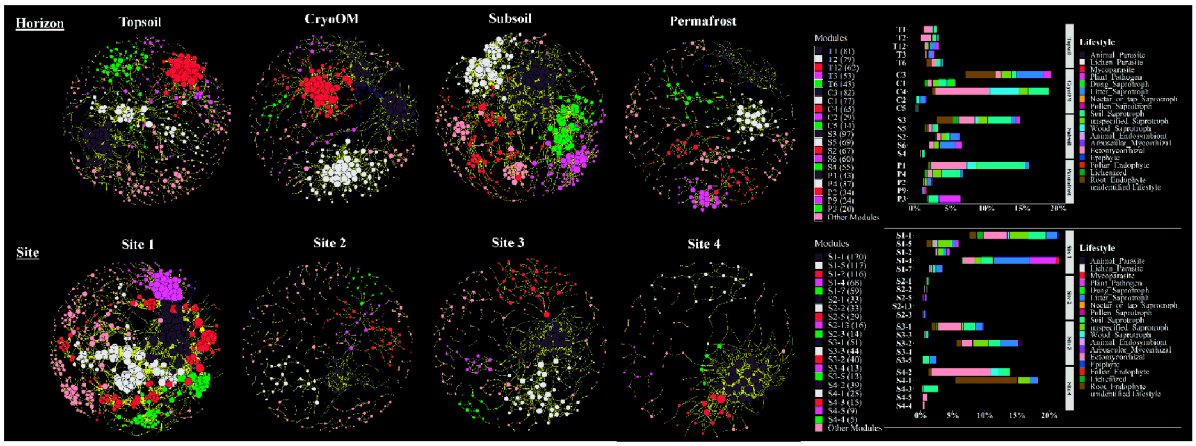

Figure 3. Co-occurrence network interaction of fungal zOTUs found in each horizon and tundra site. A connection stands for a strong Spearman's correlation ($r \geq 0.6$ and p-value ≤ 0.01). Each circle or node represented a fungal zOTU and the sizes of the circles were proportional to the values of node square-root degree. Lines connecting two fungal zOTU represented the interactions between them, yellow and blue lines represented the positive and negative significant correlations, respectively. Nodes were colored according to the top five modules. The relative proportion of fungal lifestyle from the top five modules of each horizon and tundra site are shown.

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
Empirical network	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
Random network [c]	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.

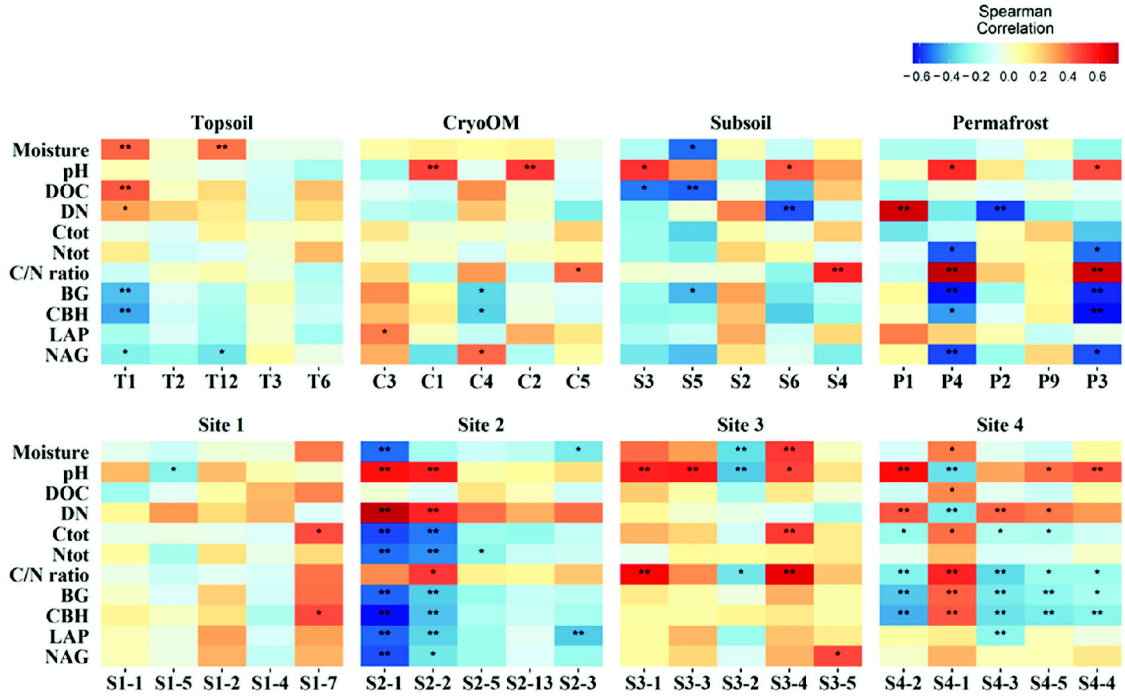

Figure 4. Spearman rank correlation coefficients of soil environmental factors and network modules (top five) for individual horizons and tundra sites. The reds represented a positive correlation and the blue represented a negative correlation. The heatmap cells marked by "*" or "**" were statistically 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., Pi = 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.

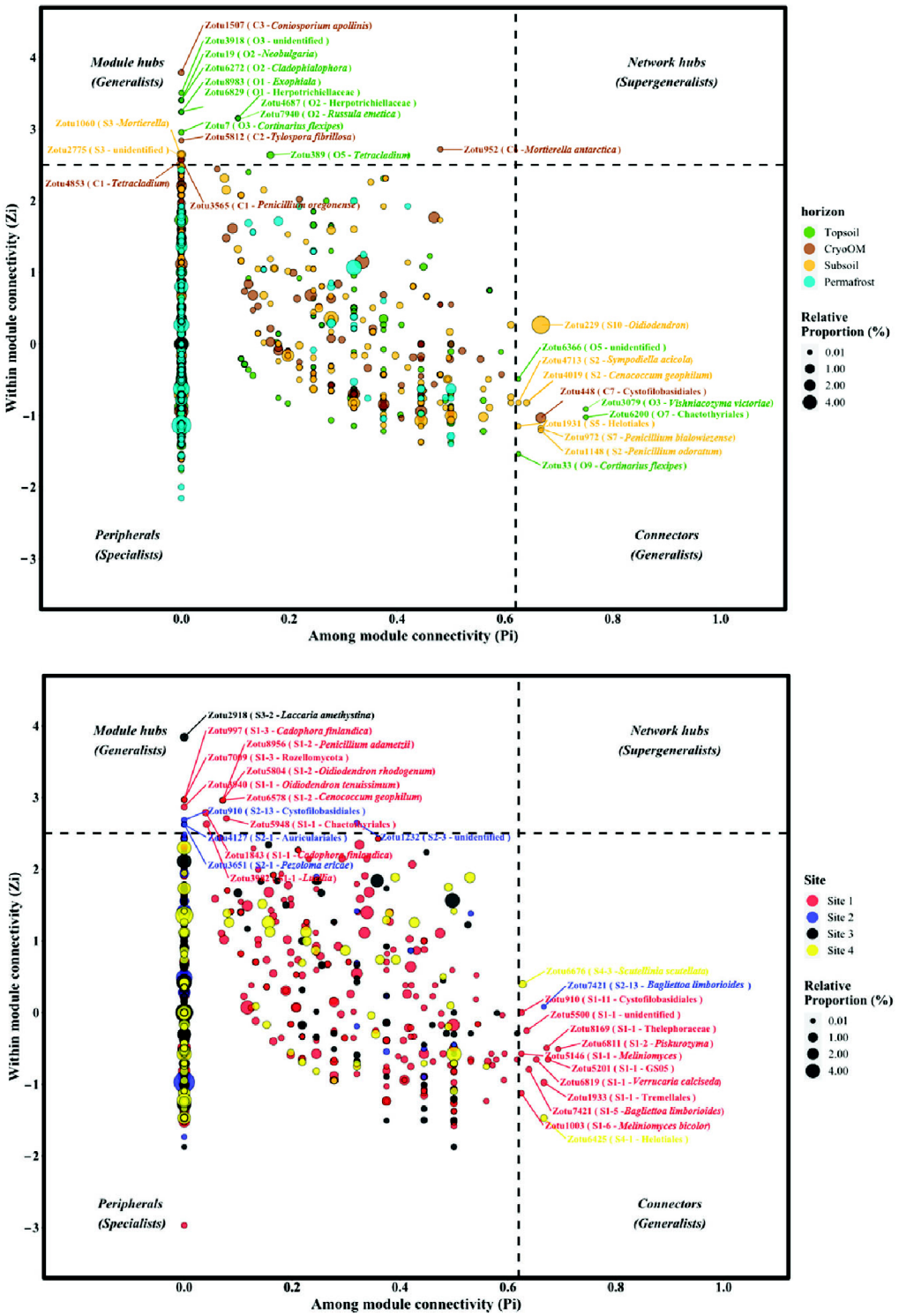

Figure 5. Zi-Pi plot showing topological roles of nodes in different horizons and tundra sites. The threshold values of Zi and Pi for categorizing nodes were 2.5 and 0.62, respectively. Generalists (connectors and module hubs) were labeled with zOTU IDs, module number, and maximum assigned taxonomy (bolded 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	*Cladophialophora*	*unidentified*	Soil_Saprotroph	Module hubs	Peripheral	Peripheral	-
Zotu7	*Cortinarius*	*Cortinarius flexipes*	Ectomycorrhizal	Module hubs	-	-	Peripheral
Zotu952	*Mortierella*	*Mortierella antarctica*	Soil_Saprotroph	Peripheral	Module hubs	Peripheral	Peripheral
Zotu1060	*Mortierella*	*unidentified*	Soil_Saprotroph	Peripheral	Peripheral	Module hubs	-
Zotu19	*Neobulgaria*	*unidentified*	Wood_Saprotroph	Module hubs	-	Peripheral	Peripheral
Zotu229	*Oidiodendron*	*unidentified*	Soil_Saprotroph	-	Peripheral	Connectors	Peripheral
Zotu972	*Penicillium*	*Penicillium bialowiezense*	unspecified_Saprotroph	Peripheral	-	Connectors	Peripheral
Zotu1148	*Penicillium*	*Penicillium odoratum*	unspecified_Saprotroph	Peripheral	Peripheral	Connectors	Peripheral
Zotu3565	*Penicillium*	*Penicillium oregonense*	unspecified_Saprotroph	Peripheral	Module hubs	Peripheral	Peripheral
Zotu7940	*Russula*	*Russula emetica*	Ectomycorrhizal	Module hubs	-	Peripheral	-
Zotu4713	*Sympodiella*	*Sympodiella acicola*	Litter_Saprotroph	-	-	Connectors	Peripheral
Zotu389	*Tetracladium*	*unidentified*	Litter_Saprotroph	Module hubs	-	-	Peripheral
Zotu4853	*Tetracladium*	*unidentified*	Litter_Saprotroph	Peripheral	Module hubs	Peripheral	Peripheral
Zotu5812	*Tylospora*	*Tylospora fibrillosa*	Ectomycorrhizal	-	Module hubs	Peripheral	-
Zotu3079	*Vishniacozyma*	*Vishniacozyma_victoriae*	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	*Cadophora*	*Cadophora finlandica*	Litter_Saprotroph	Module hubs	Peripheral	Peripheral	Peripheral
Zotu6578	*Cenococcum*	*Cenococcum geophilum*	Ectomycorrhizal	Module hubs	Peripheral	-	-
Zotu3982	*Luellia*	*unidentified*	Wood_Saprotroph	Module hubs	Peripheral	Peripheral	-
Zotu5146	*Meliniomyces*	*unidentified*	Root_Endophyte	Connectors	Peripheral	Peripheral	-
Zotu6819	*Verrucaria*	*Verrucaria calciseda*	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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References

1. Payer, D.; Barry, T.; Berteaux, D.; Bültmann, H.; Cristiansen, J.S.; Cook, J.S.; Dahlberg, A.; Daniëls, F.J.A.; Ehrich, D.; Fjeldså, J.; et al. Arctic biodiversity assessment. Status and trends in Arctic biodiversity. In *Fungi*; Meltofte, H., Ed.; Narayana Press: Odder, Denmark, 2013; pp. 303–319.
2. Barea, J.-M.; Pozo, M.J.; Azcón, R.; Azcón-Aguilar, C. Microbial co-operation in the rhizosphere. *J. Exp. Bot.* **2005**, *56*, 1761–1778. [CrossRef]
3. Hestrin, R.; Hammer, E.C.; Mueller, C.W.; Lehmann, J. Synergies between mycorrhizal fungi and soil microbial communities increase plant nitrogen acquisition. *Commun. Biol.* **2019**, *2*, 233. [CrossRef] [PubMed]

4. de Boer, W.; Folman, L.B.; Summerbell, R.C.; Boddy, L. Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiol. Rev.* **2005**, *29*, 795–811. [CrossRef] [PubMed]
5. Geml, J.; Timling, I.; Robinson, C.H.; Lennon, N.; Nusbaum, H.C.; Brochmann, C.; Noordeloos, M.E.; Taylor, D.L. An arctic community of symbiotic fungi assembled by long-distance dispersers: Phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. *J. Biogeogr.* **2012**, *39*, 74–88. [CrossRef]
6. Blaud, A.; Phoenix, G.K.; Osborn, A.M. Variation in bacterial, archaeal and fungal community structure and abundance in High Arctic tundra soil. *Polar Biol.* **2015**, *38*, 1009–1024. [CrossRef]
7. Wallenstein, M.D.; McMahon, S.; Schimel, J. Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. *FEMS Microbiol. Ecol.* **2007**, *59*, 428–435. [CrossRef]
8. Deslippe, J.R.; Hartmann, M.; Simard, S.W.; Mohn, W.W. Long-term warming alters the composition of Arctic soil microbial communities. *FEMS Microbiol. Ecol.* **2012**, *82*, 303–315. [CrossRef]
9. Perini, L.; Gostinčar, C.; Anesio, A.M.; Williamson, C.; Tranter, M.; Gunde-Cimerman, N. Darkening of the Greenland Ice Sheet: Fungal Abundance and Diversity Are Associated With Algal Bloom. *Front. Microbiol.* **2019**, *10*, 557. [CrossRef] [PubMed]
10. Meyling, N.V.; Schmidt, N.M.; Eilenberg, J. Occurrence and diversity of fungal entomopathogens in soils of low and high Arctic Greenland. *Polar Biol.* **2012**, *35*, 1439–1445. [CrossRef]
11. Gittel, A.; Bárta, J.; Kohoutová, I.; Mikutta, R.; Owens, S.; Gilbert, J.; Schnecker, J.; Wild, B.; Hannisdal, B.; Maerz, J.; et al. Distinct microbial communities associated with buried soils in the Siberian tundra. *ISME J.* **2014**, *8*, 841–853. [CrossRef]
12. Timling, I.; Walker, D.A.; Nusbaum, C.; Lennon, N.J.; Taylor, D.L. Rich and cold: Diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. *Mol. Ecol.* **2014**, *23*, 3258–3272. [CrossRef]
13. Louca, S.; Jacques, S.M.S.; Pires, A.P.F.; Leal, J.S.; Srivastava, D.S.; Parfrey, L.W.; Farjalla, V.F.; Doebeli, M. High taxonomic variability despite stable functional structure across microbial communities. *Nat. Ecol. Evol.* **2017**, *1*, 15. [CrossRef]
14. Cernansky, R. Biodiversity moves beyond counting species. *Nature* **2017**, *546*, 22–24. [CrossRef] [PubMed]
15. Moore, D.; Robson, G.D.; Trinci, A.P.J. *21st Century Guidebook to Fungi*; Cambridge University Press: Cambridge, UK, 2011; ISBN 9780511977022.
16. Alzarhani, A.K.; Clark, D.R.; Underwood, G.J.C.; Ford, H.; Cotton, T.E.A.; Dumbrell, A.J. Are drivers of root-associated fungal community structure context specific? *ISME J.* **2019**, *13*, 1330–1344. [CrossRef] [PubMed]
17. Veach, A.M.; Stokes, C.E.; Knoepp, J.; Jumpponen, A.; Baird, R. Fungal Communities and Functional Guilds Shift Along an Elevational Gradient in the Southern Appalachian Mountains. *Microb. Ecol.* **2018**, *76*, 156–168. [CrossRef] [PubMed]
18. Fahey, C.; Koyama, A.; Antunes, P.M.; Dunfield, K.; Flory, S.L. Plant communities mediate the interactive effects of invasion and drought on soil microbial communities. *ISME J.* **2020**, *14*, 1396–1409. [CrossRef]
19. Nguyen, N.H.; Song, Z.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Schilling, J.S.; Kennedy, P.G. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **2016**, *20*, 241–248. [CrossRef]
20. Wild, B.; Schnecker, J.; Bárta, J.; Čapek, P.; Guggenberger, G.; Hofhansl, F.; Kaiser, C.; Lashchinsky, N.; Mikutta, R.; Mooshammer, M.; et al. Nitrogen dynamics in Turbic Cryosols from Siberia and Greenland. *Soil Biol. Biochem.* **2013**, *67*, 85–93. [CrossRef]
21. Kohler, A.; Kuo, A.; Nagy, L.G.; Morin, E.; Barry, K.W.; Buscot, F.; Canbäck, B.; Choi, C.; Cichocki, N.; Clum, A.; et al. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat. Genet.* **2015**, *47*, 410–415. [CrossRef]
22. Wutkowska, M.; Vader, A.; Mundra, S.; Cooper, E.J.; Eidesen, P.B. Dead or Alive; or Does It Really Matter? Level of Congruency Between Trophic Modes in Total and Active Fungal Communities in High Arctic Soil. *Front. Microbiol.* **2019**, *9*, 3243. [CrossRef]
23. Mäkipää, R.; Rajala, T.; Schigel, D.; Rinne, K.T.; Pennanen, T.; Abrego, N.; Ovaskainen, O. Interactions between soil- and dead wood-inhabiting fungal communities during the decay of Norway spruce logs. *ISME J.* **2017**, *11*, 1964–1974. [CrossRef]
24. Anthony, M.A.; Frey, S.D.; Stinson, K.A. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. *Ecosphere* **2017**, *8*, e01951. [CrossRef]
25. Faust, K.; Sathirapongsasuti, J.F.; Izard, J.; Segata, N.; Gevers, D.; Raes, J.; Huttenhower, C. Microbial Co-occurrence Relationships in the Human Microbiome. *PLoS Comput. Biol.* **2012**, *8*, e1002606. [CrossRef] [PubMed]
26. Lupatini, M.; Suleiman, A.K.A.; Jacques, R.J.S.; Antoniolli, Z.I.; de Siqueira Ferreira, A.; Kuramae, E.E.; Roesch, L.F.W. Network topology reveals high connectance levels and few key microbial genera within soils. *Front. Environ. Sci.* **2014**, *2*, 10. [CrossRef]
27. Deng, Y.; Jiang, Y.-H.; Yang, Y.; He, Z.; Luo, F.; Zhou, J. Molecular ecological network analyses. *BMC Bioinform.* **2012**, *13*, 113. [CrossRef] [PubMed]
28. Coyte, K.Z.; Schluter, J.; Foster, K.R. The ecology of the microbiome: Networks, competition, and stability. *Science* **2015**, *350*, 663–666. [CrossRef]
29. Barberán, A.; Bates, S.T.; Casamayor, E.O.; Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* **2012**, *6*, 343–351. [CrossRef]
30. Wagg, C.; Schlaeppi, K.; Banerjee, S.; Kuramae, E.E.; van der Heijden, M.G.A. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nat. Commun.* **2019**, *10*, 4841. [CrossRef]
31. Banerjee, S.; Thrall, P.H.; Bissett, A.; Heijden, M.G.A.; Richardson, A.E. Linking microbial co-occurrences to soil ecological processes across a woodland-grassland ecotone. *Ecol. Evol.* **2018**, *8*, 8217–8230. [CrossRef] [PubMed]
32. Banerjee, S.; Schlaeppi, K.; van der Heijden, M.G.A. Keystone taxa as drivers of microbiome structure and functioning. *Nat. Rev. Microbiol.* **2018**, *16*, 567–576. [CrossRef]

33. Layeghifard, M.; Hwang, D.M.; Guttman, D.S. Disentangling Interactions in the Microbiome: A Network Perspective. *Trends Microbiol.* **2017**, *25*, 217–228. [CrossRef] [PubMed]
34. de Vries, F.T.; Wallenstein, M.D. Below-ground connections underlying above-ground food production: A framework for optimising ecological connections in the rhizosphere. *J. Ecol.* **2017**, *105*, 913–920. [CrossRef]
35. de Vries, F.T.; Liiri, M.E.; Bjørnlund, L.; Bowker, M.A.; Christensen, S.; Setälä, H.M.; Bardgett, R.D. Land use alters the resistance and resilience of soil food webs to drought. *Nat. Clim. Chang.* **2012**, *2*, 276–280. [CrossRef]
36. Feng, J.; Wang, C.; Lei, J.; Yang, Y.; Yan, Q.; Zhou, X.; Tao, X.; Ning, D.; Yuan, M.M.; Qin, Y.; et al. Warming-induced permafrost thaw exacerbates tundra soil carbon decomposition mediated by microbial community. *Microbiome* **2020**, *8*, 3. [CrossRef] [PubMed]
37. Newman, M.E.J. Modularity and community structure in networks. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8577–8582. [CrossRef]
38. Benedek, Z.; Jordán, F.; Báldi, A. Topological keystone species complexes in ecological interaction networks. *Community Ecol.* **2007**, *8*, 1–7. [CrossRef]
39. Berry, D.; Widder, S. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Front. Microbiol.* **2014**, *5*, 219. [CrossRef]
40. Zhang, T.; Wang, N.; Yu, L. Soil fungal community composition differs significantly among the Antarctic, Arctic, and Tibetan Plateau. *Extremophiles* **2020**, *24*, 821–829. [CrossRef]
41. Zhang, T.; Wang, N.-F.; Liu, H.-Y.; Zhang, Y.-Q.; Yu, L.-Y. Soil pH is a Key Determinant of Soil Fungal Community Composition in the Ny-Ålesund Region, Svalbard (High Arctic). *Front. Microbiol.* **2016**, *7*, 227. [CrossRef]
42. Chen, Y.-L.; Deng, Y.; Ding, J.-Z.; Hu, H.-W.; Xu, T.-L.; Li, F.; Yang, G.-B.; Yang, Y.-H. Distinct microbial communities in the active and permafrost layers on the Tibetan Plateau. *Mol. Ecol.* **2017**, *26*, 6608–6620. [CrossRef] [PubMed]
43. Sun, S.; Li, S.; Avera, B.N.; Strahm, B.D.; Badgley, B.D. Soil Bacterial and Fungal Communities Show Distinct Recovery Patterns during Forest Ecosystem Restoration. *Appl. Environ. Microbiol.* **2017**, *83*, e00966-17. [CrossRef] [PubMed]
44. Herren, C.M.; McMahon, K.D. Keystone taxa predict compositional change in microbial communities. *Environ. Microbiol.* **2018**, *20*, 2207–2217. [CrossRef] [PubMed]
45. Martín González, A.M.; Dalsgaard, B.; Olesen, J.M. Centrality measures and the importance of generalist species in pollination networks. *Ecol. Complex.* **2010**, *7*, 36–43. [CrossRef]
46. Qi, G.; Ma, G.; Chen, S.; Lin, C.; Zhao, X. Microbial Network and Soil Properties Are Changed in Bacterial Wilt-Susceptible Soil. *Appl. Environ. Microbiol.* **2019**, *85*. [CrossRef]
47. Burn, C.R. *Herschel Island Qikiqtaryuk: A Natural and Cultural History of Yukon's Arctic Island*; University of Calgary Press: Whitehorse, YT, Canada, 2012; pp. 48–53.
48. Siewert, M.B.; Lantuit, H.; Richter, A.; Hugelius, G. Permafrost Causes Unique Fine-Scale Spatial Variability Across Tundra Soils. *Glob. Biogeochem. Cycles* **2021**, *35*. [CrossRef]
49. Schoeneberger, P.J.; Wysocki, D.A.; Benham, E.C. (Eds.) *Field Book for Describing and Sampling Soils*; National Soil Survey Center, Natural Resources Conservation Service: Lincoln, NE, USA, 2012. [CrossRef]
50. Ping, C.-L.; Clark, M.H.; Kimble, J.M.; Michaelson, G.J.; Shur, Y.; Stiles, C.A. Sampling Protocols for Permafrost-Affected Soils. *Soil Horiz.* **2013**, *54*, 13. [CrossRef]
51. Siewert, M.B.; Hugelius, G.; Heim, B.; Faucherre, S. Landscape controls and vertical variability of soil organic carbon storage in permafrost-affected soils of the Lena River Delta. *Catena* **2016**, *147*, 725–741. [CrossRef]
52. Varsadiya, M.; Urich, T.; Hugelius, G.; Bárta, J. Microbiome structure and functional potential in permafrost soils of the Western Canadian Arctic. *FEMS Microbiol. Ecol.* **2021**, *97*, fiab008. [CrossRef]
53. Bárta, J.; Šlajsová, P.; Tahovská, K.; Picek, T.; Šantrůčková, H. Different temperature sensitivity and kinetics of soil enzymes indicate seasonal shifts in C, N and P nutrient stoichiometry in acid forest soil. *Biogeochemistry* **2014**, *117*, 525–537. [CrossRef]
54. Marx, M.-C.; Wood, M.; Jarvis, S. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biol. Biochem.* **2001**, *33*, 1633–1640. [CrossRef]
55. Borneman, J.; Hartin, R.J. PCR primers that amplify fungal rRNA genes from environmental samples. *Appl. Environ. Microbiol.* **2000**, *66*, 4356–4360. [CrossRef] [PubMed]
56. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols*; Elsevier: Amsterdam, The Netherlands, 1990; pp. 315–322.
57. Bengtsson-Palme, J.; Ryberg, M.; Hartmann, M.; Branco, S.; Wang, Z.; Godhe, A.; De Wit, P.; Sánchez-García, M.; Ebersberger, I.; de Sousa, F.; et al. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* **2013**, *4*, 914–919. [CrossRef]
58. Edgar, R.C. UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv* **2016**. [CrossRef]
59. Edgar, R.C.; Flyvbjerg, H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* **2015**, *31*, 3476–3482. [CrossRef] [PubMed]
60. Kõljalg, U.; Nilsson, R.H.; Abarenkov, K.; Tedersoo, L.; Taylor, A.F.S.; Bahram, M.; Bates, S.T.; Bruns, T.D.; Bengtsson-Palme, J.; Callaghan, T.M.; et al. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* **2013**, *22*, 5271–5277. [CrossRef] [PubMed]
61. Lahti, L.; Shetty, S. Tools for Microbiome Analysis in R 2017. Available online: http://microbiome.github.com/microbiome (accessed on 19 July 2021).

62. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Development Core Team: Vienna, Austria, 2011; ISBN 3900051070.
63. Põlme, S.; Abarenkov, K.; Henrik Nilsson, R.; Lindahl, B.D.; Clemmensen, K.E.; Kauserud, H.; Nguyen, N.; Kjøller, R.; Bates, S.T.; Baldrian, P.; et al. FungalTraits: A user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers.* **2020**, *105*, 1–16. [CrossRef]
64. Harrell, F.E.J. *R package*, version 4.0-1; Hmisc: Harrell Miscellaneous: Nashville, TN, USA, 2020.
65. Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* **1995**, *57*, 289–300. [CrossRef]
66. Csardi, G.; Nepusz, T. The igraph software package for complex network research. *InterJ. Complex Syst.* **2006**, *1695*, 1–9.
67. Zhou, J.; Deng, Y.; Luo, F.; He, Z.; Yang, Y. Phylogenetic Molecular Ecological Network of Soil Microbial Communities in Response to Elevated CO_2. *MBio* **2011**, *2*, e00122-11. [CrossRef]
68. Lu, L.; Yin, S.; Liu, X.; Zhang, W.; Gu, T.; Shen, Q.; Qiu, H. Fungal networks in yield-invigorating and -debilitating soils induced by prolonged potato monoculture. *Soil Biol. Biochem.* **2013**, *65*, 186–194. [CrossRef]
69. Langfelder, P.; Horvath, S. Eigengene networks for studying the relationships between co-expression modules. *BMC Syst. Biol.* **2007**, *1*, 54. [CrossRef]
70. Olesen, J.M.; Bascompte, J.; Dupont, Y.L.; Jordano, P. The modularity of pollination networks. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 19891–19896. [CrossRef] [PubMed]
71. Faust, K.; Raes, J. Microbial interactions: From networks to models. *Nat. Rev. Microbiol.* **2012**, *10*, 538–550. [CrossRef] [PubMed]
72. Oksanen, J.; Kindt, R.; Legendre, P.; O'hara, B.; Henry, M.; Maintainer, H.S. The Vegan Package Title Community Ecology Package. 2007. Available online: http://Cran.R-Project.Org/; http://R-Forge.R-Project.Org/Projects/Vegan/ (accessed on 19 July 2021).
73. Parks, D.H.; Beiko, R.G. Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* **2010**, *26*, 715–721. [CrossRef] [PubMed]
74. Guimerà, R.; Nunes Amaral, L.A. Functional cartography of complex metabolic networks. *Nature* **2005**, *433*, 895–900. [CrossRef]
75. Hoppe, B.; Purahong, W.; Wubet, T.; Kahl, T.; Bauhus, J.; Arnstadt, T.; Hofrichter, M.; Buscot, F.; Krüger, D. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in Central European forests. *Fungal Divers.* **2016**, *77*, 367–379. [CrossRef]
76. Bani, A.; Pioli, S.; Ventura, M.; Panzacchi, P.; Borruso, L.; Tognetti, R.; Tonon, G.; Brusetti, L. The role of microbial community in the decomposition of leaf litter and deadwood. *Appl. Soil Ecol.* **2018**, *126*, 75–84. [CrossRef]
77. Dickie, I.A. Host preference, niches and fungal diversity. *New Phytol.* **2007**, *174*, 230–233. [CrossRef]
78. Bascompte, J. Networks in ecology. *Basic Appl. Ecol.* **2007**, *8*, 485–490. [CrossRef]
79. Robinson, C.H.; Saunders, P.W.; Madan, N.J.; Janie Pryce-Miller, E.; Pentecost, A. Does nitrogen deposition affect soil microfungal diversity and soil N and P dynamics in a high Arctic ecosystem? *Glob. Chang. Biol.* **2004**, *10*, 1065–1079. [CrossRef]
80. Lennon, J.T.; Aanderud, Z.T.; Lehmkuhl, B.K.; Schoolmaster, D.R. Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology* **2012**, *93*, 1867–1879. [CrossRef]
81. Mundra, S.; Halvorsen, R.; Kauserud, H.; Müller, E.; Vik, U.; Eidesen, P.B. Arctic fungal communities associated with roots of Bistorta vivipara do not respond to the same fine-scale edaphic gradients as the aboveground vegetation. *New Phytol.* **2015**, *205*, 1587–1597. [CrossRef]
82. Clemmensen, K.E.; Michelsen, A.; Jonasson, S.; Shaver, G.R. Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytol.* **2006**, *171*, 391–404. [CrossRef] [PubMed]
83. Zhang, T.; Yao, Y.-F. Endophytic Fungal Communities Associated with Vascular Plants in the High Arctic Zone Are Highly Diverse and Host-Plant Specific. *PLoS ONE* **2015**, *10*, e0130051. [CrossRef]
84. Newsham, K.K.; Upson, R.; Read, D.J. Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecol.* **2009**, *2*, 10–20. [CrossRef]
85. Walker, X.J.; Basinger, J.F.; Kaminskyj, S.G.W. Endorhizal Fungi in Ranunculus from Western and Arctic Canada: Predominance of Fine Endophytes at High Latitudes. *Open Mycol. J.* **2010**, *4*, 1–9. [CrossRef]
86. Müller, M.M.; Valjakka, R.; Suokko, A.; Hantula, J. Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. *Mol. Ecol.* **2001**, *10*, 1801–1810. [CrossRef] [PubMed]
87. Korkama-Rajala, T.; Müller, M.M.; Pennanen, T. Decomposition and Fungi of Needle Litter from Slow- and Fast-growing Norway Spruce (Picea abies) Clones. *Microb. Ecol.* **2008**, *56*, 76–89. [CrossRef] [PubMed]
88. Promputtha, I.; Hyde, K.D.; McKenzie, E.H.C.; Peberdy, J.F.; Lumyong, S. Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? *Fungal Divers.* **2010**, *41*, 89–99. [CrossRef]
89. Caldwell, B.A.; Jumpponen, A.; Trappe, J.M. Utilization of Major Detrital Substrates by Dark-Septate, Root Endophytes. *Mycologia* **2000**, *92*, 230. [CrossRef]
90. Upson, R.; Read, D.J.; Newsham, K.K. Nitrogen form influences the response of Deschampsia antarctica to dark septate root endophytes. *Mycorrhiza* **2009**, *20*, 1–11. [CrossRef] [PubMed]
91. Surono; Narisawa, K. The dark septate endophytic fungus Phialocephala fortinii is a potential decomposer of soil organic compounds and a promoter of Asparagus officinalis growth. *Fungal Ecol.* **2017**, *28*, 1–10. [CrossRef]
92. Verbruggen, E.; Pena, R.; Fernandez, C.W.; Soong, J.L. Mycorrhizal Interactions With Saprotrophs and Impact on Soil Carbon Storage. In *Mycorrhizal Mediation of Soil*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 441–460.

93. Bödeker, I.T.M.; Lindahl, B.D.; Olson, Å.; Clemmensen, K.E. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. *Funct. Ecol.* **2016**, *30*, 1967–1978. [CrossRef]
94. Talbot, J.M.; Allison, S.D.; Treseder, K.K. Decomposers in disguise: Mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Funct. Ecol.* **2008**, *22*, 955–963. [CrossRef]
95. Rineau, F.; Shah, F.; Smits, M.M.; Persson, P.; Johansson, T.; Carleer, R.; Troein, C.; Tunlid, A. Carbon availability triggers the decomposition of plant litter and assimilation of nitrogen by an ectomycorrhizal fungus. *ISME J.* **2013**, *7*, 2010–2022. [CrossRef] [PubMed]
96. Colpaert, J.V.; Tichelen, K.K. Decomposition, nitrogen and phosphorus mineralization from beech leaf litter colonized by ectomycorrhizal or litter-decomposing basidiomycetes. *New Phytol.* **1996**, *134*, 123–132. [CrossRef]
97. Gadgil, R.L.; Gadgil, P.D. Mycorrhiza and Litter Decomposition. *Nature* **1971**, *233*, 133. [CrossRef]
98. Fontaine, S.; Barot, S.; Barré, P.; Bdioui, N.; Mary, B.; Rumpel, C. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **2007**, *450*, 277–280. [CrossRef]
99. Wild, B.; Schnecker, J.; Alves, R.J.E.; Barsukov, P.; Bárta, J.; Čapek, P.; Gentsch, N.; Gittel, A.; Guggenberger, G.; Lashchinskiy, N.; et al. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. *Soil Biol. Biochem.* **2014**, *75*, 143–151. [CrossRef]
100. Lindahl, B.D.; Ihrmark, K.; Boberg, J.; Trumbore, S.E.; Högberg, P.; Stenlid, J.; Finlay, R.D. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* **2007**, *173*, 611–620. [CrossRef]
101. Orwin, K.H.; Kirschbaum, M.U.F.; St John, M.G.; Dickie, I.A. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: A model-based assessment. *Ecol. Lett.* **2011**, *14*, 493–502. [CrossRef]
102. Averill, C.; Hawkes, C.V. Ectomycorrhizal fungi slow soil carbon cycling. *Ecol. Lett.* **2016**, *19*, 937–947. [CrossRef] [PubMed]
103. Kaiser, C.; Meyer, H.; Biasi, C.; Rusalimova, O.; Barsukov, P.; Richter, A. Conservation of soil organic matter through cryoturbation in arctic soils in Siberia. *J. Geophys. Res. Biogeosci.* **2007**, *112*, 1–8. [CrossRef]
104. Wild, B.; Schnecker, J.; Knoltsch, A.; Takriti, M.; Mooshammer, M.; Gentsch, N.; Mikutta, R.; Alves, R.J.E.; Gittel, A.; Lashchinskiy, N.; et al. Microbial nitrogen dynamics in organic and mineral soil horizons along a latitudinal transect in western Siberia. *Glob. Biogeochem. Cycles* **2015**, *29*, 567–582. [CrossRef]
105. Talbot, J.M.; Martin, F.; Kohler, A.; Henrissat, B.; Peay, K.G. Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry. *Soil Biol. Biochem.* **2015**, *88*, 441–456. [CrossRef]
106. Shi, S.; Nuccio, E.E.; Shi, Z.J.; He, Z.; Zhou, J.; Firestone, M.K. The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages. *Ecol. Lett.* **2016**, *19*, 926–936. [CrossRef] [PubMed]
107. Upton, R.N.; Checinska Sielaff, A.; Hofmockel, K.S.; Xu, X.; Polley, H.W.; Wilsey, B.J. Soil depth and grassland origin cooperatively shape microbial community co-occurrence and function. *Ecosphere* **2020**, *11*, e02973. [CrossRef]
108. Toju, H.; Kishida, O.; Katayama, N.; Takagi, K. Networks Depicting the Fine-Scale Co-Occurrences of Fungi in Soil Horizons. *PLoS ONE* **2016**, *11*, e0165987. [CrossRef]
109. Watts, D.J.; Strogatz, S.H. Collective dynamics of 'small-world' networks. *Nature* **1998**, *393*, 440–442. [CrossRef]
110. Albert, R.; Barabási, A.-L. Statistical mechanics of complex networks. *Rev. Mod. Phys.* **2002**, *74*, 47–97. [CrossRef]
111. Zhou, J.; Deng, Y.; Luo, F.; He, Z.; Tu, Q.; Zhi, X. Functional Molecular Ecological Networks. *MBio* **2010**, *1*, e00169-10. [CrossRef]
112. Dupont, Y.L.; Olesen, J.M. Ecological modules and roles of species in heathland plant-insect flower visitor networks. *J. Anim. Ecol.* **2009**, *78*, 346–353. [CrossRef] [PubMed]
113. Banerjee, S.; Kirkby, C.A.; Schmutter, D.; Bissett, A.; Kirkegaard, J.A.; Richardson, A.E. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biol. Biochem.* **2016**, *97*, 188–198. [CrossRef]
114. Wu, L.; Yang, Y.; Chen, S.; Zhao, M.; Zhu, Z.; Yang, S.; Qu, Y.; Ma, Q.; He, Z.; Zhou, J.; et al. Long-term successional dynamics of microbial association networks in anaerobic digestion processes. *Water Res.* **2016**, *104*, 1–10. [CrossRef]
115. Zhang, B.; Zhang, J.; Liu, Y.; Shi, P.; Wei, G. Co-occurrence patterns of soybean rhizosphere microbiome at a continental scale. *Soil Biol. Biochem.* **2018**, *118*, 178–186. [CrossRef]
116. Tao, J.; Meng, D.; Qin, C.; Liu, X.; Liang, Y.; Xiao, Y.; Liu, Z.; Gu, Y.; Li, J.; Yin, H. Integrated network analysis reveals the importance of microbial interactions for maize growth. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 3805–3818. [CrossRef]
117. Schnecker, J.; Wild, B.; Hofhansl, F.; Eloy Alves, R.J.; Bárta, J.; Čapek, P.; Fuchslueger, L.; Gentsch, N.; Gittel, A.; Guggenberger, G.; et al. Effects of Soil Organic Matter Properties and Microbial Community Composition on Enzyme Activities in Cryoturbated Arctic Soils. *PLoS ONE* **2014**, *9*, e94076. [CrossRef]
118. Jurgens, J.A.; Blanchette, R.A.; Filley, T.R. Fungal diversity and deterioration in mummified woods from the ad Astra Ice Cap region in the Canadian High Arctic. *Polar Biol.* **2009**, *32*, 751–758. [CrossRef]
119. Obase, K.; Douhan, G.W.; Matsuda, Y.; Smith, M.E. Culturable fungal assemblages growing within Cenococcum sclerotia in forest soils. *FEMS Microbiol. Ecol.* **2014**, *90*, 708–717. [CrossRef]
120. James, T.Y.; Kauff, F.; Schoch, C.L.; Matheny, P.B.; Hofstetter, V.; Cox, C.J.; Celio, G.; Gueidan, C.; Fraker, E.; Miadlikowska, J.; et al. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **2006**, *443*, 818–822. [CrossRef]

Paper III

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

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

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1. Abstract

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

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

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

2. Introduction

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

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

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

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

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

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

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

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

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

2) As N is considered main limiting nutrient in Arctic soils (Sistla et al. 2012), the activity of N enzymes will be higher than that of C enzymes and will increase with depth, where the availability of N is lower.

3) As the limitation of N and P increases from topsoil to permafrost, microbial nutrient acquisitions ratio decreases but more complex C compound increases.

3. Materials and Methods

1.1 Study sites

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

1.2 Soil physico-chemical analysis

As soil physicochemical parameters we analyzed soil pH, water content, water-extractable organic carbon (WEOC), water-extractable total nitrogen (WETN), total carbon (Ctot) and total nitrogen (Ntot). The soil pH was determined with water (ultra-pure water) at a solid to solution ratio of 1:25 using a 3151i pH meter (Fisher Scientific GmbH, Schwerte Germany). The soil water content was determined gravimetrically by drying field fresh soil at 60°C for 48 h and weighing the sample before and after. The Ctot and Ntot content were determined from dried soil samples at 60 °C using an Elementar Vario Micro cube (Elementar, Langenselbold, Germany). WEOC and WETN were 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} on a horizontal shaker). Water extracts were filtered through 0.45 µm polyether sulfone (PES) filters (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and measured by a LiquiTOCII (Elementar, Langenselbold, Germany), and expressed in µg $gram^{-1}$ dry weight. Each measured soil physico-chemical parameter is given in **Table S1.**

1.3 Soil enzyme analysis

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

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

1.4 Indicator of microbial resource limitation

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

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

$$Ec:Ep = Ln\,(BG + CBH)/Ln\,(PME) \quad (2)$$

$$En:Ep = Ln\,(LAP + NAG)/Ln\,(PME) \quad (3)$$

$$CRI = Ln\,(POX)/(Ln\,(BG) + Ln\,(CBH) + Ln(POX)) \quad (4)$$

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

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

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

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

$$Vector\ A\ (degree) = Degrees\ (Atan2(x, y)) \quad (6)$$

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

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

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

1.5 Genomic DNA extraction and gene quantification

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

1.6 Statistical analysis

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

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

4. Results

1.7 Potential enzyme activities and stoichiometry

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

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

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

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

1.8 Indicators of microbial resource limitation

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

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

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

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

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

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

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

1.9 Bacterial and fungal abundance

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

1.10 Key factors affecting soil enzyme activity and stoichiometry

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

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

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

5. Discussion

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

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

1.11 Horizon-specific enzyme activity

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

1.12 Locality-specific enzyme activity

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

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

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

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

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

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

6. Conclusions

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

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

7. References

Allison SD, Gartner TB, Holland K, et al (2007) Soil enzymes:linking proteomics and ecological process. In: Manual of environmental microbiology, 3rd edn. ASM Press, Washington, DC, pp 704–711

Ausec L, van Elsas JD, Mandic-Mulec I (2011a) Two- and three-domain bacterial laccase-like genes are present in drained peat soils. Soil Biol Biochem 43:975–983. https://doi.org/10.1016/j.soilbio.2011.01.013

Ausec L, Zakrzewski M, Goesmann A, et al (2011b) Bioinformatic analysis reveals high diversity of bacterial genes for laccase-like enzymes. PLoS One 6:. https://doi.org/10.1371/journal.pone.0025724

Čapek P, Kotas P, Manzoni S, Šantrůčková H (2016) Drivers of phosphorus limitation across soil microbial communities. Funct Ecol 30:1705–1713. https://doi.org/10.1111/1365-2435.12650

Chen H, Li D, Mao Q, et al (2019a) Resource limitation of soil microbes in karst ecosystems. Sci Total Environ 650:241–248. https://doi.org/10.1016/j.scitotenv.2018.09.036

Chen H, Zheng M, Mao Q, et al (2019b) Cropland conversion changes the status of microbial resource limitation in degraded karst soil. Geoderma 352:197–203. https://doi.org/10.1016/j.geoderma.2019.06.018

Dobiński W (2020) Permafrost active layer. Earth-Science Rev 208:103301. https://doi.org/10.1016/j.earscirev.2020.103301

Ekblad A, Nordgren A (2002) Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability? Plant Soil 242:115–122. https://doi.org/10.1023/A:1019698108838

Fontaine S, Barot S, Barré P, et al (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450:277–280. https://doi.org/10.1038/nature06275

Freedman Z, Zak DR (2014) Atmospheric N Deposition Increases Bacterial Laccase-Like Multicopper Oxidases: Implications for Organic Matter Decay. Appl Environ Microbiol 80:4460–4468. https://doi.org/10.1128/AEM.01224-14

Gittel A, Bárta J, Kohoutová I, et al (2014) Distinct microbial communities associated with buried soils in the Siberian tundra. ISME J 8:841–853. https://doi.org/10.1038/ismej.2013.219

Harden JW, Koven CD, Ping C-L, et al (2012) Field information links permafrost carbon to physical vulnerabilities of thawing. Geophys Res Lett 39:. https://doi.org/10.1029/2012GL051958

Hill BH, Elonen CM, Herlihy AT, et al (2018) Microbial ecoenzyme stoichiometry, nutrient limitation, and organic matter decomposition in wetlands of the conterminous United States. Wetl Ecol Manag 26:425–439. https://doi.org/10.1007/s11273-017-9584-5

Hill BH, Elonen CM, Seifert LR, et al (2012) Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. Ecol Indic 18:540–551. https://doi.org/10.1016/j.ecolind.2012.01.007

Hugelius G, Strauss J, Zubrzycki S, et al (2014) Estimated stocks of circumpolar permafrost carbon with quantified uncertainty ranges and identified data gaps. Biogeosciences 11:6573–6593. https://doi.org/10.5194/bg-11-6573-2014

Kaiser C, Meyer H, Biasi C, et al (2007) Conservation of soil organic matter through cryoturbation in arctic soils in Siberia. J Geophys Res Biogeosciences 112:1–8. https://doi.org/10.1029/2006JG000258

Kellner H, Luis P, Zimdars B, et al (2008) Diversity of bacterial laccase-like multicopper oxidase genes in forest and grassland Cambisol soil samples. Soil Biol Biochem 40:638–648. https://doi.org/10.1016/j.soilbio.2007.09.013

Keuper F, Wild B, Kummu M, et al (2020) Carbon loss from northern circumpolar permafrost soils amplified by rhizosphere priming. Nat Geosci 13:560–565. https://doi.org/10.1038/s41561-020-0607-0

Kivlin SN, Treseder KK (2014) Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition. Biogeochemistry 117:23–37. https://doi.org/10.1007/s10533-013-9852-2

Li Q, Liu Y, Gu Y, et al (2020) Ecoenzymatic stoichiometry and microbial nutrient limitations in rhizosphere soil along the Hailuogou Glacier forefield chronosequence. Sci Total Environ 704:135413. https://doi.org/10.1016/j.scitotenv.2019.135413

Liu J, Chen J, Chen G, et al (2020) Enzyme stoichiometry indicates the variation of microbial nutrient requirements at different soil depths in subtropical forests. PLoS One 15:e0220599. https://doi.org/10.1371/journal.pone.0220599

446 Manzoni S, Jackson RB, Trofymow JA, Porporato A (2008) The Global Stoichiometry of Litter Nitrogen
447 Mineralization. Science (80-) 321:684–686. https://doi.org/10.1126/science.1159792

448 McGuire AD, Melillo JM, Kicklighter DW, et al (1997) Equilibrium responses of global net primary production and
449 carbon storage to doubled atmospheric carbon dioxide: Sensitivity to changes in vegetation nitrogen
450 concentration. Glob. Biogeochem. Cycles 11:173–189

451 Moorhead DL, Rinkes ZL, Sinsabaugh RL, Weintraub MN (2013) Dynamic relationships between microbial
452 biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition
453 models. Front Microbiol 4:223. https://doi.org/10.3389/fmicb.2013.00223

454 Moorhead DL, Sinsabaugh RL, Hill BH, Weintraub MN (2016) Vector analysis of ecoenzyme activities reveal
455 constraints on coupled C, N and P dynamics. Soil Biol Biochem 93:1–7.
456 https://doi.org/10.1016/j.soilbio.2015.10.019

457 Nakamura K, Kawabata T, Yura K, Go N (2003) Novel types of two-domain multi-copper oxidases: possible
458 missing links in the evolution. FEBS Lett 553:239–244. https://doi.org/10.1016/S0014-5793(03)01000-7

459 Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature
460 405:299–304. https://doi.org/10.1038/35012500

461 Palmtag J, Hugelius G, Lashchinskiy N, et al (2015) Storage, Landscape Distribution, and Burial History of Soil
462 Organic Matter in Contrasting Areas of Continuous Permafrost. Arctic, Antarct Alp Res 47:71–88.
463 https://doi.org/10.1657/AAAR0014-027

464 Palmtag J, Kuhry P (2018) Grain size controls on cryoturbation and soil organic carbon density in permafrost -
465 affected soils. 112–120

466 Peng X, Wang W (2016) Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate
467 grasslands of northern China. Soil Biol Biochem 98:74–84. https://doi.org/10.1016/j.soilbio.2016.04.008

468 R Development Core Team R (2011) R: A Language and Environment for Statistical Computing

469 Revelle W (2021) psych: Procedures for Personality and Psychological Research

Schaller J, Faucherre S, Joss H, et al (2019) Silicon increases the phosphorus availability of Arctic soils. Sci Rep 9:449. https://doi.org/10.1038/s41598-018-37104-6

Schimel J (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schmidt SK, Porazinska D, Concienne B-L, et al (2016) Biogeochemical Stoichiometry Reveals P and N Limitation Across the Post-glacial Landscape of Denali National Park, Alaska. Ecosystems 19:1164–1177. https://doi.org/10.1007/s10021-016-9992-z

Schnecker J, Wild B, Hofhansl F, et al (2014) Effects of Soil Organic Matter Properties and Microbial Community Composition on Enzyme Activities in Cryoturbated Arctic Soils. PLoS One 9:e94076. https://doi.org/10.1371/journal.pone.0094076

Schnecker J, Wild B, Takriti M, et al (2015) Microbial community composition shapes enzyme patterns in topsoil and subsoil horizons along a latitudinal transect in Western Siberia. Soil Biol Biochem 83:106–115. https://doi.org/10.1016/j.soilbio.2015.01.016

Schneider T, Keiblinger KM, Schmid E, et al (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. ISME J 6:1749–1762. https://doi.org/10.1038/ismej.2012.11

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42:391–404. https://doi.org/10.1016/j.soilbio.2009.10.014

Sinsabaugh RL, Belnap J, Findlay SG, et al (2014) Extracellular enzyme kinetics scale with resource availability. Biogeochemistry 121:287–304. https://doi.org/10.1007/s10533-014-0030-y

Sinsabaugh RL, Follstad Shah JJ, Hill BH, Elonen CM (2011) Ecoenzymatic stoichiometry of stream sediments with comparison to terrestrial soils. Biogeochemistry 111:455–467. https://doi.org/10.1007/s10533-011-9676-x

Sinsabaugh RL, Gallo ME, Lauber C, et al (2005) Extracellular Enzyme Activities and Soil Organic Matter Dynamics for Northern Hardwood Forests receiving Simulated Nitrogen Deposition. Biogeochemistry

75:201–215. https://doi.org/10.1007/s10533-004-7112-1

Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798. https://doi.org/10.1038/nature08632

Sinsabaugh RL, Lauber CL, Weintraub MN, et al (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11:1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Sistla SA, Asao S, Schimel JP (2012) Detecting microbial N-limitation in tussock tundra soil: Implications for Arctic soil organic carbon cycling. Soil Biol Biochem 55:78–84. https://doi.org/10.1016/j.soilbio.2012.06.010

Smilauer P, Leps J (2014) Multivariate analysis of ecological data using Canoco 5

Smith CAS, Kennedy CE, Hargrave AE, McKenna KM (1989) Soil and vegetation of Herschel Island, Yukon Territory. Yukon Soil Survey Report, vol. 1, Land Resource Research Centre, Agriculture Canada, Ottawa

Thomas DSG, Goudie A (2000) The Dictionary of Physical Geography. Oxford: Blackwell

Tibbett M, Sanders FE, Cairney JWG (1998) The effect of temperature and inorganic phosphorus supply on growth and acid phosphatase production in arctic and temperate strains of ectomycorrhizal Hebeloma spp. in axenic culture. Mycol Res 102:129–135. https://doi.org/10.1017/S0953756297004681

van Vliet-Lanoë B (1991) Chronostratigraphy and paleoclimatic meaning of cryogenic deformations in the Central European loess. GeoJournal 24:157–163. https://doi.org/10.1007/BF00186011

Varsadiya M, Urich T, Hugelius G, 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. https://doi.org/10.3390/microorganisms9091943

Varsadiya M, Urich T, Hugelius G, Bárta J (2021b) Microbiome structure and functional potential in permafrost soils of the Western Canadian Arctic. FEMS Microbiol Ecol 97:. https://doi.org/10.1093/femsec/fiab008

Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117:101–113. https://doi.org/10.1007/s10533-013-9849-x

Warton DI, Duursma RA, Falster DS, Taskinen S (2012) smatr 3 - an R package for estimation and inference about

allometric lines. Methods Ecol Evol 3:257–259. https://doi.org/10.1111/j.2041-210X.2011.00153.x

Wild B, Schnecker J, Alves RJE, et al (2014) Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. Soil Biol Biochem 75:143–151. https://doi.org/10.1016/j.soilbio.2014.04.014

Zuo Y, Li J, Zeng H, Wang W (2018) Vertical pattern and its driving factors in soil extracellular enzyme activity and stoichiometry along mountain grassland belts. Biogeochemistry 141:23–39. https://doi.org/10.1007/s10533-018-0499-x

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9. Author Contributions

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

10. Data availability

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

Fig. 1

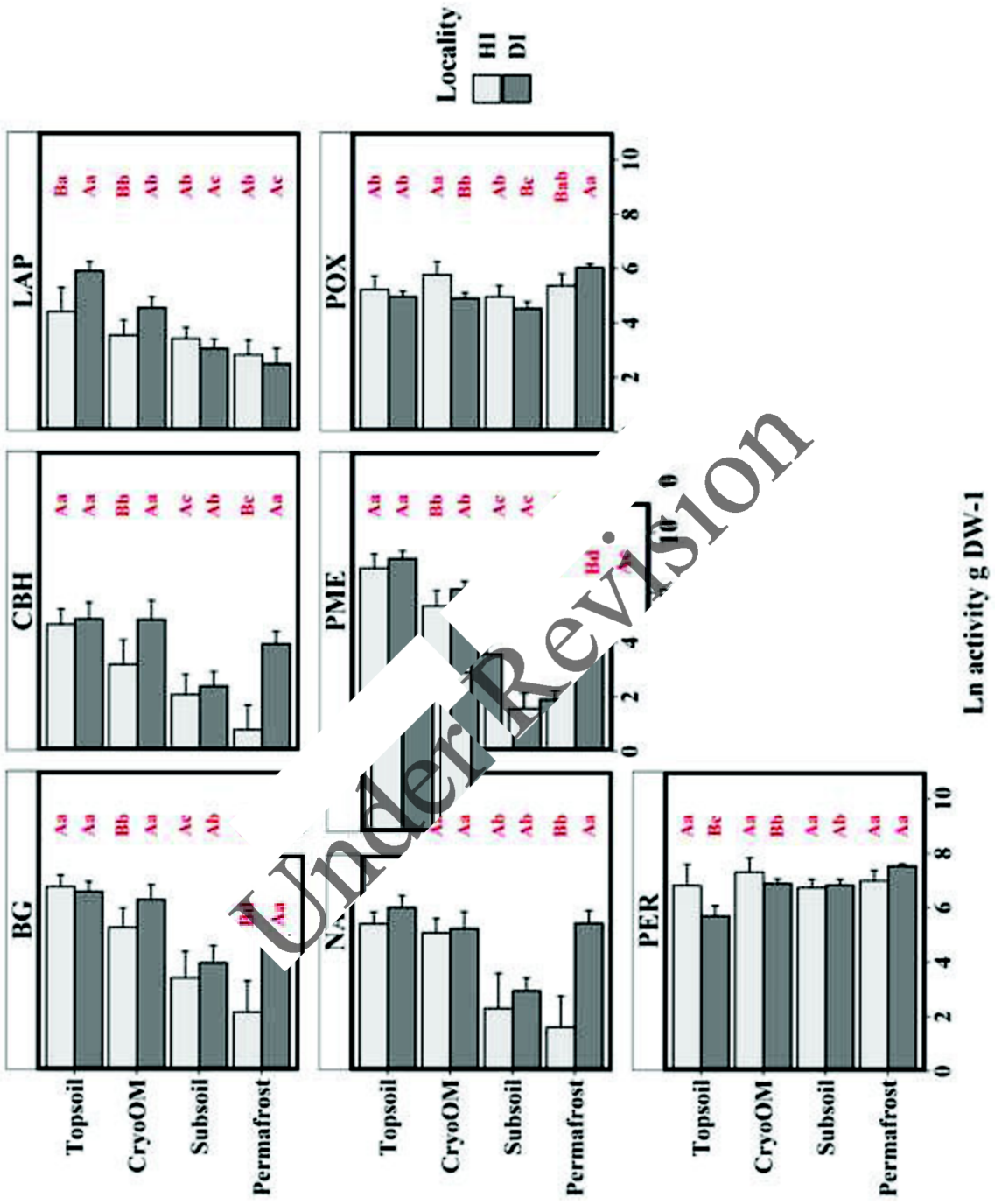

Fig. 2

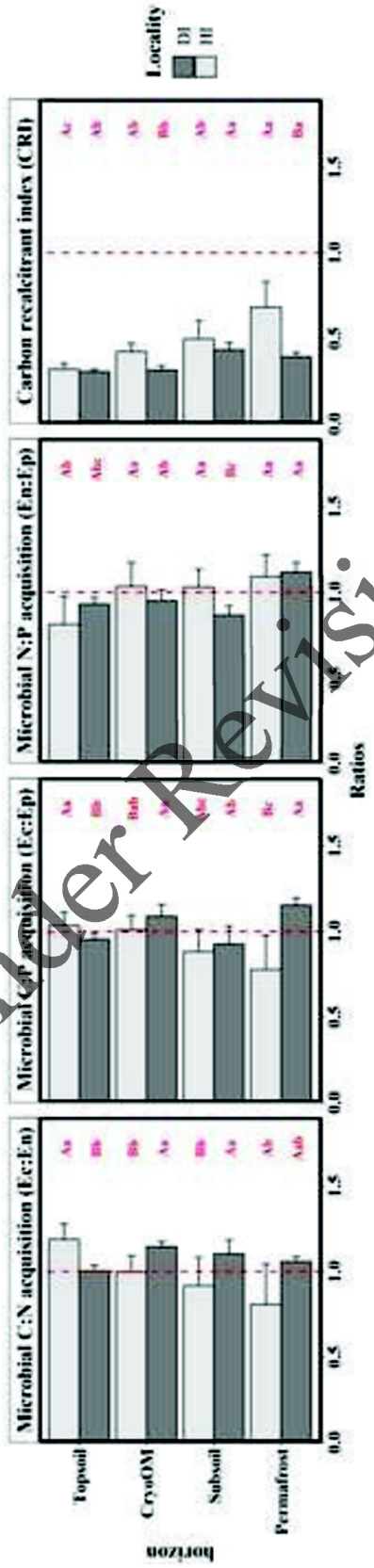

Fig. 3

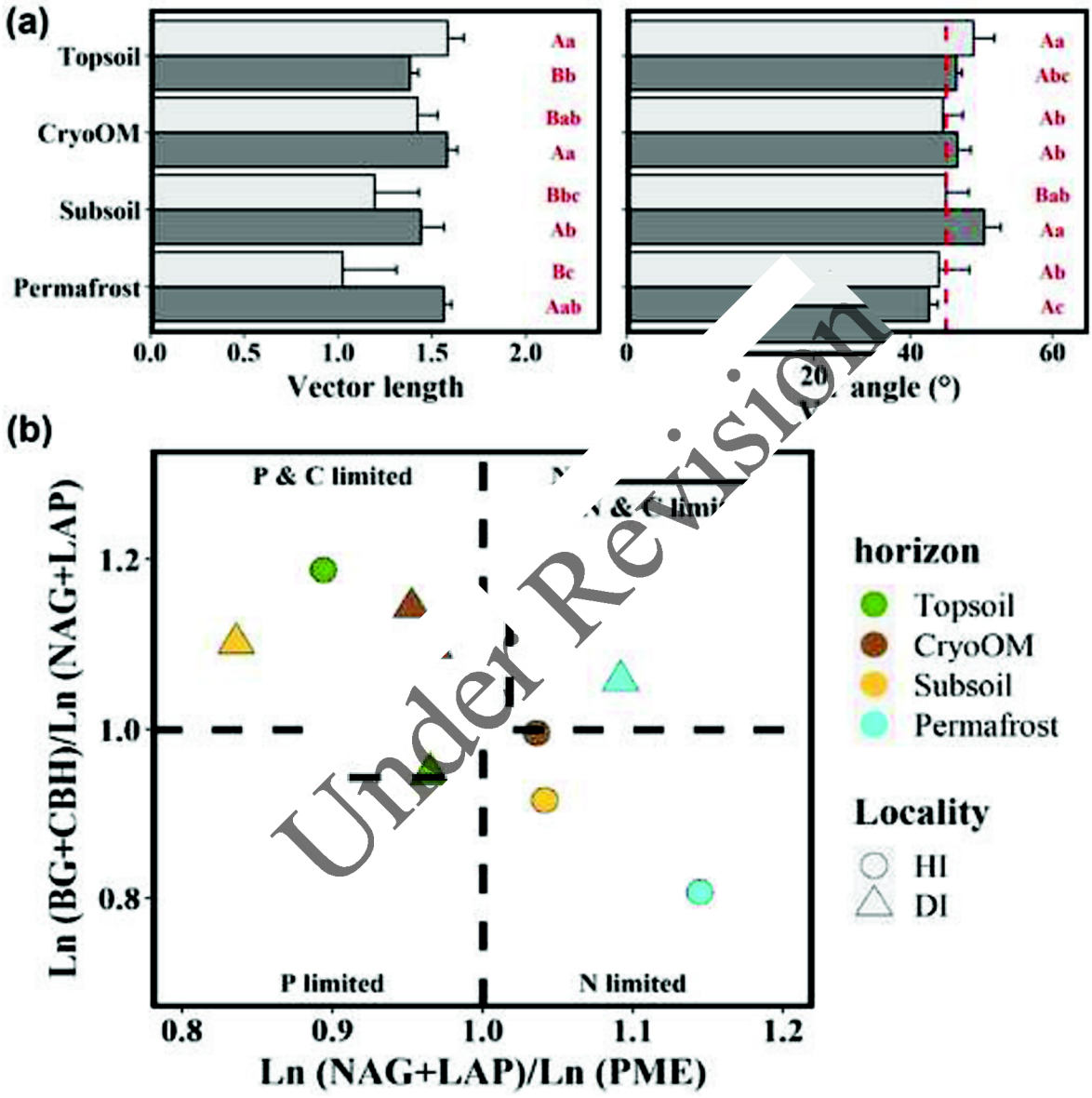

Fig. 4

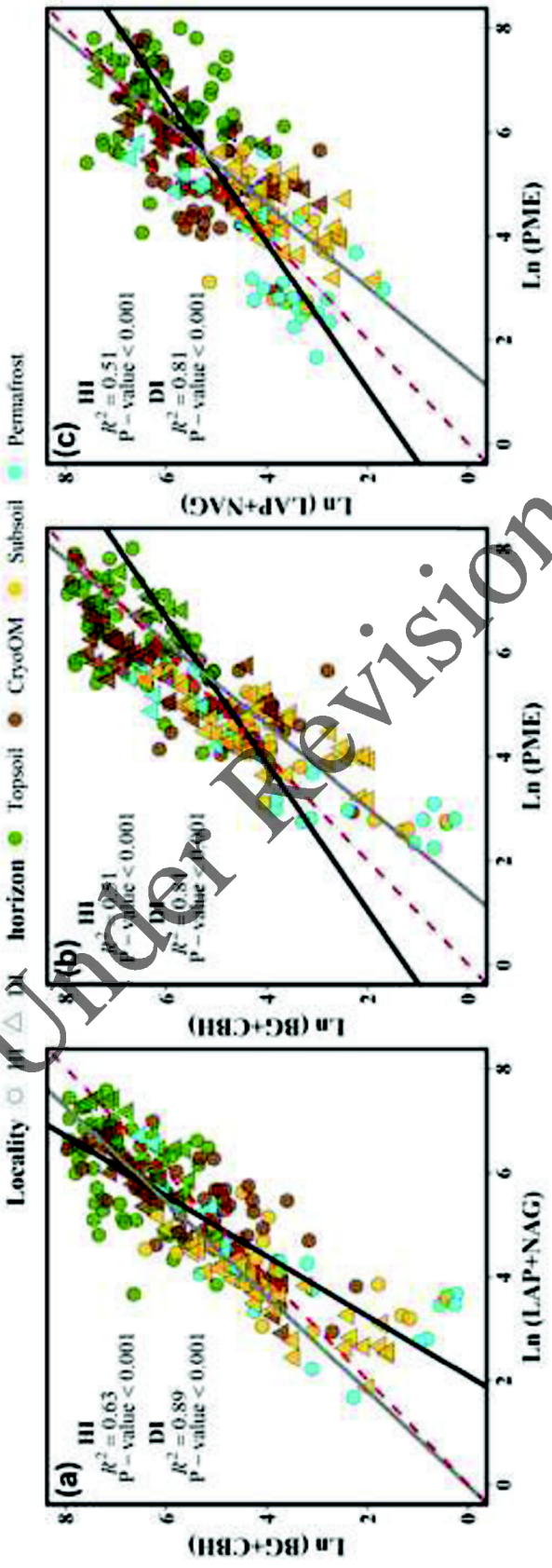

Fig. 5

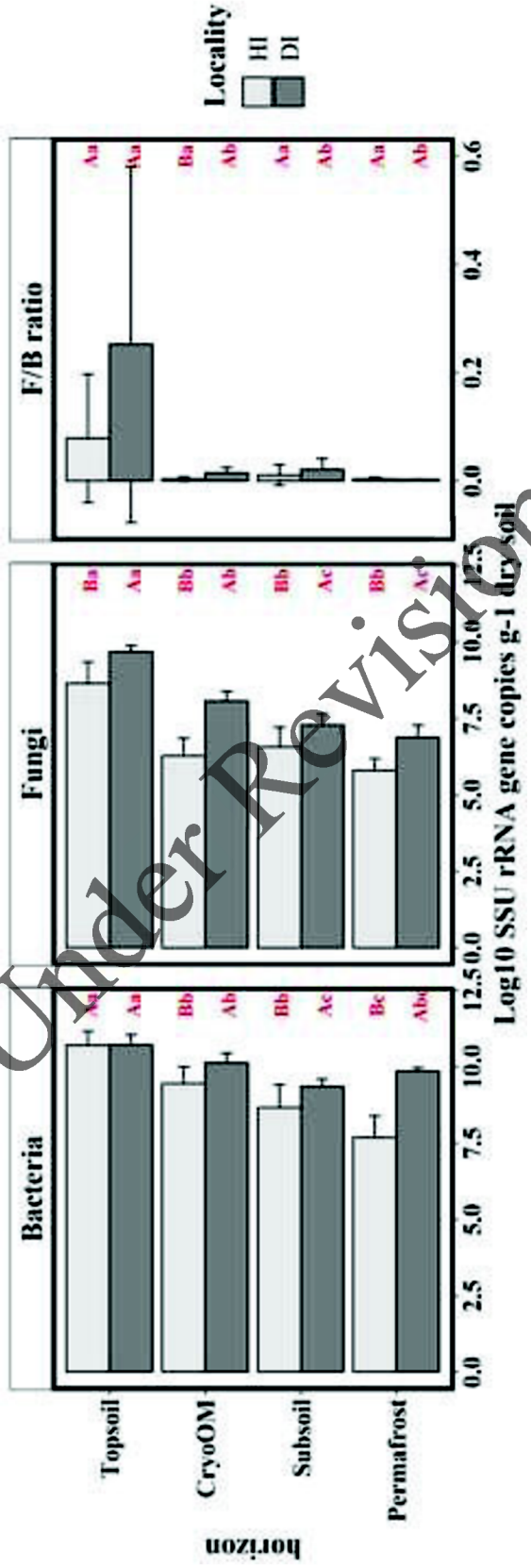

Fig. 6

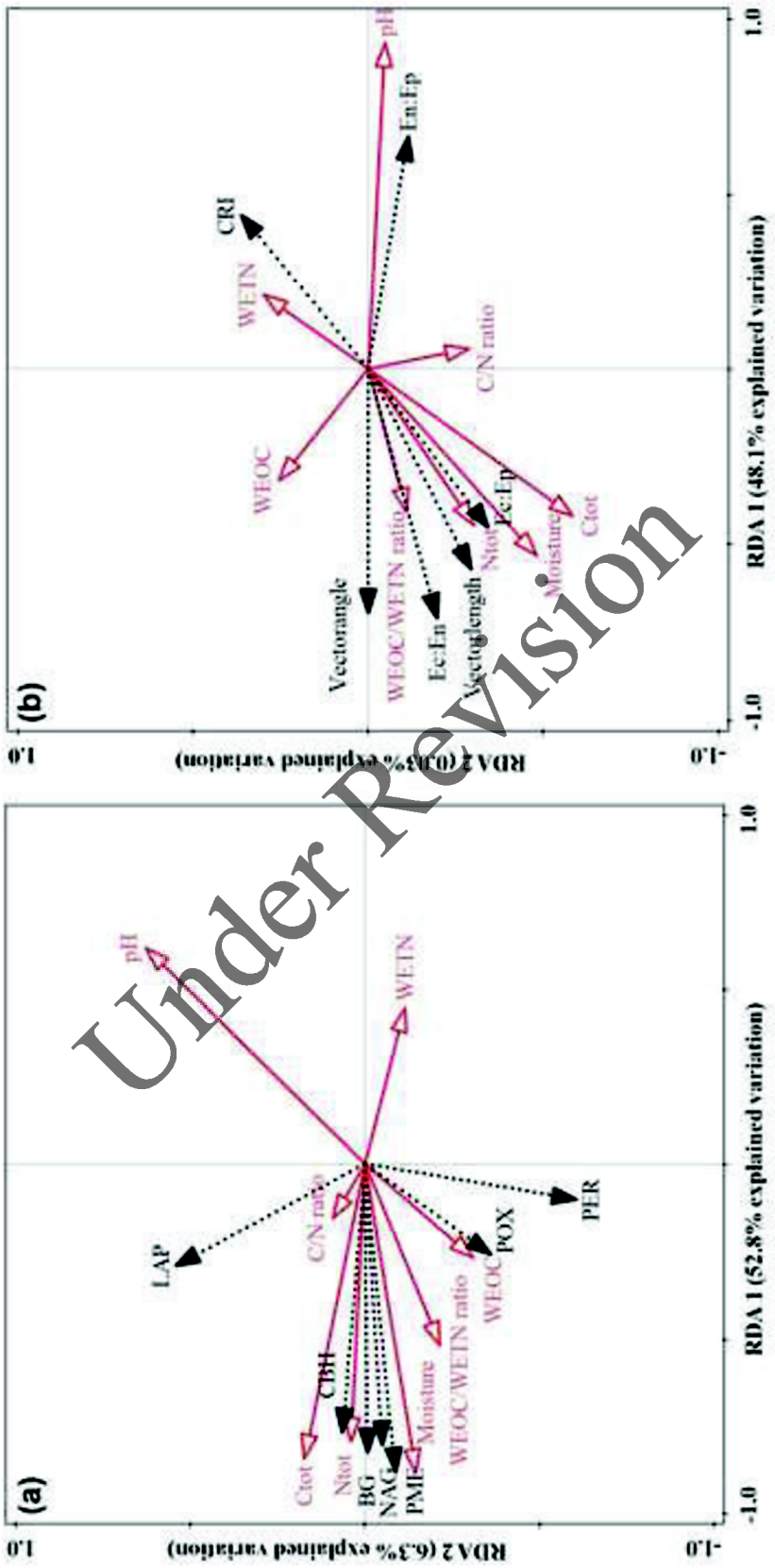

Figure legend

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 interation on enzyme

Enzymes	Horizon			Locality			Horizon*Local	
	DF	F	*p*	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 refere to materials and methods section.

activity.

ity
p
<0.001
<0.001
<0.001
<0.001
<0.05
<0.001
<0.01

Table 2. Pearson's correlation between enzyme activity, stoichiomatry 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 a

: 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.

LAP	NAG	PME	POX	PER	Ec:En	Ec:Ep	En:Ep	CRI
0.46 ***								
0.51 ***	0.79 ***							
	0.36 ***	0.24 ***						
-0.36 ***			0.66 ***					
	0.42 ***	0.51 ***		0.17 *				
0.33 ***	0.65 ***	0.29 ***			0.6 ***			
0.28 ***		-0.48 ***		-0.27 ***	-0.56 ***	0.27 ***		
-0.37 ***	-0.8 ***	-0.68 ***			-0.73 ***	-0.77 ***	0.24 ***	
0.16 *	0.67 ***	0.53 ***			0.91 ***	0.87 ***	-0.27 ***	-0.8 ***
-0.36 ***		0.33 ***		0.21 **	0.58 ***	-0.28 ***	-0.94 ***	

Vector L	Vector A
0.21 **	

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 ± 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 (μg g^{-1}dw)	WETN (μg g^{-1}dw)	WEOC/WETN ratio	Ctot (%)	Ntot (%)	C/N ratio	DNA (ng ul^{-1})
HI	Topsoil	53	6.14 ± 0.5 (Ab)	72.9 ± 6.76 (Aa)	3303.95 ± 2571.43 (Aa)	8.93 ± 5.18 (Ab)	392.83 ± 140.58 (Aa)	33.5 ± 5.18 (Aa)	1.39 ± 0.28 (Aa)	29.41 ± 10.42 (Aa)	56.29 ± 23.99 (Aa)
	CryoOM	37	6.15 ± 0.38 (Ab)	57.89 ± 8.34 (Ab)	1147.13 ± 749.55 (Ab)	16.64 ± 14.89 (Ab)	122.33 ± 68.63 (Ab)	17.1 ± 4.28 (Ab)	1.01 ± 0.25 (Ab)	17.18 ± 2.23 (Ab)	13.3 ± 11.84 (Bb)
	Subsoil	23	6.56 ± 0.59 (Ab)	28.43 ± 4.86 (Ac)	345.49 ± 110.38 (Ab)	25.56 ± 9.66 (Ab)	18.28 ± 6.5 (Bb)	4.42 ± 0.93 (Ac)	0.25 ± 0.06 (Ac)	18.26 ± 2.29 (Ab)	11.2 ± 7 (Ab)
	Permafrost	23	7.47 ± 0.56 (Aa)	37.13 ± 8.66 (Bc)	488.57 ± 298.44 (Ab)	49.83 ± 24.8 (Aa)	22.78 ± 14.64 (Ab)	4.41 ± 1.5 (Bc)	0.26 ± 0.13 (Bc)	21.17 ± 3.87 (Aab)	0.5 ± 0.29 (Bb)
DI	Topsoil	16	6.15 ± 0.19 (Ab)	69.4 ± 4.79 (Aa)	331.17 ± 50.33 (Ba)	11.54 ± 4.65 (Aa)	37.32 ± 7.2 (Ba)	27.39 ± 4.68 (Ba)	1.29 ± 0.18 (Aa)	20.8 ± 1.64 (Aa)	61 ± 15.45 (Aa)
	CryoOM	38	6.39 ± 0.2 (Ab)	54.39 ± 4.82 (Ab)	166.24 ± 26.94 (Bc)	6.33 ± 2.65 (Bb)	36.47 ± 8.15 (Ba)	14 ± 2.88 (Ab)	0.88 ± 0.16 (Ab)	15.81 ± 0.67 (Ab)	32.16 ± 9.56 (Ab)
	Subsoil	40	6.73 ± 0.2 (Aa)	27.45 ± 3.96 (Ac)	87 ± 16.34 (Bd)	2.73 ± 1.12 (Bc)	40.08 ± 8.98 (Aa)	2.71 ± 0.86 (Bc)	0.19 ± 0.06 (Bc)	15.18 ± 0.99 (Bb)	13.33 ± 6.5 (Ac)
	Permafrost	8	6.21 ± 0.05 (Ab)	52.62 ± 5.53 (Ab)	225.73 ± 34.79 (Ab)	8.57 ± 2.1 (Bab)	30.34 ± 5.3 (Aa)	9.67 ± 2.17 (Ab)	0.63 ± 0.15 (Ab)	15.64 ± 0.57 (Bb)	15.28 ± 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
			Cellulose degradation: hydrolyses cellobiose dimers from non-reducing ends of cellulose molecules	
Cellobiohydrolase	CHB	3.2.1.91	Hydrolytic, Releases disaccharides from cellulose	4-MUB-β-D-cellobioside
			Chitin and peptidoglycan degradation: hydrolyses glucosamine from chitobiose	
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
			Proteolysis: hydrolyses leucine and other hydrophobic amino acids from the N terminus of polypeptides	
Phosphatase	PME	3.1.3.1	Hydrolytic, Releases phosphate ions from phosphate group	4-MUB-phosphate
Peroxidase	PER	1.11.1.7	Oxidative, Oxidize phenols using oxygen	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 interation 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 interation 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 ***
	HI	Topsoil (n= 53)	y= 0.9 LN(LAP+NAG) 1.59	0.3 ***
		CryoOM (n= 37)	y= 1.85 LN(LAP+NAG) -4.62	0.51 ***
		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 **
	DI	Topsoil (n= 16)	y= 1.34 LN(LAP+NAG) -2.28	0.74 ***
		CryoOM (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 ***
	HI	Topsoil (n= 53)	y= 0.87 LN(PME) 1.04	0.22 ***
		CryoOM (n= 37)	y= 1.25 LN(PME) -1.28	0.68 ***
		Subsoil (n= 23)	y= 1.73 LN(PME) -3.63	0.79 ***
		Permafrost (n= 23)	y= 2.31 LN(PME) -5.53	0.66 ***
	DI	Topsoil (n= 16)	y= 1.47 LN(PME) -3.62	0.56 ***
		CryoOM (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 ***
	HI	Topsoil (n= 53)	y= 0.95 LN(PME) -0.54	0.02
		CryoOM (n= 37)	y= 0.65 LN(PME) 1.97	0.27 ***
		Subsoil (n= 23)	y= 0.68 LN(PME) 1.29	0.31 **
		Permafrost (n= 23)	y= 0.63 LN(PME) 1.53	0.32 **
	DI	Topsoil (n= 16)	y= 1.15 LN(PME) -1.41	0.63 ***
		CryoOM (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, stoichiomatry ratios, vector analysis, soil biotic and abiotic factors from HI locality only.

Biotic and abiotic	pH	Moisture	WEOC	WETN	WEOC/WETN ratio	Ctot	Ntot	C/N ratio	DNA	Bacteria	Fungi	F/B ratio	BG	CBH	LAP	NAG	PME	POX	PER	Ec:En	Ec:Ep	En:Ep	CRI	Vector L	Vector A
pH																									
Moisture	-0.53 ***																								
WEOC	-0.44 ***	0.71 ***																							
WETN	0.21 *	-0.51 ***																							
WEOC/WETN	-0.47 ***	0.89 ***	0.7 ***	-0.68 ***																					
Ctot	-0.46 ***	0.87 ***	0.59 ***	-0.68 ***	0.92 ***																				
Ntot	-0.45 ***	0.82 ***	0.56 ***	-0.58 ***	0.82 ***	0.91 ***																			
C/N ratio		0.21 *		-0.31 ***	0.32 ***	0.32 ***																			
DNA	-0.37 ***	0.5 ***	0.25 **	-0.45 ***	0.51 ***	0.64 ***	0.64 ***																		
Bacteria	-0.36 ***	0.54 ***	0.29 ***	-0.43 ***	0.52 ***	0.65 ***	0.6 ***	0.21 *	0.92 ***																
Fungi		0.43 ***	0.21 *	-0.47 ***	0.51 ***	0.58 ***	0.41 ***	0.49 ***	0.79 ***	0.82 ***															
F/B ratio		0.2 *	0.28 **								0.24 **														
BG	-0.55 ***	0.77 ***	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 ***	0.32 ***	0.68 ***	0.72 ***	0.7 ***		0.97 ***												
LAP	0.31 ***	0.19 *		-0.35 ***	0.28 **	0.38 ***	0.34 ***		0.55 ***	0.53 ***	0.57 ***		0.4 ***	0.45 ***											
NAG	-0.53 ***	0.75 ***	0.42 ***	-0.48 ***	0.65 ***	0.74 ***	0.78 ***		0.7 ***	0.72 ***	0.47 ***		0.89 ***	0.8 ***	0.3 ***										
PME	-0.62 ***	0.83 ***	0.6 ***	-0.51 ***	0.81 ***	0.84 ***	0.82 ***		0.73 ***	0.71 ***	0.67 ***	0.19 *	0.88 ***	0.83 ***	**0.35 *****	0.76 ***									
POX	-0.28 ***	0.35 ***	0.3 ***		0.19 *		0.26 **	-0.35 ***			-0.29 **					0.25 ***	0.24 **								
PER	-0.37 ***	0.24 **	0.33 ***					-0.29 ***			-0.29 **				-0.34 ***		0.24	0.72 ***							
Ec:En	-0.65 ***	0.55 ***	0.37 ***	-0.28 **	0.48 ***	0.51 ***	0.47 ***	0.19 *	0.46 ***	0.46 ***	0.34 ***		0.76 ***	0.7 ***		0.56 ***	0.65 ***								
Ec:Ep		0.29 ***		-0.34 ***	0.29 ***	0.39 ***	0.4 ***		0.47 ***	0.52 ***	0.21 *		0.69 ***	**0.58 *****	**0.31 *****	0.65 ***	0.3 ***			0.62 ***					
En:Ep	0.7 ***	-0.51 ***	-0.49 ***		-0.46 ***	-0.41 ***	-0.37 ***		-0.23 **		-0.2 *	-0.26 **	-0.39 ***	**-0.3 *****	**0.29 *****	-0.21 * *	-0.64 ***	-0.18 *	-0.37 ***	-0.64 ***					
CRI	0.54 ***	-0.61 ***	-0.34 ***	0.43 ***	-0.56 ***	-0.65 ***	-0.67 ***		-0.75 ***	-0.73 ***	-0.68 ***		-0.91 ***	**-0.87 *****	-0.28 **	**-0.82 *****	-0.69 ***			-0.79 ***	-0.8 ***	0.31 ***			
Vector L	-0.56 ***	0.57 ***	0.33 ***	-0.34 ***	0.48 ***	0.57 ***	0.57 ***		0.63 ***	0.65 ***	0.35 ***		**0.84 *****	**0.74 *****		0.61 ***	0.62 ***			0.93 ***	0.87 ***	-0.37 ***	-0.81 ***		
Vector A	-0.7 ***	0.46 ***	0.43 ***		0.37 ***	0.32 ***	0.3 ***		0.19 *		0.19 *	0.23 *	**0.36 *****	**0.32 *****	**0.32 *****	0.18 *	0.6 ***	0.18 *	0.36 ***	0.71 ***		-0.96 ***	-0.3 ***	0.4 ***	

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 abbreviations refer to materials and methods section.

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

Biotic and abiotic fac	pH	Moisture	WEOC	WETN	WEOC/WETN ratio	Ctot	Ntot	C/N ratio	DNA	Bacteria	Fungi	F/B ratio	BG	CBH	LAP	NAG	PME	POX	PER	Ec:En	Ec:Ep	En:Ep	CRI	Vector L	Vector A
pH																									
Moisture	-0.54 ***																								
WEOC	-0.6 ***	0.89 ***																							
WETN	-0.52 ***	0.75 ***	0.83 ***																						
WEOC/WETN ratio		-0.23 *	-0.24 *	-0.74 ***																					
Ctot	-0.56 ***	0.94 ***	0.86 ***	0.73 ***	-0.23 *																				
Ntot	-0.56 ***	0.94 ***	0.83 ***	0.72 ***	-0.24 *	0.99 ***																			
C/N ratio		0.37 ***	0.51 ***	0.34 ***		0.41 ***	0.28 **																		
DNA	-0.32 *	0.56 ***	0.53 ***	0.45 ***		0.59 ***	0.56 ***	**0.4 *****																	
Bacteria	-0.45 **	0.7 ***	0.65 ***	0.46 ***		0.71 ***	0.68 ***	**0.44 *****	**0.82 *****																
Fungi	-0.38 **	0.59 ***	0.58 ***	0.4 ***		0.63 ***	**0.58 *****	0.52 ***	**0.73 *****	0.77 ***															
F/B ratio		0.22 *	0.3 **	-0.25 *				**0.28 *****																	
BG	-0.32 *	0.76 ***	0.68 ***	0.57 ***		0.78 ***	**0.77 *****	**0.39 *****	**0.69 *****	0.77 ***	0.57 ***														
CBH	-0.3 *	0.76 ***	0.64 ***	0.57 ***	-0.22 *	**0.75 *****	**0.74 *****	**0.35 *****	**0.64 *****	0.74 ***	0.5 ***		0.96 ***												
LAP	-0.3 *	0.7 ***	0.61 ***	0.48 ***		**0.74 *****	**0.7 *****	**0.54 *****	0.68 ***	0.73 ***	0.77 ***	0.26 *	0.8 ***	0.78 ***											
NAG	-0.38 **	0.81 ***	0.76 ***	0.65 ***	-0.22 *	**0.82 *****	**0.79 *****	0.45 ***	0.67 ***	0.78 ***	0.58 ***		0.95 ***	0.93 ***	0.77 ***										
PME	-0.48 ***	0.87 ***	0.8 ***	0.69 ***	-0.23 *	**0.85 *****	**0.87 *****	**0.47 *****	0.69 ***	0.74 ***	0.73 ***	0.28 **	0.84 ***	0.81 ***	0.88 ***	0.85 ***									
POX		0.48 ***	0.53 ***	0.53 ***	-0.29 **	**0.43 *****	**0.43 *****	0.18	0.34 ***	0.42 ***			0.59 ***	0.53 ***	0.24 *	0.63 ***	0.37 ***								
PER			-0.23 *			-0.28 ***	-0.22 *	-0.51 ***			-0.46 ***	-0.31 **			-0.4 ***		-0.37 ***	0.38 ***							
Ec:En								-0.24 *					0.32 **	0.24 *					0.39 ***						
Ec:Ep		0.29 **	0.23 *			**0.51 *****	**0.37 *****		0.4 ***	0.48 ***			0.75 ***	0.69 ***	0.37 ***	0.65 ***	0.28 **	0.61 ***	0.43 ***	0.56 ***					
En:Ep		0.32 **	0.33 ***	0.27 **		**0.3 *****	0.27 **	0.28 **	0.35 ***	0.46 ***			0.59 ***	0.59 ***	0.35 ***	0.67 ***	0.21 *	0.64 ***	0.29 **		0.79 ***				
CRI	0.3 *	-0.65 ***	-0.55 ***	-0.4 ***		**-0.71 *****	-0.7 ***	-0.34 ***	-0.66 ***	-0.73 ***	-0.61 ***		-0.94 ***	-0.86 ***	-0.79 ***	-0.84 ***	-0.79 ***	-0.34 ***		-0.43 ***	-0.71 ***	-0.45 ***			
Vector L						0.27 **	0.31 **						0.63 ***	0.55 ***	0.2 *	0.45 ***		0.45 ***	0.47 ***	0.85 ***	0.91 ***	0.49 ***	-0.66 ***		
Vector A		-0.42 ***	-0.4 ***	-0.32 **		-0.32 ***	-0.4 ***	-0.35 ***	-0.47 ***	-0.58 ***	-0.28 **		-0.72 ***	-0.68 ***	-0.51 ***	-0.76 ***	-0.38 ***	-0.66 ***	-0.22 *		-0.83 ***	-0.95 ***	0.61 ***	-0.53 ***	

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 abbreviations refer to materials and methods section.

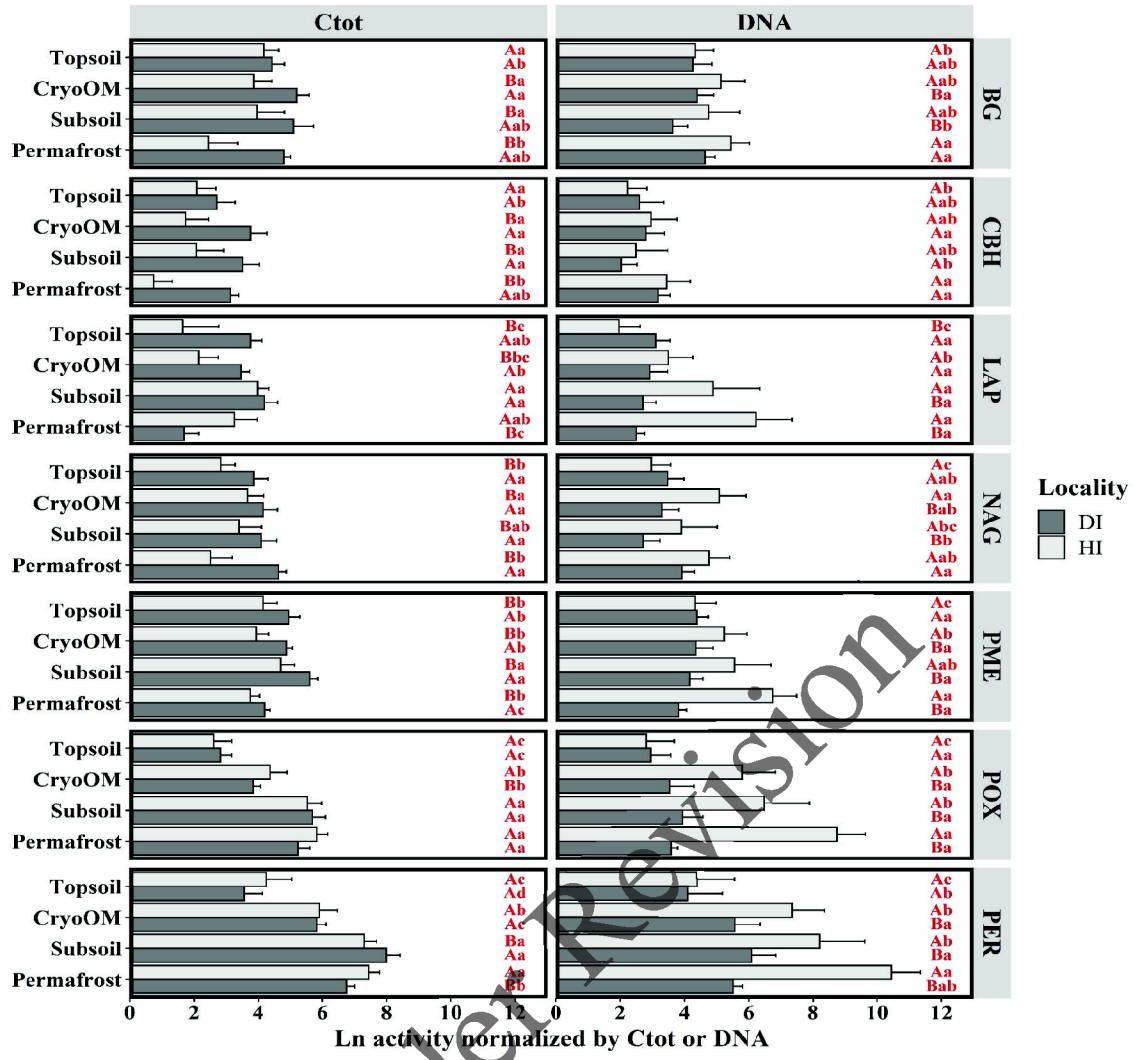

Figure S1. Variation in enzyme activity normalized to Ctot and microbial biomass (DNA).

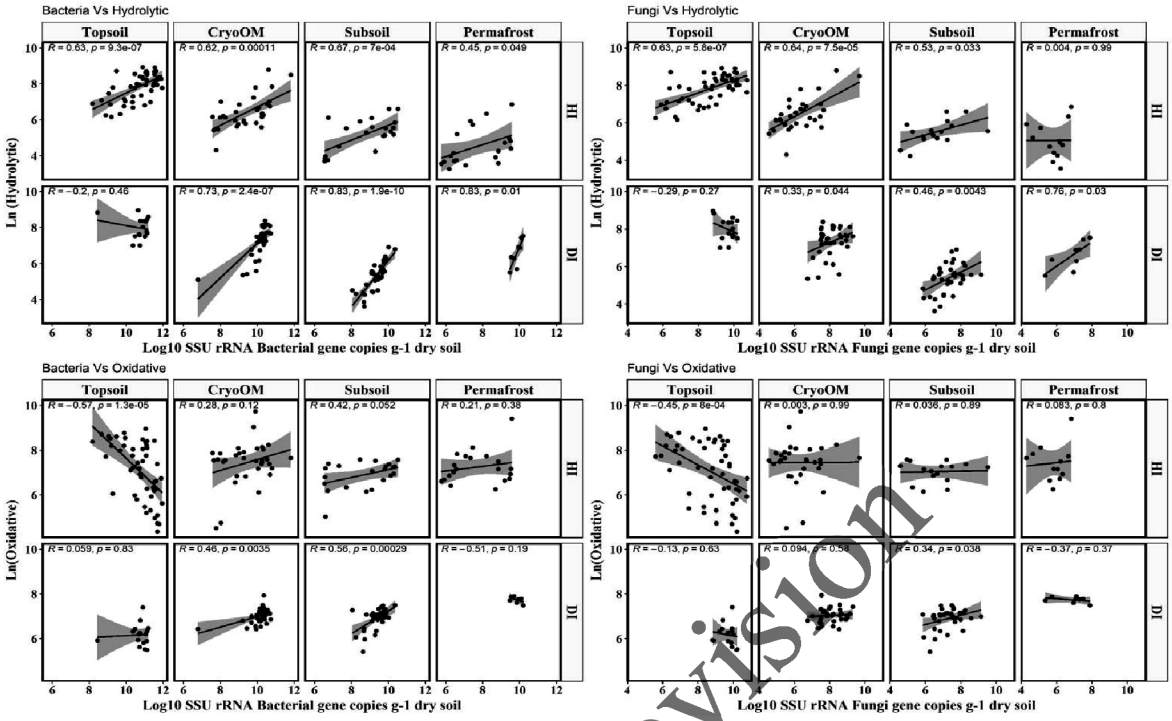

Figure S2. Pearson's correlation between enzyme activity and microbial gene abundance.

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

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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

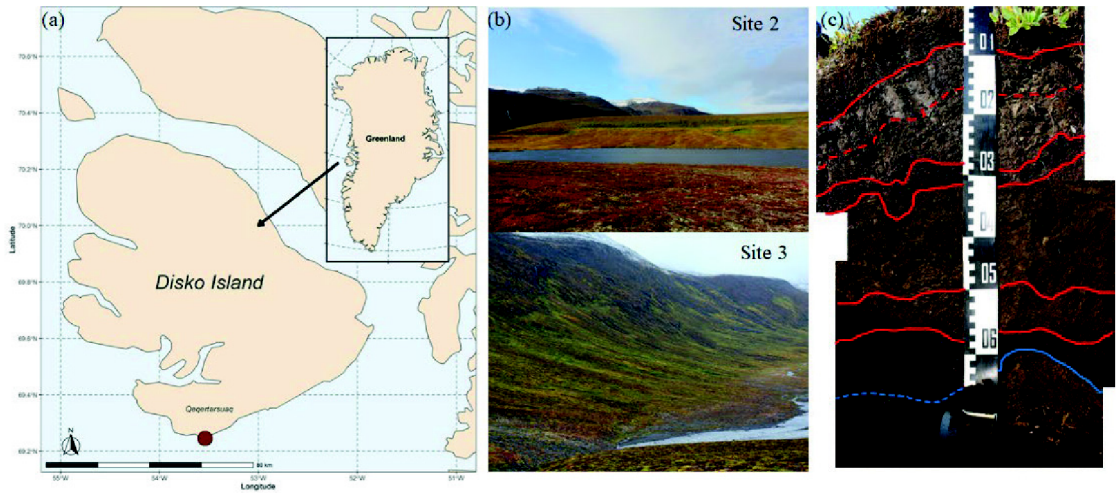

Figure 1. *(a) Sampling location Disko Island, Greenland, (b) Sampling sites, and (c) Cryoturbated soil profile.*

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 (Ctot) and N (Ntot) 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). Ctot and Ntot 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 (Biomatrica, 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 silvamod128 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, Ctot content was highest in the top O layer with 24.3% and the Ctot 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 Ctot and DOC content (Figure 2). The O horizon samples with high Ctot 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 Ctot (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	jj	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	jj	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	jj	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	jj	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	jj	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	jj	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	jj	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	jj	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).

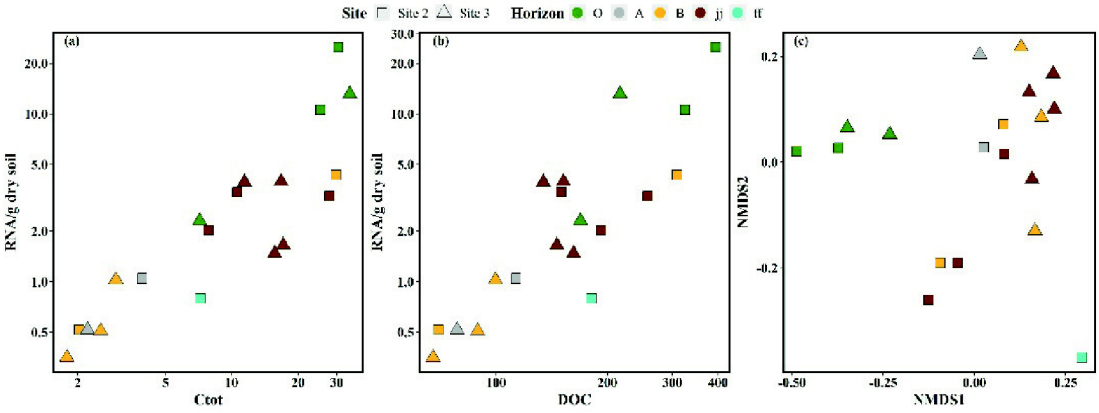

Figure 2. *RNA yield and phylogenetic dissimilarity. (a) RNA yield per gram soil against total C, (b) RNA yield per gram soil against DOC, and (c) the phylogenetic dissimilarity between soil samples by NMDS plot. Different colors and shapes represented distinct horizons and sites, respectively. Green, 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.

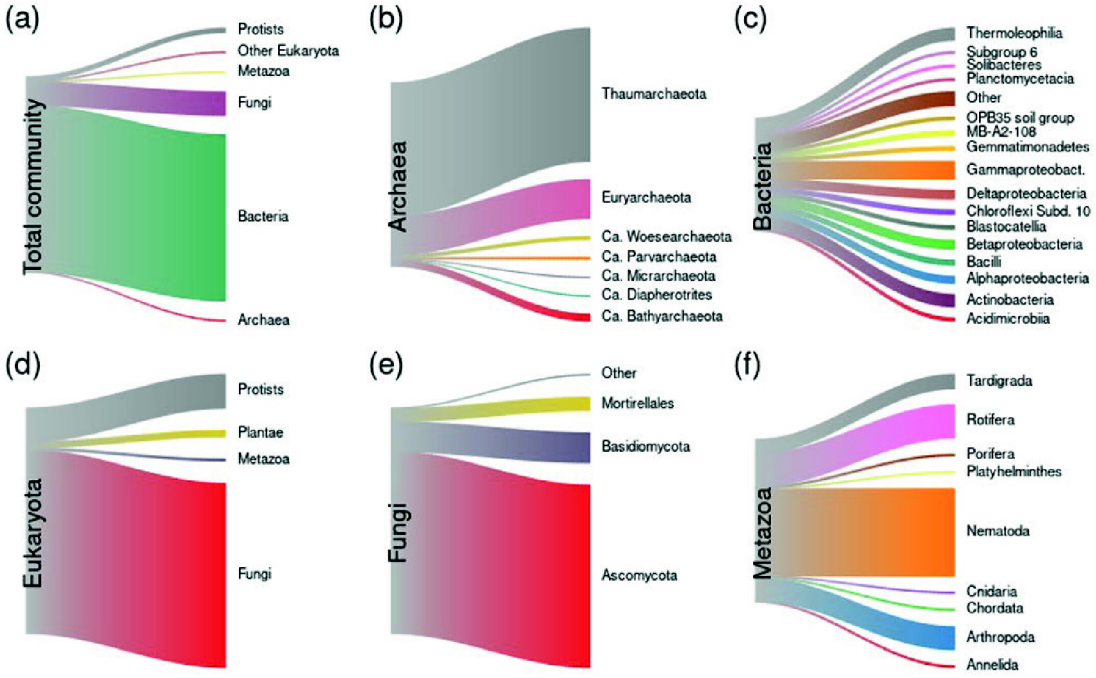

Figure 3. *SSU rRNA abundance of the Arctic soil community. The size of the edges displays the number of SSU rRNA read counts. (a) Total community, (b) Archaea, (c) Bacteria, (d) Eukaryota, (e) Fungi, (f) Metazoa.*

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).

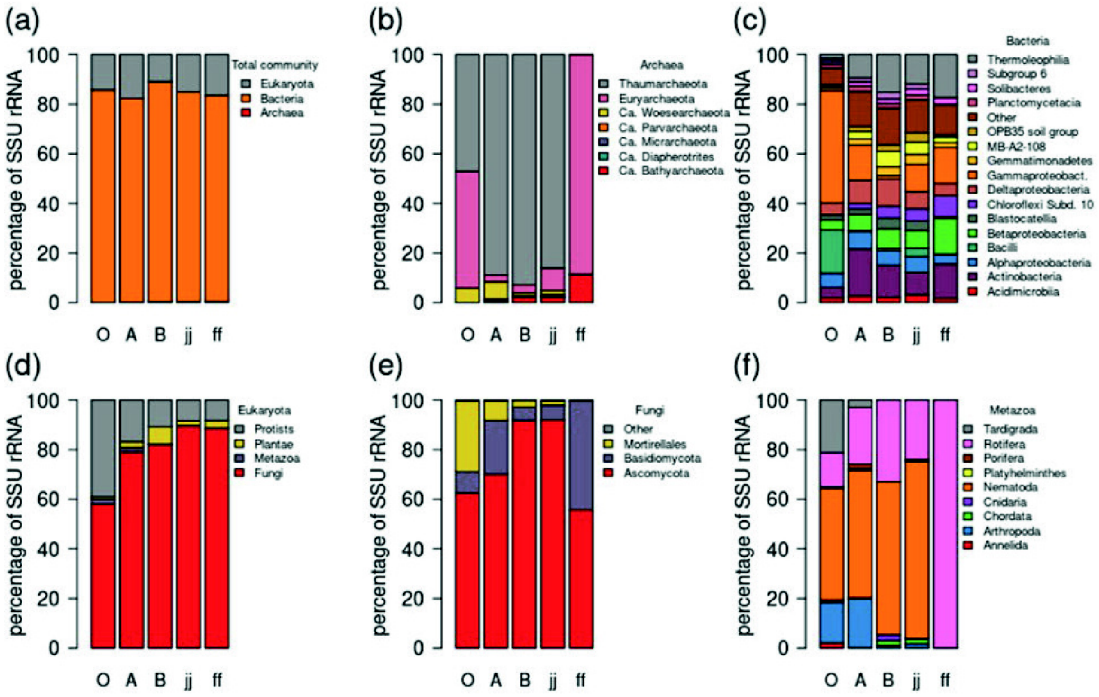

Figure 4. *Horizon resolved community composition. Read counts normalized to 100%. O: organic top layer. A: mineral topsoil. B: mineral subsoil. jj: buried organic layer. ff: permafrost. (a) Total community. (b) Archaea. (c) Bacteria. (d) Eukaryota. (e) Fungi. (f) Metazoa.*

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 ($p = 0.18$).

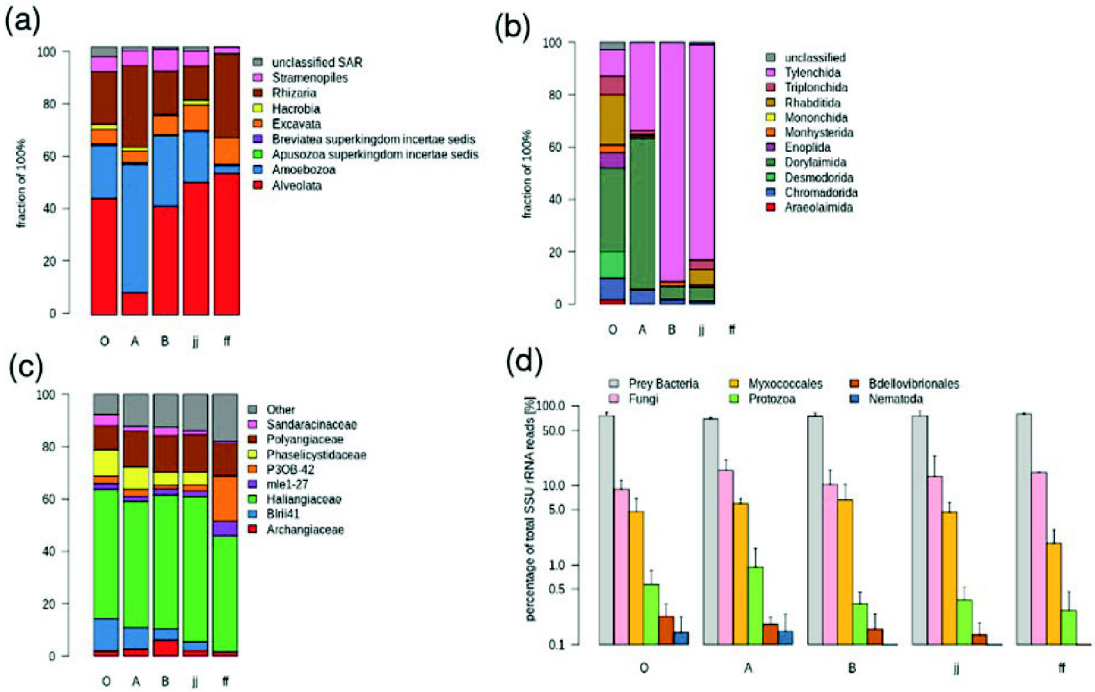

Figure 5. *The abundance of prey and predatory organisms in different soil layers. O: organic top layer. A: mineral topsoil. B: mineral subsoil. jj: buried organic layer. ff: permafrost. (a) Prey and predatory organisms. (b) Myxococcales. (c) Nematoda. (d) Protists.*

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.

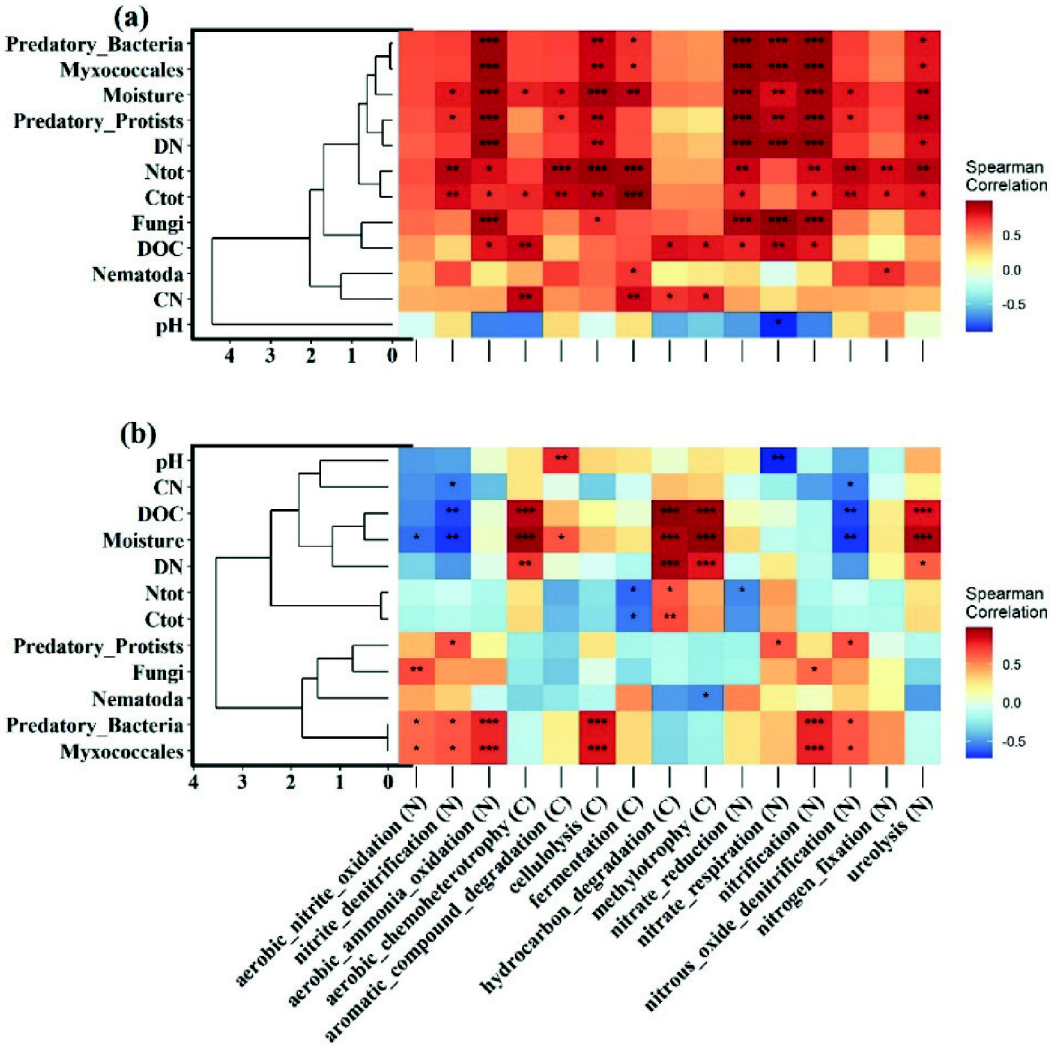

Figure 6. *Correlation matrix of functional groups and different biotic (taxonomic groups) and abiotic factors. Functional groups were assigned by FAPROTAX pipeline using SSU rRNA abundances. Abundances of both functional and taxonomic groups were based on quantified SSU rRNA abundances. (a) Organic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

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References

Altschul SF, Gish W, Miller W, et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Beare MH, Coleman DC, Crossley DA, et al (1995) A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. Plant Soil 170:5–22. https://doi.org/10.1007/BF02183051

Bonkowski M (2004) Protozoa and plant growth: the microbial loop in soil revisited. New Phytol 162:617–631. https://doi.org/10.1111/j.1469-8137.2004.01066.x

Bonkowski M, Cheng W, Griffiths BS, et al (2000) Microbial-faunal interactions in the rhizosphere and effects on plant growth. Eur J Soil Biol 36:135–147. https://doi.org/10.1016/S1164-5563(00)01059-1

Christensen S, Griffiths BS, Ekelund F, Rønn R (1992) Huge increase in bacterivores on freshly killed barley roots. FEMS Microbiol Lett 86:303–310. https://doi.org/10.1111/j.1574-6968.1992.tb04822.x

Clarholm M (1985) Possible roles for roots, bacteria, protozoa and fungi in supplying nitrogen to plants. Ecol Interact soil 4:355–365

Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is governed by accessibility not recalcitrance. Glob Chang Biol 18:1781–1796. https://doi.org/10.1111/j.1365-2486.2012.02665.x

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Geisen S, Tveit AT, Clark IM, et al (2015) Metatranscriptomic census of active protists in soils. ISME J 9:2178–2190. https://doi.org/10.1038/ismej.2015.30

Gittel A, Bárta J, Kohoutová I, et al (2014a) Distinct microbial communities associated with buried soils in the Siberian tundra. ISME J 8:841–853. https://doi.org/10.1038/ismej.2013.219

Gittel A, Bárta J, Kohoutová I, et al (2014b) Site- and horizon-specific patterns of microbial community structure and enzyme activities in permafrost-affected soils of Greenland. Front Microbiol 5:541. https://doi.org/10.3389/fmicb.2014.00541

Griffiths BS, Caul S (1993) Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. Biol Fertil Soils 15:201–207. https://doi.org/10.1007/BF00361612

Griffiths BS, Ekelund F, Rønn R, Christensen S (1993) Protozoa and nematodes on decomposing barley roots. Soil Biol Biochem 25:1293–1295. https://doi.org/10.1016/0038-0717(93)90228-4

Huson DH, Auch AF, Qi J, Schuster SC (2007) MEGAN analysis of metagenomic data. Genome Res 17:377–386. https://doi.org/10.1101/gr.5969107

Jurkevitch E (2007) Predatory Behaviors in Bacteria — Diversity and Transitions. Microbe Mag 2:67–73. https://doi.org/10.1128/microbe.2.67.1

Kaiser C, Meyer H, Biasi C, et al (2007) Conservation of soil organic matter through cryoturbation in arctic soils in Siberia. J Geophys Res Biogeosciences 112:1–8. https://doi.org/10.1029/2006JG000258

Keane R, Berleman J (2016) The predatory life cycle of Myxococcus xanthus. Microbiology 162:1–11. https://doi.org/10.1099/mic.0.000208

Kopylova E, Noé L, Touzet H (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28:3211–3217. https://doi.org/10.1093/bioinformatics/bts611

Lanzén A, Jørgensen SL, Huson DH, et al (2012) CREST – Classification Resources for Environmental Sequence Tags. PLoS One 7:e49334. https://doi.org/10.1371/journal.pone.0049334

Louca S, Parfrey LW, Doebeli M (2016) Decoupling function and taxonomy in the global ocean microbiome. Science (80-). https://doi.org/10.1126/science.aaf4507

Magoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. https://doi.org/10.1093/bioinformatics/btr507

McGuire AD, Anderson LG, Christensen TR, et al (2009) Sensitivity of the carbon cycle in the Arctic to climate change. Ecol Monogr 79:523–555. https://doi.org/10.1890/08-2025.1

Moore JC, Walter DE, Hunt HW (1988) Arthropod Regulation of Micro- and Mesobiota in Below-Ground Detrital Food Webs. Annu Rev Entomol 33:419–435. https://doi.org/10.1146/annurev.en.33.010188.002223

Petters S, Groß V, Söllinger A, et al (2021) The soil microbial food web revisited: Predatory myxobacteria as keystone taxa? ISME J 15:2665–2675. https://doi.org/10.1038/s41396-021-00958-2

Reichenbach H (1999) The ecology of the myxobacteria. Environ Microbiol 1:15–21. https://doi.org/10.1046/j.1462-2920.1999.00016.x

Rosenberg E, DeLong EF, Lory S, et al (eds) (2014) The Prokaryotes. Springer Berlin Heidelberg, Berlin, Heidelberg

Schädel C, Schuur EAG, Bracho R, et al (2014) Circumpolar assessment of permafrost C quality and its vulnerability over time using long-term incubation data. Glob Chang Biol 20:641–652. https://doi.org/10.1111/gcb.12417

Scheu S, Setälä H (2002) Multitrophic interactions in decomposer food-webs. In: Multitrophic Level Interactions. Cambridge University Press, pp 223–264

Schmidt MWI, Torn MS, Abiven S, et al (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56. https://doi.org/10.1038/nature10386

Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10.1093/bioinformatics/btr026

Schnecker J, Wild B, Hofhansl F, et al (2014) Effects of Soil Organic Matter Properties and Microbial Community Composition on Enzyme Activities in Cryoturbated Arctic Soils. PLoS One 9:e94076. https://doi.org/10.1371/journal.pone.0094076

Schoeneberger, P.J., D.A. Wysocki, E.C. Benham and SSS (2012) Field Book for Describing and Sampling Soils. Nat Resour Conserv Serv Natl Soil Surv Center, Lincoln, NE. https://doi.org/10.1111/j.1600-0587.2009.05973.x

Söllinger A, Tveit AT, Poulsen M, et al (2018) Holistic Assessment of Rumen Microbiome Dynamics through Quantitative Metatranscriptomics Reveals Multifunctional Redundancy during Key Steps of Anaerobic Feed Degradation. mSystems 3:e00038-18. https://doi.org/10.1128/mSystems.00038-18

Tarnocai C, Canadell JG, Schuur EAG, et al (2009) Soil organic carbon pools in the northern circumpolar permafrost region. Global Biogeochem Cycles 23:1–11. https://doi.org/10.1029/2008GB003327

Treuner-Lange A, Bruckskotten M, Rupp O, et al (2017) Whole-Genome Sequence of the Fruiting Myxobacterium Cystobacter fuscus DSM 52655. Genome Announc 5:. https://doi.org/10.1128/genomeA.01196-17

Urich T, Lanzén A, Qi J, et al (2008) Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome. PLoS One 3:e2527. https://doi.org/10.1371/journal.pone.0002527

Varsadiya M, Urich T, Hugelius G, Bárta J (2021a) Microbiome structure and functional potential in permafrost soils of the Western Canadian Arctic. FEMS Microbiol Ecol 97:. https://doi.org/10.1093/femsec/fiab008

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. https://doi.org/10.3390/microorganisms9091943

Wild B, Schnecker J, Alves RJE, et al (2014) Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. Soil Biol Biochem 75:143–151. https://doi.org/10.1016/j.soilbio.2014.04.014

Wild B, Schnecker J, Bárta J, et al (2013) Nitrogen dynamics in Turbic Cryosols from Siberia and Greenland. Soil Biol Biochem 67:85–93. https://doi.org/10.1016/j.soilbio.2013.08.004

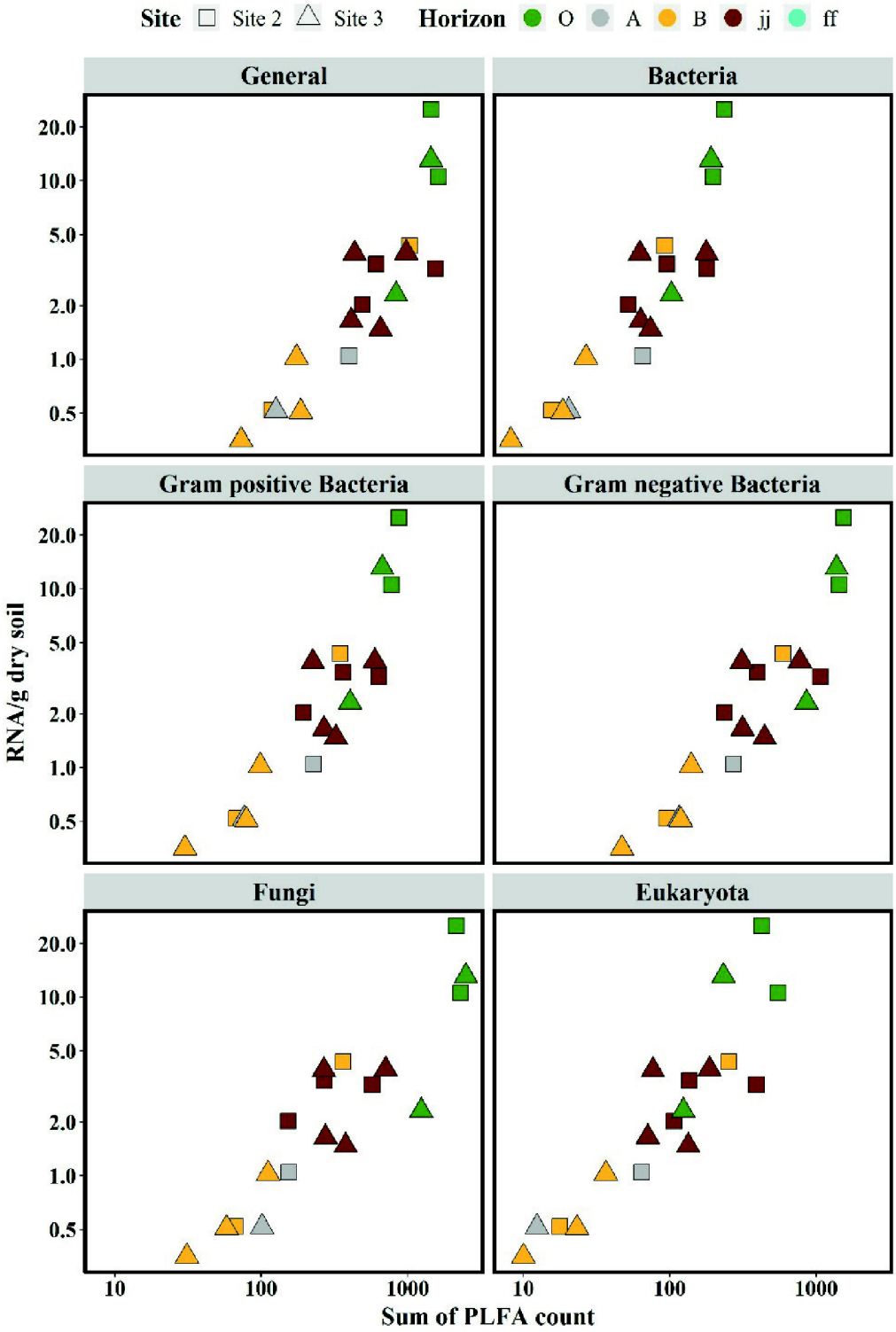
Yeates GW, Bongers T, De Goede RG, et al (1993) Feeding habits in soil nematode families and genera-an outline for soil ecologists. J Nematol 25:315–331

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

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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
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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.

Varsadiya, M., Urich, T., Hugelius, G., and Bárta, J. (2021b). Microbiome structure and functional potential in permafrost soils of the Western Canadian Arctic. FEMS Microbiol. Ecol. 97. doi:10.1093/femsec/fiab008.

Gałązka, A., Marzec-Grządziel, A., **Varsadiya, M.**, Niedźwiecki, J., Gawryjołek, 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

Publication under revision or submitted

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 (Under major revision in Biogeochemistry).

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)

Varsadiya, M., Walter, D., Liebman, P., Petters, S., Šantrůčková, H., Urich, T., Guggenberger, G., Bárta, J. Microbial functional diversity and enzyme activity patterns of permafrost affected (PAS) soil from Greenland. 17th International Symposium on Microbial Ecology (ISME) 2018, Leipzig, Germany. (poster)

Bárta, J., Urich, T., Šantrůčková, H., **Varsadiya, M**., Petters, S., Liebman, P., Gentsch, N., Guggenberger, G., Richter, A. Vulnerability of subducted carbon and nitrogen to permafrost thaw. 17th International Symposium on Microbial Ecology (ISME) 2018, Leipzig, Germany. (poster)

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, Srni, Czech Republic. (poster).

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