Jihočeská univerzita v Českých Budějovicích
Přírodovědecká fakulta

Faculty of Science
University of South Bohemia in České Budějovice

Effect of different water regimes on microorganisms of nitrogen cycle in permafrost

Bachelor's thesis

Sára Turková

Supervisor: Doc. Ing. Jiří Bárta, PhD.

České Budějovice, Czech Republic 2022

Turková S. (2022). Effect of different water regimes on microorganisms of nitrogen cycle in permafrost. Bc. Thesis, in English. – 30 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech republic

Annotation: The gene abundances and community structures of nitrogen-fixing and denitrifying bacteria in permafrost-affected soils with different moisture level were analysed. Additionally, DNA- and RNA-based analysis was used to evaluate the variations in gene copy numbers and community composition between the total and active bacteria. The gene abundances of nitrogen-fixing and denitrifying bacteria were quantified by performing qPCR of the *nifH* and *nosZ* genes, and the gene copy numbers were compared between the soils with different moisture. Furthermore, the bacterial community structure variation was evaluated by analysis of the taxonomic classification obtained from sequencing data.

I hereby declare that I have worked on my bachelor's thesis independently and used only the sources listed in the bibliography. I hereby declare that, in accordance with Article 47b of Act No. 111/1998 in the valid wording, I agree with the publication of my bachelor thesis, in full / in shortened form resulting from deletion of indicated parts to be kept in the Faculty of Science archive, in electronic form in publicly accessible part of the STAG database operated by the University of South Bohemia in České Budějovice accessible through its web pages. Further, I agree to the electronic publication of the comments of my supervisor and thesis opponents and the record of the proceedings and results of the thesis defence in accordance with aforementioned Act No. 111/1998. I also agree to the comparison of the text of my thesis with the Theses.cz thesis database operated by the National Registry of University Theses and a plagiarism detection system.

Date, Signature

6.5.2022 Turková

Acknowledgment

I would like to express my gratitude to my supervisor Doc. Ing. Jiří Bárta, PhD. and consultant MSc. Milan Varsadiya for their guidance throughout the work on my bachelor thesis and for teaching me the procedures of DNA/RNA extraction and qPCR. I would also like to thank you for providing the samples and all the information to the soil properties and sequencing.

I am also very grateful to my family and friends for their support and encouragement throughout the study.

Abstract

Climate warming and subsequent permafrost thaw may result in changes in soil moisture, generally creating either wet or dry soil conditions. In the Alaskan subarctic, the consequences of permafrost thaw have not been thoroughly addressed, and predictions of its impact on the nitrogen cycle are therefore still highly uncertain. The changes in soil moisture can also result in fluctuations of microbial activity and the emissions of nitrous oxide (N_2O), a powerful greenhouse gas produced in the process of denitrification. Therefore, our study aimed to determine the effect of moisture on total and active bacteria and the bacterial community structure in permafrost-affected soils. We investigated two sites in central Alaska with thawing permafrost and one with intact permafrost. Samples collected from the three different sites were used to quantify the abundances of nitrogen-fixing (*nifH* gene) and denitrifying bacteria (*nosZ* gene) by qPCR and to investigate bacterial community structure harbouring these genes. Soil moisture had a significant impact on total gene abundance and microbial community structure. However, the activity of bacteria was not affected by moisture content and active *nosZ* genes were quantified only in one sample. This finding may point to an incomplete conversion of N_2O to N_2, which may potentially lead to an increase in N_2O emissions. Differences in the results between DNA- and RNA-based analysis demonstrated that DNA results alone can lead to overestimation of active bacteria, highlighting the importance of using this complementary approach.

Our findings provide an extension of the understanding of nitrogen cycling in thawing permafrost soils and suggest that the permafrost thaw and resulting soil moisture changes may affect both the abundance of the *nifH* and *nosZ* genes and the bacterial community structure, with possible repercussions on the global climate.

List of abbreviations

ANOVA	Analysis of variance
DN	Dissolved nitrogen
DOC	Dissolved organic carbon
NMDS	Non-metric multidimensional scaling
PERMANOVA	Permutational multivariate analysis of variance
qPCR	Quantitative polymerase chain reaction
SOM	Soil organic matter
WHC	Water holding capacity

Table of contents

1 Introduction

1.1 Subarctic ecosystems

The Subarctic is an area of the Northern Hemisphere at latitudes from 50° to 70°. Areas with subarctic climate can be found in North America from Newfoundland to Alaska, and in northern Eurasia from Scandinavia through most of Siberia (Peel, Finlayson, and McMahon 2007). The subarctic climate is characterized by short summers, in which temperatures can exceed 26°C, and cold winters, with temperatures occasionally dropping below -50°C. The frost-free periods in the subarctic last on average from 50-90 days. Annual precipitation totals are generally less than 500 mm, with most precipitation falling as rain between the months of May and September (Boggs et al. 2019).

Due to the cold and long winters, the soil is often underlain by perennially frozen ground, permafrost. Permafrost impacts subarctic ecosystems, as it restricts vertical water flow from the annually thawing surface layer (active layer), which leads to the formation of peatlands (Andresen et al. 2020). The subarctic ecosystem is shaped by cold weather, short growing season, and the presence of permafrost. The typical biomes of the Subarctic include coniferous taiga forests, grasslands, moors, and peat bogs.

Although the richness of plant and animal species in the subarctic is generally low, terrestrial ecosystems host diverse microbial communities. Microbes play major roles in biogeochemical cycling of all major macro-elements including carbon (C) and nitrogen (N), which includes breaking down soil organic C, nutrient recycling, and the remineralization of carbon and nitrogen (Malard and Pearce 2018). The microbial communities thus significantly affect the primary production in the subarctic.

The Arctic and Subarctic regions are vulnerable to climate change, and the rise in temperatures in these regions is twice as fast as the global average (Agnan et al. 2019; Pascual et al. 2021). Hence, the rising temperatures are expected to affect ecological communities, water balance, and nutrient cycling (Schaeffer et al. 2013).

1.2 Permafrost

Permafrost is ground material that remains frozen (<0°C) for at least two successive years and can be present in soil, rock, and sediment (van Everdingen 1998). Present estimates indicate that approximately 17% of the exposed land surface on Earth is underlain by permafrost (Biskaborn et al. 2019) which can be found predominantly in the Arctic, Subarctic, and Antarctic regions and to a lesser degree in alpine regions (Zubrzycki, Kutzbach, and Pfeiffer 2014).

Based on the spatial extent of perennially frozen ground, permafrost regions can be divided into three zones – the continuous, the discontinuous, and sporadic. The continuous permafrost zone is present primarily in the high Arctic; discontinuous and sporadic, which include numerous permafrost-free areas, is more common in the Subarctic (Osterkamp and Burn 2015).

Depending on the conditions, the thickness of permafrost can vary from several meters to more than 1000 meters (Steven et al. 2008). The upper layer (active layer) of permafrost thaws and freezes seasonally and its thickness can range from 10 cm to 5 m (Altshuler, Goordial and Whyte 2017). In the active layer, most biological, physical, chemical, and pedogenic processes occur (Smith and Brown 2009).

Permafrost-affected soils store vast amounts of carbon and nitrogen (Ramm et al. 2020). In estimation, 1330-1580 Pg of C (Salmon et al. 2018) and 67 Pg of N are stored in permafrost-affected soils, cryosols (Harden et al. 2012). Due to permafrost thaw induced by raising temperatures, these C and N stocks will eventually get available to microbial degradation, which will increase methane, carbon dioxide, and nitrous oxide emissions. Moreover, a change in nitrogen availability also influences plant productivity and decomposition of soil organic matter (Salmon et al. 2018).

1.2.1 Hydrological impact of thawing permafrost

The surface temperature warming rates in high latitudes are greater than the global average. The increase of surface temperature significantly affects the hydrologic processes and conditions of the cryosphere, including permafrost (Walvoord and Kurylyk 2016).

Hydrology plays an important role in permafrost landscapes by regulating interactions among biogeochemical cycling, geomorphology, and ecosystem structure (Andresen et al. 2020). The permafrost in cryosols prevents vertical water flow, which often leads to water-saturated soil conditions at the boundary of the active layer and the permafrost. In the arctic and subarctic ecosystems, the mean annual precipitation is generally low. Therefore, while deeper soil is generally waterlogged, the surface soil can dry out quickly (Hicks Pries et al. 2013). As the permafrost thaws, the moisture levels in the surface soil are expected to change, with some areas becoming either drier or wetter.

Topography variation also plays an important role in soil moisture patterns in Arctic landscapes (Stewart et al. 2014). In lowland regions, the permafrost thaw can increase soil moisture as a result of ground slumping, which is caused by melting of ground ice (Lawrence et al. 2015). Furthermore, the thaw can create thermokarst terrain, an uneven ground feature formed as a result of soil subsidence followed by the loss of ground ice. Thermokarst changes the height of the water table perched on top of the permafrost, making subsided areas wetter while funnelling water from adjacent areas (Hicks Pries et al. 2013).

In contrast, permafrost thaw can also cause drying of the near-surface soil, as the active layer deepens and water infiltrates deeper soil layers or as the permafrost thaws completely. Surface drying could be counteracted by an increase in precipitation in the arctic and subarctic caused by global warming. However, the majority of hydrological models project a long-term drying of the surface soil despite increases in the net air-surface water flux (Rousk, Sorensen, and Michelsen 2018; Schuur et al. 2015).

The moisture content in soil affects the physiological state of microorganisms. The soil water content is essential for processes such as regulating oxygen diffusion and maintaining the catalytically active state of soil enzymes. Usually, soils with moisture levels between 50-70% water holding capacity (WHC) harbour more functionally diverse microbial communities. Nevertheless, excessive soil moisture can lead to a lower biomass of microorganisms due to the formation of oxygen conditions that are unfavourable for aerobic bacteria (Borowik and Wyszkowska 2016). On the other hand, low soil moisture also promotes unfavourable

conditions for the life of microorganisms, by reducing diffusion of soluble substrates, microbial mobility, and intracellular water potential (Stres et al. 2008).

The alternations in soil hydrologic conditions induced by thawing of the permafrost will thus affect organic matter decomposition and the biogeochemical cycling. Soil moisture is one of the key factors controlling the nitrogen cycle. In areas with high levels of soil moisture, the rate of processes that occur primarily under anaerobic conditions, such as nitrogen fixation and denitrification, is expected to increase (Stewart et al. 2014).

1.2.2 Microbial life in permafrost

Permafrost provides a highly challenging environment for soil microorganisms. The microbes must cope with environmental stressors, such as low temperatures, low nutrient availability, or limited water accessibility. Yet, many microorganisms thrive in these harsh conditions. In estimation, a microbial community of 10^5-10^9 cells per gram of soil lives in the Arctic permafrost (Altshuler, Goordial, and Whyte 2017).

The dominant groups that can be found in permafrost soils are Actinobacteria, Bacterioidetes, Proteobacteria, Firmicutes, Chloroflexi, and Acidobacteria (Ernakovich and Wallenstein 2015; Wilhelm et al. 2011). The aforementioned phyla are dominant not only in permafrost soils but also in all of the world's biomes (Thompson et al. 2017).

Most microorganisms in the permafrost are psychrotolerant with optimum growth temperatures at or above 20°C, that are able to survive long-time subzero temperature in nonviable or dormant state (Steven et al. 2008). However, a distinct community of psychrophilic organisms able to grow and reproduce at temperatures of 15°C and lower is present in permafrost. Psychrophilic microorganisms developed various molecular adaptations for life in a constantly cold environment. These adaptations include alpha-helix-rich enzymes functional in cold cytoplasmic membranes with a higher content of unsaturated and shorter-chain fatty acids, 'cold shock' proteins, and cryoprotectants, which serve to prevent the formation of ice crystals (D'Amico et al. 2006). Focusing on bacteria involved in the nitrogen cycle, psychrophilic species can be found in the "usual" genera *Psychromonas*, *Pseudomonas*, and *Shewanella,* but also in newly described *Arcobacter* in the Campylobacterota phylum (Canion et al. 2013).

An important factor that affects the microbial abundance and diversity is the permafrost thaw induced by climatic change. Thawing causes changes in the microbial community not

only in terms of diversity, but also functional potential in response to warmer temperatures and increased carbon and water availability (Altshuler et al. 2017).

1.3 Nitrogen cycle

Nitrogen (N) is an indispensable nutrient for plants and soil microorganisms. This element is a component of numerous essential molecules, such as nucleic acids, proteins, and membrane lipids. Nevertheless, dinitrogen gas (N_2) in the atmosphere is inaccessible to organisms and must first be converted to ammonium ions (NH_4^+) to become available to microorganisms and plants. Hence, nitrogen is generally the primary limiting nutrient that plays an important role in the decomposition of soil organic matter (SOM) by soil microorganisms, as well as in the regulation of plant productivity in the tundra and boreal communities overlying the permafrost zone (Penton et al. 2016; Salmon et al. 2018).

Throughout the N cycle, N undergoes diverse transformations in ecosystems, where it exists in both inorganic and organic forms, and enters various oxidation states. The transformation of nitrogen throughout the nitrogen cycle is particularly dependent on the activities of microorganisms. The three major steps mediated by microbes are nitrification, denitrification, and N_2 fixation (Figure 1).

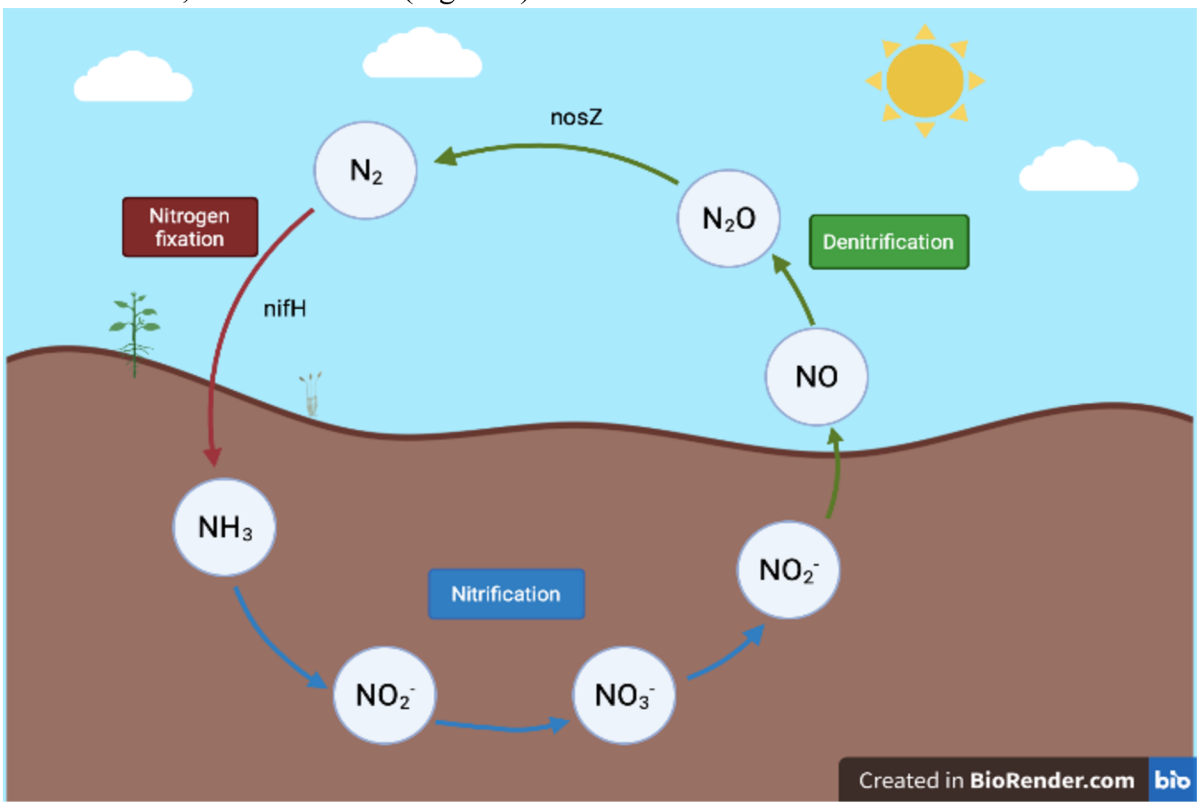

Figure 1: Simplified scheme of nitrogen cycle with highlighted *nifH* and *nosZ* genes, which are involved in nitrogen fixation and the last step of denitrification process, respectively

The main nitrogen input for the pristine ecosystems of the subarctic originates from biological nitrogen fixation. In this process, atmospheric nitrogen (N_2) is converted to biologically available ammonium ions (NH_4^+) via the enzyme nitrogenase (Rousk, Sorensen, and Michelsen 2018)

The reduced nitrogen compounds - ammonium (NH_4^+) and nitrite (NO_2^-) - are then oxidized to nitrate (NO_3^-) by aerobic nitrifying bacteria in the process of nitrification. In nitrification, two different sets of reactions occur. The first set of reactions catalyzes the oxidation of ammonia to nitrite, and the second set induces the conversion of NO_2 to NO_3^-. Most nitrifying microorganisms can perform only one set of these reactions (Madigan et al. 2019).

In addition, a bacterium capable of one-step oxidation of ammonium ions to nitrate has recently been discovered. This process has been named commamox, i.e. complete ammonia oxidation (Kits et al. 2019).

In the process of denitrification, a series of reactions catalysed by oxidoreductases reduce nitrate (NO_3^-) back to the form of nitrogen gas (N_2). Depending on abiotic factors, such as water, oxygen availability, and pH, denitrification can be incomplete and result in significant production of nitrous oxide (N_2O), a potent greenhouse gas (Altshuler et al. 2019).

The key drivers that control N cycling in the Arctic and subarctic are soil moisture, light, temperature, SOM, and N and C substrate availability (Stewart et al. 2014). In this study, we investigate the influence of soil moisture variation on nitrogen cycling, with a focus on nitrogen input and output represented as nitrogen fixation and denitrification.

In high-latitude ecosystems the nitrogen availability in soils is generally low (Schimel and Bennett 2004) However, due to changes induced by global warming, such as increasing temperature and moisture level of Arctic soils, the N-availability is expected to increase as a result of enhanced nitrogen fixation rates (Stewart et al. 2014). Furthermore, the active layer of permafrost was reported to harbour a more active community of nitrogen fixers than the underlying permafrost (Hultman et al. 2015). As the active layer deepens, nitrogen-fixing communities can become a considerable source of nitrogen input to permafrost soils and further stimulate microbial metabolism and degradation of frozen SOM (Altshuler et al. 2019). The enhanced N-fixation would also increase the rate of nitrogen cycling and thus the potential for higher N_2O emissions (Penton et al. 2016).

Furthermore, it has been shown that the bacteria involved in the nitrogen cycle are affected by temperature, anaerobiosis, and the availability of nitrate (Stres et al. 2008). As nitrogen-fixing and denitrifying bacteria contain both aerobic and anaerobic species, the

different soil water content may cause shifts in community structure. With increasing moisture, the proportion of bacteria that belong to the phyla *Verrucomicrobia*, *Gemmatimonadetes*, and *Proteobacteria* is expected to increase, while the proportion of *Acidobacteria*, *Actinobacteria*, *Firmicutes,* and *Bacteroidota* would decrease (Li et al. 2021; Siebielec et al. 2020). Therefore, permafrost thaw and the resulting changes in soil moisture level could be an important determinant of the activity and community composition of nitrogen-fixing and denitrifying bacteria, but its impact has not been thoroughly evaluated so far.

1.3.1 Nitrogen fixation

Nitrogen fixation is considered to be the primary source of nitrogen in terrestrial subarctic environments. The process of nitrogen fixation is mediated by bacteria and archaea called diazotrophs. Microorganisms that carry out nitrogen fixation exhibit great diversity in physiological attributes; they can be autotrophic, heterotrophic, chemolithotrophic, photoheterotrophic, and methanogenic (Reed 2011). Despite their differences in physiology, they all possess a similar enzyme complex – nitrogenase – which catalyses the reduction of nitrogen in air (N_2) to ammonia. Nitrogenase consists of two protein subunits, the electron transfer Fe subunit (nitrogenase reductase), which is encoded by the *nifH* gene, and the MoFe protein (dinitrogenase reductase), which is encoded by the *nifD* and *nifK* gene (Stewart, Coxson, and Siciliano 2011). *Nif* genes, in particular the *nifH*, are used as a functional marker to determine the abundance of N-fixing microorganisms, as they are highly conserved and are present in numerous phylogenetically divergent bacteria and archaea (Ortiz et al. 2020). In nitrogenase, the reduction of N_2 occurs at the iron-molybdenum cofactor site (FeMo-co) in dinitrogenase reductase. The reduction of the triple bond in N_2 requires a high amount of energy, by converting 1 mole of N_2 into NH_4^+ 8 electrons and 16 ATP molecules are consumed.

Furthermore, microorganisms need to establish anoxic conditions for the nitrogen fixation process, since dinitrogenase reductase is inactivated by O_2. Yet many nitrogen-fixing bacteria are obligate aerobes. These bacteria protect nitrogenase from deactivation by various mechanisms, for example, by rapid removal by respiration, production of slime layers, or by the formation of differentiated cells with nitrogenase cyanobacterial heterocysts (Madigan et al. 2019).

Among nitrogen fixers, there are both free-living species and species that form a symbiotic relationship with plants and lichens. In the Arctic ecosystems, most N_2 fixation is carried out by phototrophic cyanobacteria (Solheim and Zielke 2007; Zielke et al. 2005).

Nitrogen (N) fixation is the predominant source of nitrogen to terrestrial ecosystems in the Arctic environments, providing in estimation 50-80% of total landscape annual N inputs. Therefore, any changes in nitrogen input can affect ecosystem productivity (Stewart et al. 2014).

Due to the diverse types of respiration among nitrogen-fixing bacteria, soil moisture is an important factor in the variability of N fixation among vegetative communities of the Arctic (Penton et al. 2016). Nitrogen-fixing bacteria favour conditions with increased soil moisture (Rousk, Sorensen, and Michelsen 2018; Zielke et al. 2005). Thus, an elevation in soil moisture in permafrost may lead to an increase in nitrogen fixation rates and vice versa.

1.3.2 Denitrification

Denitrification is a pathway in which bioavailable nitrogen in the form of nitrate (NO_3^-) is converted to gaseous end products, which are then lost from the soil. In the process, nitrate (NO_3^-) is reduced stepwise to nitrite (NO_2^-) or further to gaseous forms such as nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) under anaerobic conditions (Madigan et al. 2019).

The gaseous products of denitrification, predominantly N_2O, contribute to global climate change. N_2O is a strong greenhouse gas, with a long-term global warming potential 298 times that of carbon dioxide (Di et al. 2014). Nitrous oxide is also involved in the depletion of the ozone layer through photochemical oxidation to nitric oxide (Henry et al. 2006).

There are several enzymes involved in the process of denitrification. The first step of nitrate reduction is catalysed by nitrate reductase, an oxygen repressed molybdenum-containing enzyme. The next steps are mediated by metalloenzymes nitrite reductase, nitric oxide reductase, and nitrous oxide reductase, which is involved in the last stage of the denitrification process (Henry et al. 2006). Nitrous oxide reductase is a multicopper homodimeric enzyme encoded by the *nosR* and *nosZ* genes. *NosZ* encodes the catalytic subunit of N_2O reductase and is widely used as a microbial marker gene (Grzyb, Wolna-Maruwka, and Niewiadomska 2021).

Several studies show that permafrost-affected soils already harbour organisms and genes involved in denitrification (Hultman et al. 2015; Yergeau et al. 2010). The genera with bacteria capable of denitrification found in Arctic soils include *Thiobacillus*, *Denitrovibrio*, *Pseudomonas*, *Azospirillum,* and *Azorhizobium* (Altshuler et al. 2019).

Changes resulting from global warming, such as elevated temperature and moisture level in permafrost-affected soils, are expected to cause an increase in the denitrification rate (Altshuler et al. 2019). In general, denitrification genes are more abundant in thawed permafrost compared to intact permafrost. However, the genes for nitrite and nitrous oxide reductases are less abundant in thawed permafrost soils, indicating a potential for incomplete denitrification and release of N_2O after thaw (Elberling, Christiansen, and Hansen 2010; Taş et al. 2014). This effect may be counteracted by an increase in water moisture to the point of complete saturation, allowing nitrous oxide reductase to be fully active and cause the conversion of more N_2O into N_2 (Di et al. 2014).

1.4 Aims and hypotheses

The main objective of the thesis was to evaluate the impact of permafrost thaw and subsequent soil moisture changes in the active layer on bacteria of nitrogen cycle, particularly on nitrogen-fixing and denitrifying bacterial communities. In our study, we used DNA- and RNA-based analysis to estimate the differences in the total and active bacterial communities. The abundance of total and active bacteria was quantified by qPCR of the *nifH* and *nosZ* genes, representing diazotrophs and denitrifiers, respectively. Furthermore, the bacterial community structure variation between three permafrost thaw scenarios (control, dry, wet) was evaluated by analysis of the taxonomic classification obtained from sequencing data.

Our main hypothesis was that the diazotrophic bacteria would be more abundant in the wet scenario, and together with denitrifying bacteria would be the main drivers of the nitrogen cycle in the active layer. We have also hypothesized that soil moisture level in the different scenarios of permafrost thaw could be an important determinant of the bacterial community structure, as both diazotrophs and denitrifiers contain aerobic and anaerobic specimens.

2 Materials and methods

2.1 Location

Soil samples were collected from three selected sites in Fairbanks, Alaska, USA. Wet, dry, and control sites were selected based on the depth of permafrost. At the control site, the depth was less than 1 m, and at both the "wet" and "dry" scenario sites, the permafrost depth was greater than 1 m. Three soil profiles (approximately 1x1m) were excavated at each site and samples of the active layer were taken from different depths. Basic soil chemical parameters were also determined.

2.2 Soil chemical parameters

Soil moisture was measured by drying the soil at 103°C for 24h and reweighting the dried soil sample. Soil pH was measured on calibrated pH meter. The dissolved organic carbon (DOC) and dissolved nitrogen (DN) were determined by mixing the sieved soil and water (ultra-pure) at a 1:5 ratio (w/v) for an hour on an orbital shaker (150 rpm) and filtered soil solution (approx. 10-15ml) was used for measurements on LiquiTOCII (Elementar, Germany).

2.3 DNA/RNA extraction

Total genomic RNA was extracted from 2 g of soil using Total RNA Isolation Kit (Qiagen, Germany) and DNA was parallelly extracted using DNA elution Accessory Kit (Qiagen, Germany) according to manufacturer protocol. The final elution volume for DNA and RNA was 50ul and stored at -20°C and -80°C, respectively. Total RNA was latter reverse transcribed to complementary DNA (cDNA) using Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany) according to manufacture protocol and stored at -20°C until further analysis.

2.4 qPCR

Real-time quantitative PCR was used to quantify the total and active population sizes of nitrogen-fixing (*nifH*) and denitrifying bacteria (*nosZ*) in the DNA and cDNA from a total of 56 soil samples extracted from three different types of soil. The qPCR assays were carried out in 48-well plates on the StepONE RealTime PCR System (Applied Biosystems).

Each of the reactions was performed with 20 µL of reaction mixture containing 3 µL of DNA from the soil samples. To quantify the *nifH* gene, 10 µL of a 1 µM solution of each PCR primer IGK3 (GCIWTHTAYGGIAARGGIGGIATHGGIAA) and DVV (ATIGCRAAICCICCRCAIACIACRTC) were added to the PCR mix, resulting in a product of 383 bp (Gaby and Buckley 2012). The cycling conditions were 95°C for 3 min, followed by 40 cycles of 15 s at 95°C, then 58, 72, and 77°C for 30 s each. Quantification of *nosZ* fragments (700 bp) was carried out using the primer pair nosZ-F-1181 (CGCTGTTCITCGACAYCAG) and nosZ-R-1880 (ATGTGCAKIGCRTGGCAGAA) of which 20 µL (1 µM each) was added to the reaction mix (Rich et al. 2003). The cycling conditions were 40 cycles of 95°C for 45 s, followed by 58°C for 30 s, and 72°C for 1 min. Briefly, the PCR mixtures contained Fast Start Sybr Green Mix, forward and reverse primer (1 µM), DMSO and BSA (20 mg/mL), and water.

The specificity of the amplified products was confirmed by melting point analysis by observing a single melting peak. The right size of amplicons was confirmed by agarose gel electrophoresis. Standards for qPCR were prepared by serial dilution of stocks of a known number of plasmids containing the respective functional gene. Serial dilution of plasmids harbouring respective genes with a known concentration ranging from 10^3 – 10^7 copies μL^{-1} served as controls. The plasmids with *nifH* and *nosZ* genes were obtained from genomic DNA of *Methylocystis heyeri* and *Pseudomonas aeruginosa*, respectively.

Furthermore, a negative control was added in all quantifications for the monitoring of contamination. The samples, standards, and non-template controls were run in duplicates. The abundances of the *nifH* and *nosZ* genes were then calculated as the copy number g^{-1} of the soil. The amplification efficiencies were calculated in the Step One software and accounted on average for 61.9% for *nifH* and 87.7% for *nosZ*.

2.5 Sequencing

The aliquots of DNA/RNA extracts were sent to SEQme (Prague, CZ) for the preparation of a library and sequencing using the MiSeq platform. The Earth Microbiome Project (EMP) protocol was used for library preparation with modified universal primers 515FB/806RB45 prokaryotic 16S rDNA amplicons (Caporaso et al. 2011). The coverage of prokaryotic primer pair 515FB/806RB was additionally tested in-silico using ARB Silva database release 132. The primer pair 515FB/806RB covers almost uniformly all major bacterial and archaeal phyla. 16SrDNA raw pair-end reads (250 bp) were joined using ea-utils to obtain reads of approx. 350 bp length (Caporaso et al. 2010). After quality filtering, the sequences were trimmed to a uniform length of 350 bp to avoid spurious OTU clusters (Caporaso et al. 2011). Bacterial reads were clustered zOTUs (zero radius OTUs) using an usearch11 pipeline (Edgar 2013). Taxonomy was assigned to each read by accepting the Silva132 taxonomy string of the best matching Silva132 sequence.

The lists of bacterial and archaeal genera containing *nifH* (diazotrophs) and *nosZ* (denitrifiers) metabolism were downloaded from the FunGene database (Fish et al. 2013). The list of these genera was used for functional annotation in our OTU table from which we were able to identify the main taxa in N transformation pathways.

2.6 Statistical analysis

All statistical analyses were performed using R (v 4.0.2) (R Core Team 2020) and the script was also used to produce the figures. Prior to one-way analysis of variance (ANOVA), the Shapiro-Wilk test was used to examine whether the conditions of normality and homogeneity of variance were met by nontransformed data sets as well as transformed data sets. The log-transformed data of soil properties and the gene abundance were subjected one-way ANOVA and the significance of difference between the three different permafrost thaw scenarios was identified using Tukey's HSD test (Tukey 1949). A significant difference was considered at P-values<0.05. Next, the correlations between the abundance of each nitrogen cycle gene and the chemical properties of the soil were tested using Spearman's rank-order correlation (Spearman, 1904). Non-normal data were log-transformed before analysis.

The effects of soil moisture levels on the structure of the bacterial community were assessed with permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001).The relative abundances of zOTUs were used to perform PERMANOVA using the 'adonis' function of the 'Vegan' package with 999 permutations in R (Oksanen et al. 2020). Bray-Curtis distance (Bray and Curtis 1957) was used in these tests. Furthermore, all of the boxplots and bar charts were created using the ‘ggplot2’ R package (Wickham 2016).

3 Results

A total of 56 samples were collected from three sites with permafrost affected soils with different moisture. For each of the permafrost thaw scenario, the soil samples were collected from different depths ranging from 0 to 120 cm. The soils from the three scenarios significantly differed in temperature, pH, moisture, DOC, and DN showed. Total organic carbon, total nitrogen, and moisture were significantly higher in the control scenario compared to the other two scenarios, while the temperature was highest in soil of the dry scenario and the pH in the wet scenario. Generally, the top 15 cm of soil had the highest moisture, DOC and DN. The physical and chemical parameters of the soil samples are shown in Table 1.

Table 1: Selected physical and chemical properties of soil

Scenario	Number of samples	Moisture (%)	pH	Temperature (°C)	DOC (g/g dw)	DN (g/g dw)
Control	15	43.8 (b)	5.8 (c)	0.9 (b)	42.2 (b)	1.4 (b)
Dry	20	19.1 (a)	4.8 (a)	11.7 (a)	14.5 (a)	0.7 (ab)
Wet	21	23.9 (a)	7.6 (b)	1.6 (b)	12.2 (a)	0.4 (a)

The values represent averages of soil samples classified into each permafrost thaw scenario. Letters in brackets indicate significant differences as determined by One-Way ANOVA and Tukey's HSD test.

3.1 Gene abundance

To evaluate the abundance of *nifH* and *nosZ* genes, qPCR analyses were conducted with DNA and cDNA samples. The abundances were then compared between the soil with intact permafrost (control) and the two different scenarios of permafrost thaw with different soil moisture level (dry, wet). Furthermore, the number of gene copies from soils of each scenario was classified according to the soil depth range.

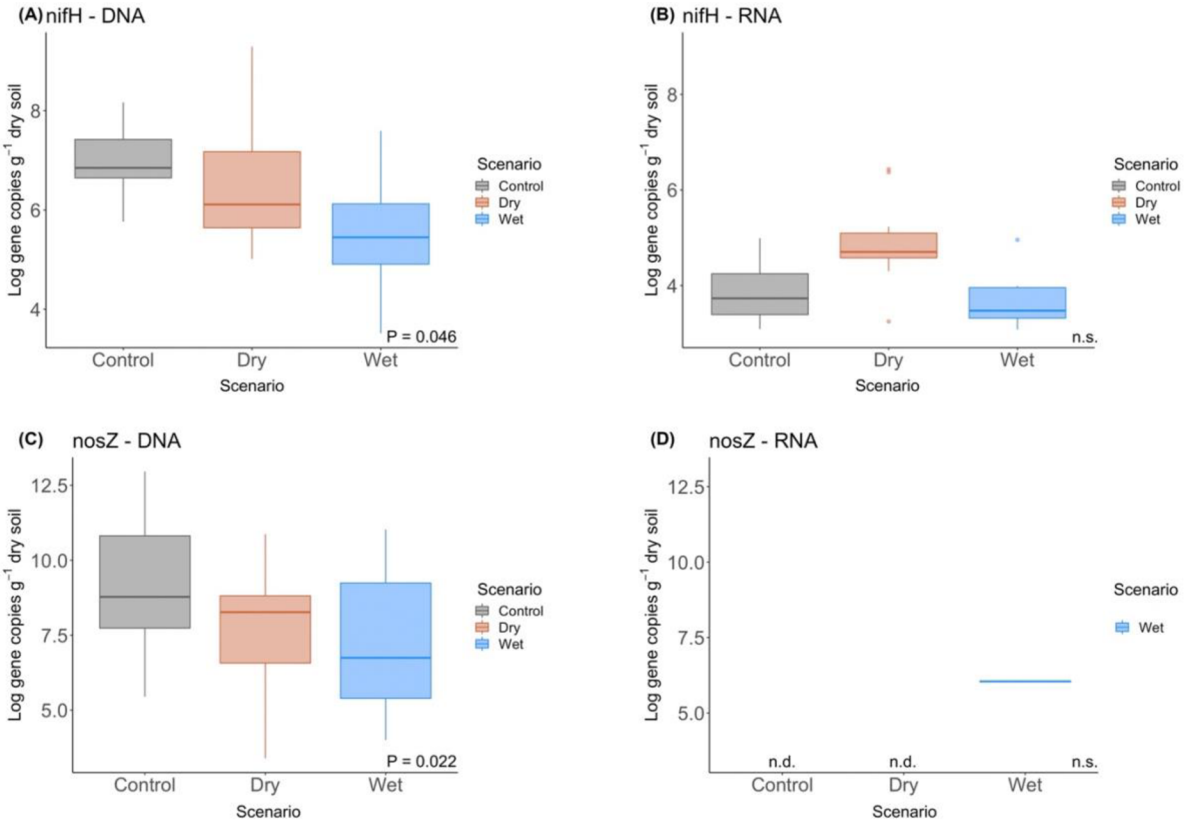

Figure 2: Log10-transformed abundances of nitrogen-fixing (A,B) and denitrifying (C,D) bacteria shown as *nifH* and *nosZ* gene copy numbers per gram dry soil. The n.d. denotes not detected.

The abundance of the average total genes ranged from 3.61×10^6 to 1.43×10^8 copies per gram of dry soil for *nifH* and from 5.87×10^9 to 6.48×10^{11} for *nosZ* within soils of the three different scenarios. Figure 2 shows the gene abundances in soils of the three different scenarios of permafrost thaw. The highest average *nifH* gene abundance was in dry scenario, followed by control and wet scenario (Figure 2A). For *nifH*, the number of active genes was 1×10^3 fold lower on average than the number of total *nifH* harbouring bacteria. The numbers of active *nifH* genes followed a similar pattern, with the highest average abundance present in dry scenario and the lowest number of gene copies in wet scenario (Figure 2B).

Overall, a significant effect of soil water content on the abundance of *nifH* genes was observed (P-value=0.046), nevertheless, the Tukey HSD test did not reveal significant difference between the three scenarios. On the contrary, the abundance of potentially active *nifH* genes did not differ significantly (P-value=0.164).

The *nosZ* genes in the scenarios were on average $4x10^3$ times more abundant than the *nifH* genes. In contrast to *nifH* gene abundance distribution, the highest average *nosZ* gene copies were present in control scenario, followed by dry and wet scenarios (Figure 2C). Active *nosZ* genes were quantified only in one sample from the soil of wet scenario, and accounted for $1.12x10^6$ genes per g^{-1} dry soil (Figure 2D). The values from the other samples were under the detection limit of the qPCR assay. The number of gene copies differed significantly between scenarios (P-value=0.022), with abundance significantly higher in the control scenario.

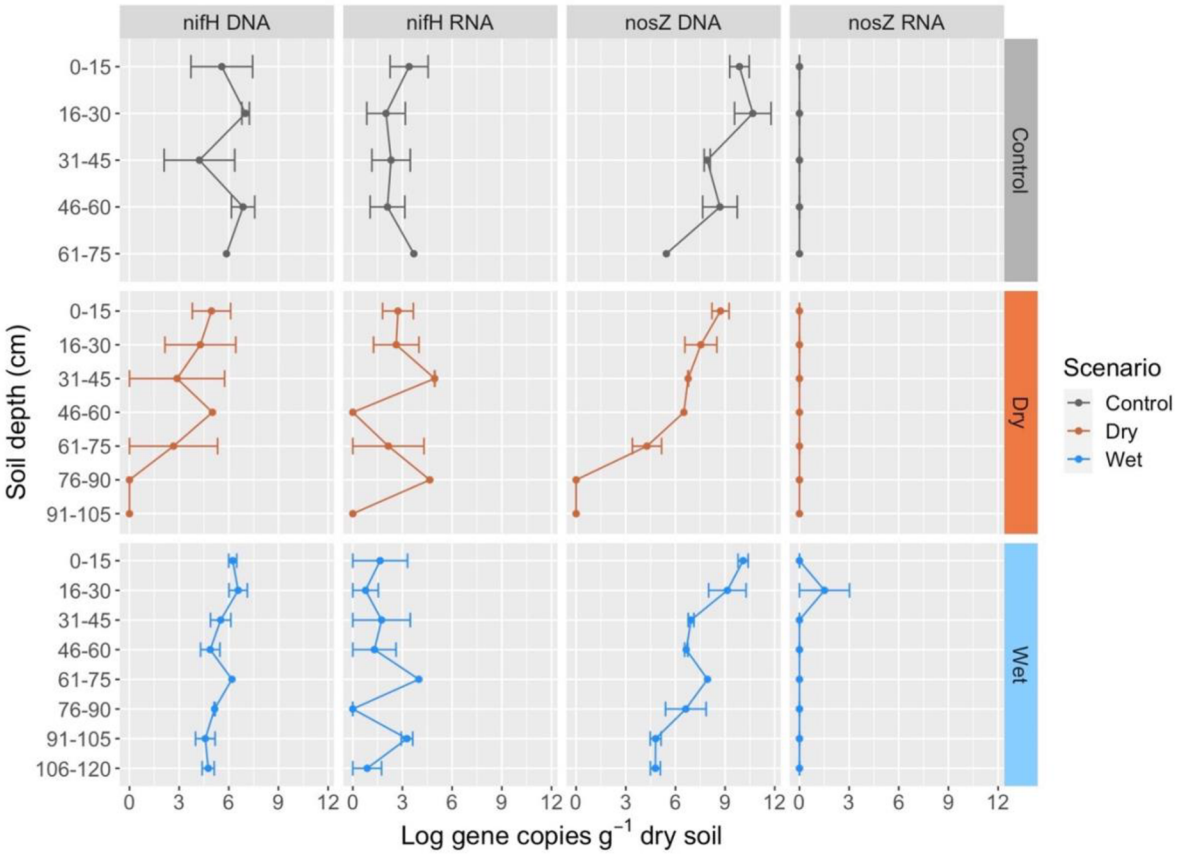

Figure 3: Log10-transformed number of *nifH* and *nosZ* gene copies categorized according to the soil depth range. The points represent averages ± standard errors.

In addition, the depth profiling showed a variation of *nifH* gene abundance ranging from 195.15 to $1.04x10^7$, with the highest average gene copy numbers in the depth range of 16-30 cm (Figure 3). However, the number of gene copy numbers did not follow any trend and there was no significant difference in gene copy numbers in the different soil depth ranges.

The depth profiling of *nosZ* abundance revealed a decreasing trend in the number of genes with increasing depth in the case of DNA-based analysis. Also in this case, no significant impact was observed on the number of gene copies in the different soil depth ranges.

Furthermore, the copy numbers of the *nifH* and *nosZ* genes were correlated with abiotic soil conditions. A significant positive correlation (P-value<0.001) was indicated between dissolved nitrogen (DN), dissolved organic carbon (DOC), and soil moisture. In the case of soil moisture, the *nosZ* gene showed a greater positive correlation than the *nifH* genes. Overall, both *nifH* and *nosZ* show the highest positive correlations with dissolved organic carbon.

Table 2:Correlation coefficients (*r*) of soil properties versus total *nifH* and *nosZ* gene copy numbers

	Moisture	pH	Temperature	DN	DOC
nifH	0.48***	ns	ns	0.46***	0.52***
nosZ	0.64***	-0.32*	ns	0.69***	0.72***

Correlation coefficients are given and their significances are marked by asterisks as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *ns* not significant

3.2 Community structure

We analyzed the composition of the total bacterial community of nitrogen-fixers and denitrifiers in control, dry, and wet scenarios of permafrost thaw by sequencing the 16S rRNA gene. A total of 5826 zOTU were identified with 157 zOTUs assigned to nitrogen-fixing bacteria with the *nifH* gene and with 177 zOTUs assigned to denitrifiers with the *nosZ* gene. The results indicate, that soils with different water content contained similar populations in terms of the numbers of phyla and genera with differences in their abundances among soil types.

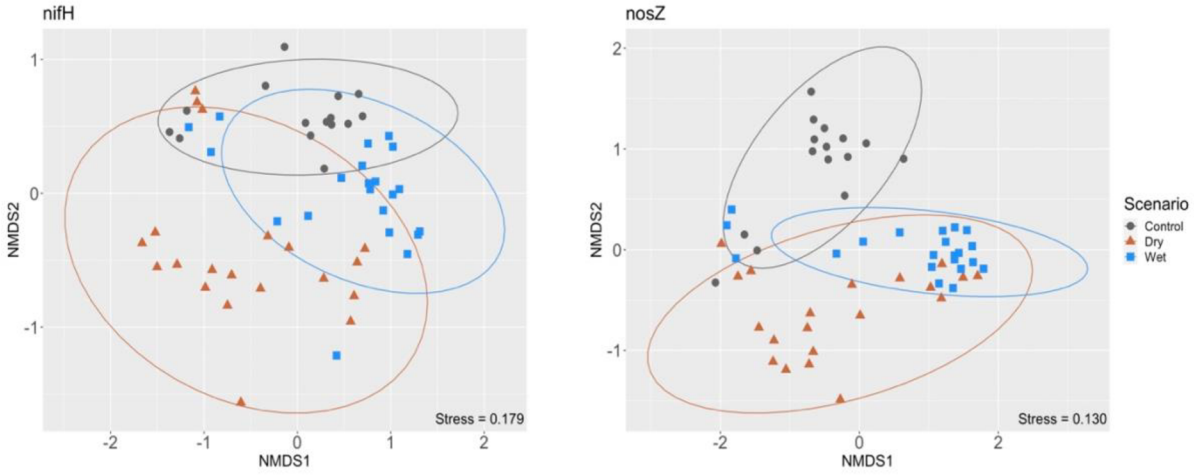

Figure 4: Non-metric multidimensional scaling (NMDS) ordination plot of OTU-level community composition based on Bray-Curtis distance illustrating differences between control, dry and wet scenario. Ellipses indicate 95% confidence intervals.

To test whether the communities in soils of the three different scenarios harbour a different composition, a Permutational multivariate analysis of variance (PERMANOVA) was conducted. The results indicate significant differences in the taxonomic distribution among the soils of the different scenarios for both the *nifH* (P-value=0.001) and *nosZ* (P-value=0.001) communities. The non-metric multidimensional scaling (NMDS) ordination of the zOTUs confirmed the differences in the composition of bacterial communities between the three scenarios (Figure 4).

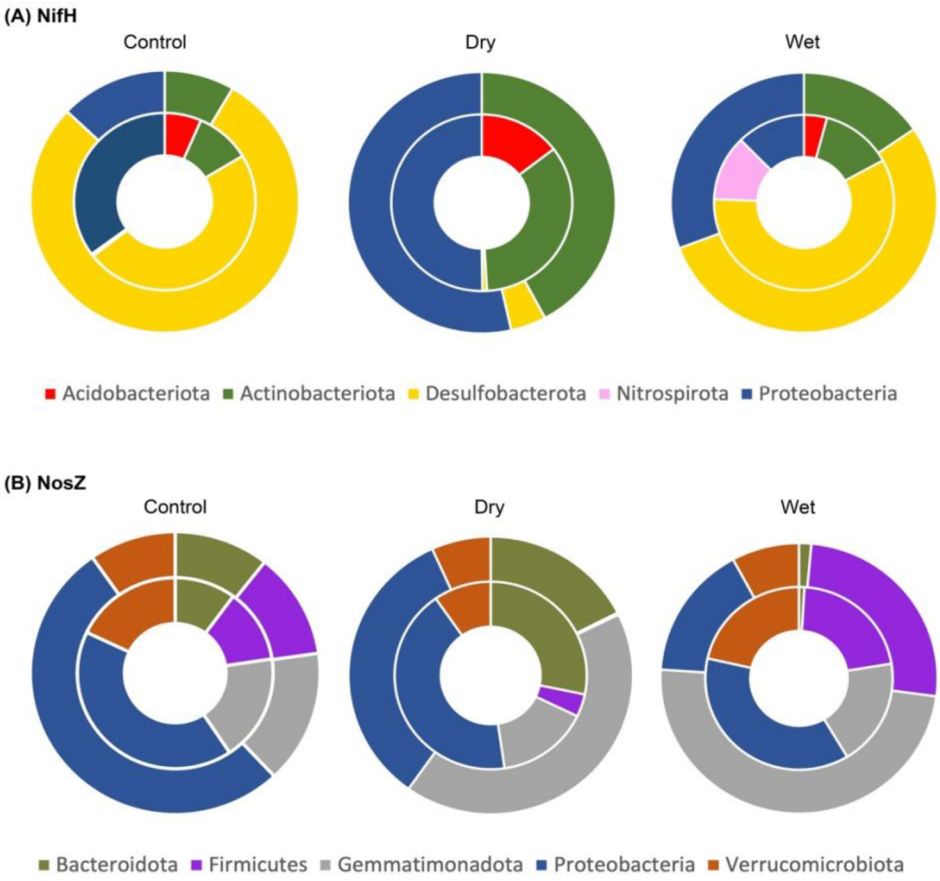

Figure 5: Relative abundances of phyla of *nifH* **(A)** and *nosZ* **(B)** harbouring bacterial community between the three different scenarios. Outer rings of the charts show the total community composition and inner rings represent the composition of the active bacterial community

The relative abundances of the phyla detected in each scenario varied between DNA- and RNA-based analysis. The nitrogen-fixer community was distributed within the phyla *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Desulfobacterota*, and *Nitrospirota* (Figure 5A). The dominant phylum in control and wet scenarios was *Desulfurobacterota* (control-78.6% DNA, 48.1% RNA; wet-53.9% DNA, 58.6% RNA), while the prevalent phylum in dry scenario was *Proteobacteria* (53.5% DNA; 50.1% RNA). Furthermore, members of the phyla *Nitrospirota* and *Acidobacteria* were present only among the active bacterial community.

The community of denitrifying bacteria comprised of the phyla *Proteobacteria*, *Bacteroidota*, *Firmicutes*, *Gemmatimonadota*, *Proteobaceria*, and *Verrucomicrobiota* (Figure 5B). The most abundant phylum in both dry and wet scenarios was *Gemmatimonadotas* (dry – 42.0% DNA, 15.6% RNA; wet – 48.9% DNA, 19.9% RNA), while Proteobacteria showed the highest abundance in control scenario (52.3% DNA; 41.4% RNA).

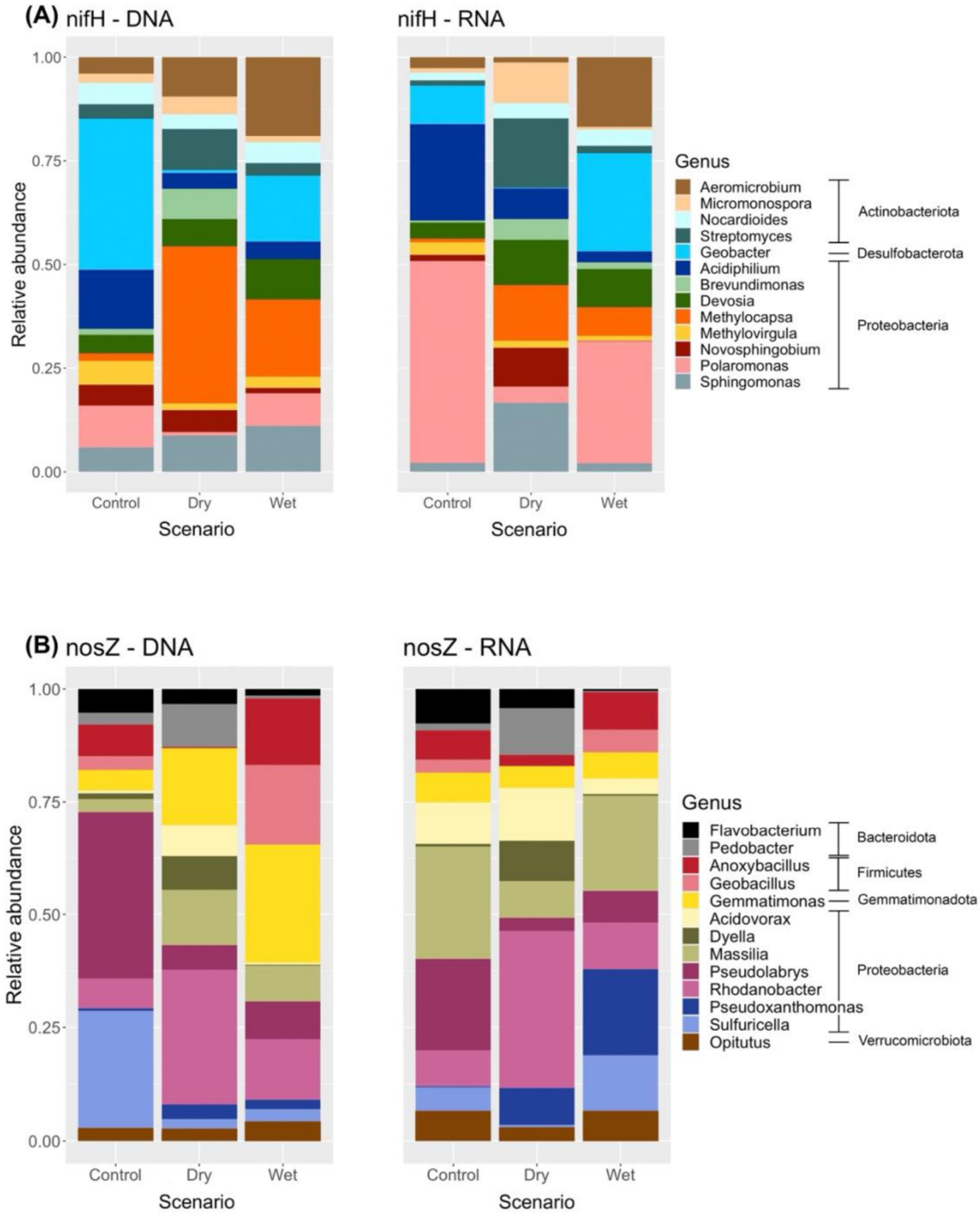

Figure 6: Relative abundances of major genera of *nifH* **(A)** and *nosZ* **(B)** harbouring bacterial community between the three different scenarios. Left panels show the total community composition and right panels show the composition of the active bacterial community.

The results of genus level analysis indicate, that both *nifH* and *nosZ* communities have similar genus diversity with differences in their abundance between the three different scenarios. Among nitrogen-fixing bacteria with the *nifH* gene, the three most abundant genera were *Methylocapsa*, *Geobacter* and *Aeromicrobium* with a relative abundance of 16.1%, 14.2%, and 9.1%, respectively.

However, their abundance fluctuated depending on the variation in soil moisture. The genus *Nocardioides* showed similar abundances in the total and active communities. The differences in relative abundance of genera between the three scenarios are shown in Figure 6A. The top ten most abundant genera in dry scenario also included specimens of the genus *Brevundimonas,* and wet scenario exhibited the presence of genera *Beijerinckia*. In terms of activity, the genus with psychrotolerant and psychrophilic species *Polaromonas* dominated in control and wet scenarios among nitrogen fixers, with relative abundances of 43.9% and 25.6%.

In the community of denitrifiers with *nosZ* gene, *Pseudolabrys*, *Rhodanobacter* and *Gemmatimonas* represented the three dominant genera, with relative abundances of 15.3%, 14.8%, and 14.3%, respectively. The most abundant genera of dry scenario also include *Duganella,* and in wet scenario, the genus *Brevibacillus* is fairly represented. The prevailing genus of RNA-based analysis was *Massilia* in control and wet scenarios, and *Rhodanobacter* in dry scenario (Figure 6B).

4 Discussion

4.1 Abundance of nitrogen-fixing and denitrifying bacteria

The soil moisture content is considered a factor of great importance related to nitrogen fixation and denitrification (Denardin et al. 2020). However, soil moisture is never stable in the natural environment. It has been the subject of focus of several former arctic studies such as of Rousk et al. (2018), Lawrence et al. (2015), and Walwoord and Kurylyk (2016).

As demonstrated in the results, our hypothesis of a higher abundance of nitrogen-fixing bacteria in the wet scenario was disproven. Instead, bacteria with the *nifH* gene showed a higher abundance in dry scenario, contrary to the results of other studies (Rousk, Sorensen, and Michelsen 2018; Zielke et al. 2005). This result can be explained by inspecting the community composition which shows that more of the nitrogen-fixing bacteria in dry scenario are from genera that contain mainly aerobic species. Another reason for a higher abundance of nitrogen fixers in the dry scenario was the soil temperature, which was higher on average than in the other two soil types. The temperature ranged from 8.5 to 14.3°C, with the latter approaching the estimated optimum temperature of 15-30°C for N_2 fixation in the Arctic. Above 10°C, the nitrogen fixation rates were shown to increase rapidly (Hobara et al. 2006). As a consequence, in the dry scenario of permafrost thaw, more nitrogen may be fixed and cause a higher nitrogen influx to permafrost affected soils. Nitrogen is a limiting nutrient in subarctic ecosystems, therefore, increased N availability would further stimulate microbial metabolism and degradation of frozen SOM (Altshuler et al. 2019). This may result in higher emissions of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O).

The number of gene copies of bacteria with *nosZ* was significantly higher in the control scenario. This result is in line with the study of Altshuler et al. (2019), which indicated a higher abundance of denitrifiers in soils with higher soil moisture level. Furthermore, the control scenario appears to contain higher amounts of dissolved inorganic nitrogen. According to the study by Kemp and Dodds (2002), higher levels of nitrate were shown to stimulate denitrification; thus, this factor may also contribute to the increased abundance of *nosZ* in the control scenario. Regarding the activity of the *nosZ* gene harbouring bacteria, the *nosZ* genes were quantified only in one sample. Hence, these results suggest a potential for incomplete denitrification in permafrost soils, which would lead to a lower amount of N_2O being

converted to N_2 and cause higher N_2O emissions to the atmosphere, especially if enhanced nitrogen fixation in the dry scenario would increase the overall rate of nitrogen cycling.

Nevertheless, the overall abundances of total and potentially active *nifH* and *nosZ* gene of microbial communities estimated by qPCR have suggested that the size of active microbial communities does not respond significantly to changes in soil moisture (Stres et al. 2008). These findings may likely result from the fact that the activity of bacteria shows both temporal and spatial fluctuations. The nitrogen fixation and denitrification rates also show great temporal variability with seasonal variation in moisture, temperature, and light (Solheim et al. 2007). The soil characteristics also exhibit great spatial variability and heterogeneity (Stres et al. 2008). These factors make it difficult to accurately assess the importance of different environmental factors in the abundance of bacteria. Hence, further quantification of the input and output of N through the nitrogen fixation and denitrification processes in Arctic landscapes would provide more precise results.

4.2 Bacterial communities in soils of different permafrost thaw scenarios

As permafrost in the Arctic thaws, different conditions for the life of bacteria arise as a result of altered moisture levels in soils. A higher water content creates conditions unfavourable to aerobic bacteria and also increases the reducing potential of soils. In contrast, the dry scenario would create oxygen-rich conditions in which aerobic bacteria will thrive. Therefore, permafrost thaw can result in a change in microbial community structure and activity (Kim et al. 2008).

The numbers of phyla and genera of nitrogen fixing and denitrifying bacteria were similar in all of the scenarios. The bacteria with *nifH* gene were represented by 20 genera, while the *nosZ* bacterial community was represented by 18 genera. Our hypothesis, according to which the soil moisture level could be an important determinant of the community structure, was supported for both nitrogen-fixing and denitrifying bacteria. PERMANOVA analysis demonstrated that the soils with different moisture level significantly influenced the taxonomic distribution of the *nifH* (F-value=6.560, P-value=0.001) and *nosZ* (F-value=9.694, P-value=0.001) gene harbouring community. At the phylum level, the dominance of *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, and *Gemmatimonades* is in accordance with previous studies by Schostag et al. (2015).

The analysis at the genus level revealed a great variation in relative abundances between the different scenarios and enabled to point out genera with interesting life strategies and possible biotechnological uses.

The Gram-negative genera of bacteria identified in the samples, such as *Burkholderia*, *Pedobacter*, *Duganella*, *Dyella*, *Pseudomonas,* and *Sphingomonas,* are typical members of the arctic soil microbial communities (Margesin 2008). Our findings also confirm the results of earlier studies (Li et al. 2021; Siebielec et al. 2020), namely the increase of relative abundance of the phyla *Verrucomicrobiota* and decrease of *Acidobacteria*, *Actinobacteria,* and *Bacteroidota* with increasing soil moisture. The genus of nitrogen-fixing bacteria *Nocardioides* remained at a relatively similar level of abundance regardless of scenario, which is in accordance with the results of Siebielec et al. (2020). Among the genera of nitrogen-fixing bacteria with *nifH* gene, the genus *Methylocapsa* was also present. *Methylocapsa* are methane oxidizing bacteria able to metabolize significant amounts of methane produced by methanogens in deeper anoxic layers of cryosols, and act as a biological sink for this greenhouse gas (Tveit et al. 2019). This genus encompasses at least 98 published species that are Gram-positive, aerobic, mesophilic, and have a broad habitat adaptability (Chen et al. 2020). Additionally, the members of *Methylocapsa* along with *Acidovorax* were particularly sensitive to high soil moisture. In contrast, the relative abundance of *Geobacter* and *Polaromonas* increased with increasing soil moisture level. Bacteria of the former genus are known to possess unusual electron transfer capabilities in the form of electrically conductive protein nanowires and were also found to be effective in the bioremediation of organic and metal contaminants in subsurface environments (Lovley and Walker 2019).

The genera with psychrotolerant and psychrophilic species, such as *Polaromonas, Acidovorax, Massilia,* and *Sphingomonas* dominated among the active community of nitrogen-fixers and denitrifiers. This could probably be caused by their adaptation to low temperatures in subarctic environments. Psychrophiles are capable of biodegradation of organic contaminants, including petroleum and phenols, and they have been used to treat petroleum-contaminated sites in Alaska, Canada, Greenland, or Norway (Margesin and Feller 2010). The cold adapted hydrocarbon-degraders contain monooxygenases and dioxygenases, the key enzymes in the aerobic degradation of broad range of environmental pollutants (Margesin and Feller 2010). Two bacterial species able to degrade organic contaminants were revealed by inspecting the species-level of the taxonomic classification, and namely the *Sphingomonas polyaromaticivorans* and *Sphingomonas fennica*.

The genera *Anoxybacillus* and *Geobacillus,* which are predominantly thermophilic (i.e. bacteria with optimum growth temperatures of 50-70 °C) were present in the total and surprisingly even among active genera. The presence of these bacteria in Arctic soils has already been reported (Boyd, Onn, and Boyd 1990). The origin of thermophilic bacteria in cold environments remains unclear, although some studies point to the existence of both short- and long-distance dispersal from hot environments by wind or water currents (Hubert et al. 2009) or the possibility of onsite speciation and adaptation (Portillo, Santana and Gonzalez 2012).

The differences in the community structure as well as the gene abundance of total and active bacteria also revealed that the widely used DNA-based approach may generate unreliable results, which overestimate or underestimate the number of active bacteria in the community.

5 Conclusion

This study provides comprehensive information about the abundance of nitrogen-fixing bacteria with *nifH* gene and denitrifiers with the *nosZ* gene and their bacterial community structure. The abundance of these two bacterial functional guilds varied significantly between the three different scenarios of permafrost thaw, with the highest abundance of *nifH* genes in dry scenario, while *nosZ* in control scenario. The abundance of active bacteria did not significantly vary with different soil moisture. In the case of the *nosZ* gene, active genes were quantified only in one sample. This finding may indicate an incomplete conversion of N_2O to N_2, which would subsequently lead to an increase in N_2O emissions. Furthermore, a significant difference was observed between the three scenarios for both the *nifH* and *nosZ* harbouring bacterial community structure. Variations between the total and active bacterial community structure as well as between gene abundances were also demonstrated. These results highlight the importance of performing both DNA- and RNA-based analysis to obtain a more comprehensive characterization of the active bacterial community.

6 Literature

Agnan, Yannick et al. 2019. "Distribution of Trace and Major Elements in Subarctic Ecosystem Soils: Sources and Influence of Vegetation." *Science of the Total Environment* 682: 650–62.

Altshuler, Ianina et al. 2019. "Denitrifiers, Nitrogen-Fixing Bacteria and N2O Soil Gas Flux in High Arctic Ice-Wedge Polygon Cryosols." *FEMS Microbiology Ecology* 95(5).

Altshuler, Ianina, Jacqueline Goordial, and Lyle G. Whyte. 2017. "Microbial Life in Permafrost." In *Psychrophiles: From Biodiversity to Biotechnology: Second Edition*, Springer International Publishing, 153–79.

Andresen, Christian G. et al. 2020. "Soil Moisture and Hydrology Projections of the Permafrost Region – a Model Intercomparison." *The Cryosphere* 14(2): 445–59.

Biskaborn, Boris K. et al. 2019. "Permafrost Is Warming at a Global Scale." *Nature Communications* 10(1).

Boggs, Keith et al. 2019. *Alaska Ecosystems of Conservation Concern: Biophysical Settings and Plant Associations*.

Borowik, A., and J. Wyszkowska. 2016. "Soil Moisture as a Factor Affecting the Microbiological and Biochemical Activity of Soil." *Plant, Soil and Environment* 62(6): 250–55.

Boyd, William L., David R. Onn, and Josephine W. Boyd. 1990. "Thermophilic Bacteria among Arctic, Subarctic, and Alpine Habitats." *Arctic and Alpine Research* 22(4): 401.

Canion, Andy et al. 2013. "Isolation and Physiological Characterization of Psychrophilic Denitrifying Bacteria from Permanently Cold Arctic Fjord Sediments (Svalbard, Norway)." *Environmental Microbiology* 15(5): 1606–18.

Caporaso, J Gregory et al. 2010. "QIIME Allows Analysis of High-Throughput Community Sequencing Data." *Nature Methods* 7(5): 335–36.

Caporaso, J. Gregory et al. 2011. "Global Patterns of 16S RRNA Diversity at a Depth of Millions of Sequences per Sample." *Proceedings of the National Academy of Sciences* 108(supplement_1): 4516–22.

Chen, Ping, Yiran Fu, Yi Cai, and Zhanglin Lin. 2020. "Nocardioides Guangzhouensis Sp. Nov., an Actinobacterium Isolated from Soil." *International Journal of Systematic and Evolutionary Microbiology* 70(1): 112–19.

D'Amico, Salvino et al. 2006. "Psychrophilic Microorganisms: Challenges for Life." *EMBO reports* 7(4): 385–89.

Denardin, Luiz Gustavo de Oliveira et al. 2020. "How Different Soil Moisture Levels Affect the Microbial Activity of a Lowland Soil Used by Paddy Fields?" *Ciencia Rural* 50(6).

Di, Hong J., Keith C. Cameron, Andriy Podolyan, and Aimee Robinson. 2014. "Effect of Soil Moisture Status and a Nitrification Inhibitor, Dicyandiamide, on Ammonia Oxidizer and Denitrifier Growth and Nitrous Oxide Emissions in a Grassland Soil." *Soil Biology and Biochemistry* 73: 59–68.

Edgar, Robert C. 2013. "UPARSE: Highly Accurate OTU Sequences from Microbial Amplicon Reads." *Nature Methods* 10(10): 996–98.

Elberling, Bo, Hanne H. Christiansen, and Birger U. Hansen. 2010. "Erratum: High Nitrous Oxide Production from Thawing Permafrost." *Nature Geoscience* 3(7): 506–506.

Ernakovich, Jessica G., and Matthew D. Wallenstein. 2015. "Permafrost Microbial Community Traits and Functional Diversity Indicate Low Activity at in Situ Thaw Temperatures." *Soil Biology and Biochemistry* 87: 78–89.

Fish, Jordan A. et al. 2013. "FunGene: The Functional Gene Pipeline and Repository." *Frontiers in Microbiology* 4.
Gaby, John Christian, and Daniel H. Buckley. 2012. "A Comprehensive Evaluation of PCR Primers to Amplify the NifH Gene of Nitrogenase." *PLoS ONE* 7(7): e42149.
Grzyb, Aleksandra, Agnieszka Wolna-Maruwka, and Alicja Niewiadomska. 2021. "The Significance of Microbial Transformation of Nitrogen Compounds in the Light of Integrated Crop Management." *Agronomy* 11(7).
Harden, Jennifer W. et al. 2012. "Field Information Links Permafrost Carbon to Physical Vulnerabilities of Thawing." *Geophysical Research Letters* 39(15).
Henry, S. et al. 2006. "Quantitative Detection of the NosZ Gene, Encoding Nitrous Oxide Reductase, and Comparison of the Abundances of 16S RRNA, NarG, NirK, and NosZ Genes in Soils." *Applied and Environmental Microbiology* 72(8): 5181–89.
Hicks Pries, Caitlin E., E. A.G. Schuur, Jason G. Vogel, and Susan M. Natali. 2013. "Moisture Drives Surface Decomposition in Thawing Tundra." *Journal of Geophysical Research: Biogeosciences* 118(3): 1133–43.
Hobara, Satoru & Mccalley, Carmody & Koba, Keisuke & Giblin, Anne & Weiss, Marissa & Gettel, Gretchen & Shaver, Gaius. (2006). Nitrogen Fixation in Surface Soils and Vegetation in an Arctic Tundra Watershed: A Key Source of Atmospheric Nitrogen. Arctic Antarctic and Alpine Research - ARCT ANTARCT ALP RES. 38. 363-372. 10.1657/1523-0430(2006)38[363:NFISSA]2.0.CO;2.
Hubert, Casey et al. 2009. "A Constant Flux of Diverse Thermophilic Bacteria into the Cold Arctic Seabed." *Science* 325(5947): 1541–44.
Hultman, Jenni et al. 2015. "Multi-Omics of Permafrost, Active Layer and Thermokarst Bog Soil Microbiomes." *Nature* 521(7551): 208–12.
Kim, Seon-Young et al. 2008. "Comparative Analysis of Soil Microbial Communities and Their Responses to the Short-Term Drought in Bog, Fen, and Riparian Wetlands." *Soil Biology and Biochemistry* 40(11): 2874–80.
Kits, K. Dimitri et al. 2019. "Low Yield and Abiotic Origin of N 2 O Formed by the Complete Nitrifier Nitrospira Inopinata." *Nature Communications* 10(1).
Lawrence, D. M. et al. 2015. "Permafrost Thaw and Resulting Soil Moisture Changes Regulate Projected High-Latitude CO2 and CH4 Emissions." *Environmental Research Letters* 10(9).
Li, Haoran et al. 2021. "Irrigation Has a Higher Impact on Soil Bacterial Abundance, Diversity and Composition than Nitrogen Fertilization." *Scientific Reports* 11(1).
Lovley, Derek R., and David J.F. Walker. 2019. "Geobacter Protein Nanowires." *Frontiers in Microbiology* 10.
Madigan, Michael et al. 2019. *Brock Biology of Microorganisms*. 15th ed. Pearson.
Malard, Lucie A., and David A. Pearce. 2018. "Microbial Diversity and Biogeography in Arctic Soils." *Environmental Microbiology Reports* 10(6): 611–25.
Margesin, Rosa. 2008. *Psychrophiles: From Biodiversity to Biotechnology*. eds. Rosa Margesin, Franz Schinner, Jean-Claude Marx, and Charles Gerday. Berlin, Heidelberg: Springer Berlin Heidelberg.
Margesin, Rosa, and Georges Feller. 2010. "Biotechnological Applications of Psychrophiles." *Environmental Technology* 31(8–9): 835–44.
Ortiz, Max et al. 2020. "Microbial Nitrogen Cycling in Antarctic Soils." *Microorganisms* 8(9): 1–17.
Osterkamp, T.E., and C.R. Burn. 2015. "Cryosphere: Permafrost." In *Encyclopedia of Atmospheric Sciences*, Elsevier, 208–16.
Pascual, Didac et al. 2021. "The Missing Pieces for Better Future Predictions in Subarctic Ecosystems: A Torneträsk Case Study." *Ambio* 50(2): 375–92.

Peel, M. C., B. L. Finlayson, and T. A. McMahon. 2007. "Updated World Map of the Köppen-Geiger Climate Classification." *Hydrology and Earth System Sciences* 11(5): 1633–44.
Penton, C. Ryan et al. 2016. "NifH-Harboring Bacterial Community Composition across an Alaskan Permafrost Thaw Gradient." *Frontiers in Microbiology* 7(NOV).
Portillo, M. C., M. Santana, and J. M. Gonzalez. 2012. "Presence and Potential Role of Thermophilic Bacteria in Temperate Terrestrial Environments." *Naturwissenschaften* 99(1): 43–53.
Ramm, Elisabeth et al. 2020. "The Forgotten Nutrient—The Role of Nitrogen in Permafrost Soils of Northern China." *Advances in Atmospheric Sciences* 37(8): 793–99.
Reed, Sasha. 2011. "Functional Biology of Heterotrophic Nitrogen-Fixing Bacteria." *Annual Review of Ecology, Evolution, and Systematics* 42.
Rich, J. J. et al. 2003. "Community Composition and Functioning of Denitrifying Bacteria from Adjacent Meadow and Forest Soils." *Applied and Environmental Microbiology* 69(10): 5974–82.
Rousk, Kathrin, Pernille Laerkedal Sorensen, and Anders Michelsen. 2018. "What Drives Biological Nitrogen Fixation in High Arctic Tundra: Moisture or Temperature." *Ecosphere* 9(2).
Salmon, Verity G. et al. 2018. "Adding Depth to Our Understanding of Nitrogen Dynamics in Permafrost Soils." *Journal of Geophysical Research: Biogeosciences* 123(8): 2497–2512.
Schaeffer, Sean M., Elizabeth Sharp, Joshua P. Schimel, and Jeffery M. Welker. 2013. "Soil-Plant N Processes in a High Arctic Ecosystem, NW Greenland Are Altered by Long-Term Experimental Warming and Higher Rainfall." *Global Change Biology* 19(11): 3529–39.
Schimel, Joshua P., and Jennifer Bennett. 2004. "Nitrogen Mineralization: Challenges of a Changing Paradigm." *Ecology* 85(3): 591–602.
Schostag, Morten et al. 2015. "Distinct Summer and Winter Bacterial Communities in the Active Layer of Svalbard Permafrost Revealed by DNA- and RNA-Based Analyses." *Frontiers in Microbiology* 6.
Schuur, E. A. G. et al. 2015. "Climate Change and the Permafrost Carbon Feedback." *Nature* 520(7546): 171–79.
Siebielec, Sylwia et al. 2020. "Impact of Water Stress on Microbial Community and Activity in Sandy and Loamy Soils." *Agronomy* 10(9).
Solheim, Bjørn, and Matthias Zielke. "Associations Between Cyanobacteria and Mosses." In *Cyanobacteria in Symbiosis*, Dordrecht: Kluwer Academic Publishers, 137–52.
Steven, Blaire, Wayne H. Pollard, Charles W. Greer, and Lyle G. Whyte. 2008. "Microbial Diversity and Activity through a Permafrost/Ground Ice Core Profile from the Canadian High Arctic." *Environmental Microbiology* 10(12): 3388–3403.
Stewart, Katherine J., Darwyn Coxson, and S. D. Siciliano. 2011. "Small-Scale Spatial Patterns in N2-Fixation and Nutrient Availability in an Arctic Hummock-Hollow Ecosystem." *Soil Biology and Biochemistry* 43(1): 133–40.
Stewart, Katherine J., Paul Grogan, Darwyn S. Coxson, and Steven D. Siciliano. 2014. "Topography as a Key Factor Driving Atmospheric Nitrogen Exchanges in Arctic Terrestrial Ecosystems." *Soil Biology and Biochemistry* 70: 96–112.
Stres, Blaž et al. 2008. "Influence of Temperature and Soil Water Content on Bacterial, Archaeal and Denitrifying Microbial Communities in Drained Fen Grassland Soil Microcosms." In *FEMS Microbiology Ecology*, , 110–22.

Taş, Neslihan et al. 2014. “Impact of Fire on Active Layer and Permafrost Microbial Communities and Metagenomes in an Upland Alaskan Boreal Forest.” *The ISME Journal* 8(9): 1904–19.
Thompson, Luke R. et al. 2017. “A Communal Catalogue Reveals Earth’s Multiscale Microbial Diversity.” *Nature* 551(7681): 457–63.
Tveit, Alexander T. et al. 2019. “Widespread Soil Bacterium That Oxidizes Atmospheric Methane.” *Proceedings of the National Academy of Sciences of the United States of America* 116(17): 8515–24.
van Everdingen, R. O. (ed.), 1998. Multi-Language Glossary of Permafrost and Related Ground-ice Terms. International Permafrost Association. The Arctic Institute of North America, University of Calgary, Calgary, 268 pp.
Walvoord, Michelle A., and Barret L. Kurylyk. 2016. “Hydrologic Impacts of Thawing Permafrost-A Review.” *Vadose Zone Journal* 15(6): vzj2016.01.0010.
Wilhelm, Roland C., Thomas D. Niederberger, Charles Greer, and Lyle G. Whyte. 2011. “Microbial Diversity of Active Layer and Permafrost in an Acidic Wetland from the Canadian High Arctic.” *Canadian Journal of Microbiology* 57(4): 303–15.
Yergeau, Etienne, Hervé Hogues, Lyle G Whyte, and Charles W Greer. 2010. “The Functional Potential of High Arctic Permafrost Revealed by Metagenomic Sequencing, QPCR and Microarray Analyses.” *The ISME Journal* 4(9): 1206–14.
Zielke, Matthias, Bjørn Solheim, Sigmund Spjelkavik, and Rolf A. Olsen. 2005. “Nitrogen Fixation in the High Arctic: Role of Vegetation and Environmental Conditions.” *Arctic, Antarctic, and Alpine Research* 37(3): 372–78.
Zubrzycki, S., L. Kutzbach, and E. M. Pfeiffer. 2014. “Permafrost-Affected Soils and Their Carbon Pools with a Focus on the Russian Arctic.” *Solid Earth* 5(2): 595–609.