Anthropogenic disturbance alters the microbial biodiversity of permafrost soils

by

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Abstract

Anthropogenic climate change and increasing industrial activity is impacting Northern Canada and accelerating permafrost thaw. While research into the impact of permafrost thaw on microbial community dynamics is burgeoning, there has been little investigation into how human activities alter the resident microbial communities of permafrost. To examine the effect of anthropogenically-induced permafrost thaw on living microbial communities, I surveyed a site where permafrost thaw was induced by stripping the area's vegetation and topsoil in preparation for gold mining near Dominion Creek, Yukon, Canada. I analysed a set of permafrost cores, as well as surface soil samples, across a disturbance gradient from undisturbed forest active layer to disturbed soils, composed of recently thawed permafrost, to a newly formed thermokarst pond. I identified three distinct community groupings within the dataset: (1) undisturbed active layer, (2) lower active layer, disturbed active layer, and disturbed permafrost, and (3) intact permafrost. These groupings indicate that disturbance alters the microbial community of surface soils. Biotic interactions drove differences across these groupings, while within group variation was controlled primarily by pH. This study suggests a strong microbial community response to anthropogenic permafrost disturbance under field conditions and that this response occurs prior to shifts in the measured soil edaphic parameters. Both anthropogenic and natural disturbances to permafrost may induce significant microbial community changes, impacting carbon budgets and carbon feedbacks in permafrost-affected soils.

Preface

This thesis is an original work by Patrick Neuberger. No part of this thesis has been previously published.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Literature Review

1.1 Introduction

Permafrost is soil which has been below 0 ℃ for at least two years and is overlain by a seasonally thawed active layer (Dobinski, 2011). Approximately 50% of the world's soil organic carbon is locked in permafrost, while only 16% of Earth's land area is underlain by permafrost (Tarnocai et al., 2009). The Arctic is at particular risk of warming due to polar amplification wherein average surface temperatures in the Arctic are increasing at twice the global average (Richter-Menge et al., 2016). Yukon surface temperatures have risen by ~2 ℃ over the last 50 years and, combined with annual permafrost temperatures ranging between 0 and -2 ℃, this region is at particularly risk of extensive permafrost thaw (Smith et al., 2010). Additionally, milder temperatures are set to be accompanied by increasing tourism and industry in circumpolar regions (Elias, 2014).

Permafrost thaw is complex and dynamic. Thaw initiation events are the sum of both localized anthropogenic events, which remove insulating surface soil and vegetation, and climate change, altering mean annual precipitation and mean annual temperature (Lin et al., 2016). Removal of the active layer increases the rate of permafrost thaw by removing the insulating cover of active layer soils (Lin et al., 2016). Anthropogenic disturbance, most notably through excavation, will enhance the impacts of increasing Arctic temperatures. As permafrost soils thaw, increased microbial activity and degradation of both preserved carbon and recently deposited carbon can lead to the production and release of potent greenhouse gases (*e.g.* Cooper et al., 2017; Deng et al., 2015; Schaefer et al., 2011; Schuur et al., 2015

…). Exposure to atmospheric and terrestrial cycling upon disturbance and thaw will allow for the exchange of microorganisms, nutrients, and greenhouse gases from permafrost (Jansson & Taş, 2014). Soil processes are intimately controlled by microbial community structure and diversity, and so the changing permafrost microbiome requires comprehensive examination to predict future soil function.

A large amount of soil organic carbon is sequestered from biogeochemical cycling in deep frozen permafrost. However, much of this locked carbon is vulnerable to release upon permafrost thaw through microbial mineralization. Approximately 120-160 Pg of carbon is vulnerable to mineralization over the next century, representing one of the most substantial biospheric inputs of carbon to the atmosphere in recent history, resulting in an additional 0.13-0.26 °C of warming by 2100 (Schuur et al., 2015). Carbon release from permafrost is projected to be on a similar scale as land-use processes (totalling 2.2 Pg C yr^{-1}, including logging, fuelwood harvest, cultivation, soils, and deforestation), but permafrost emissions will represent a significant source of methane, thereby contributing as much as 2.5 times more to climate change (Baccini et al., 2012; Schuur & Abbott, 2011). Thaw of permafrost can result in active layer thickening or abrupt thaw events, such as retrogressive thaw slumps and other thermokarst formations, which can remove active layer soil horizons and thereby alter soil chemistry, biodiversity, carbon balance and thermal regime of soils in the Arctic (Streicker, 2016). The impact of thermokarst formation on carbon cycling is poorly understood. While gas flux and geochemistry of abrupt thaw sites are becoming increasingly studied, there is a lack of information regarding the resident microbial community which control these processes (Abbott & Jones, 2015; Loiko et al., 2017; Mu et al., 2017; Mu et al., 2018). Biodiversity and assembly in these changing periglacial systems will identify whether

these systems are susceptible to future perturbation and how biogeochemical cycling is changing in permafrost soils.

Microbial communities undertake essential processes for biogeochemical cycling. These processes can include those involved in carbon cycling (methanogenesis, methane oxidation, respiration, fermentation, carbon fixation, organic carbon consumption and production), nitrogen cycling (nitrogen fixation, NO and N_2O oxidation), phosphorus turnover, sulfate turnover, as well as numerous others (Fierer, 2017). Of particular importance in northern soils is the degradation of vast amounts of stored and deposited carbon. Permafrost soils harbour microorganisms responsible for methane oxidation, methanogenesis, and organic carbon decomposition (Coolen & Orsi, 2015; Hultman et al., 2015; Mackelprang et al., 2011; Singleton et al., 2018; Woodcroft et al., 2018). Understanding how the function of these microbiomes turnover is essential to future carbon budgeting in northern soils.

Microorganisms of permafrost are adapted to and capable of growth in persistently cold temperatures, but responses of these communities to warming temperatures are largely unknown (Hansen et al., 2007; Hultman et al., 2015; Tuorto et al., 2014). Laboratory experiments have shown that the microbial community of permafrost shifts to become similar to communities of the active layer with rapid thaw (Coolen & Orsi, 2015; Mackelprang et al., 2011), while *in situ* warming experiments have shown little to no change with permafrost thaw (Biasi et al., 2008; Lamb et al., 2011; Rinnan et al., 2008). Interpretations of current climate models are further muddled by the use of laboratory thaw conditions to predict current and future greenhouse gas fluxes (Schuur et al., 2015; van Huissteden & Dolman, 2012). If microbial community dynamics are not replicated *in situ*, it is also unknown if

laboratory thaw experiments are relevant to environmental systems. With increasing importance of abrupt thaw in northern landscapes, decidedly little has been done to characterize the microbial communities of these thawing permafrost soils.

I aimed to characterize how the microbial community of permafrost changes in response to anthropogenic disturbance. This research could have implications regarding the changing structure of microbial communities in response to permafrost thaw, and better predict the impacts abrupt thaw and associated physical disturbance of soils may have on biogeochemical cycling in the Arctic. This chapter will outline: (1) The geochemistry of Arctic landscapes and how these landscapes are changing with increasing global temperatures, (2) The ecological processes governing microbial community processes in soils, (3) How microbial communities survive stressors and diversify in permafrost-affected soils, and (4) How these microbial communities are responding to changing climate in the Arctic.

1.2 Permafrost and Active Layer as Periglacial Soils

Canadian Arctic soils with permafrost within 1-2 metres of the soil surface are defined as cryosols (gelisols) within the Canadian soil classification system (Canadian Agricultural Services Coordinating Committee, 1998). Permafrost soils maintain sub-zero temperatures for at least two years, while the upper active layer seasonally thaws in the summer and freezes in the winter (Berner & Heal, 2005). The dynamic between active layer and permafrost soil horizons creates a system not found in temperate soils. These regions are shaped by periglacial processes occurring within the active layer and are driven by freeze/thaw cycles. However, most permafrost-affected soils exist in regions which are

predicted to undergo the most rapid rates of climate change, at high latitudes and high altitudes (Richter-Menge et al., 2016). The overlying active layer thickens into permafrost horizons with increasing temperatures; however, the rate of this degradation is dependent upon both climatic and geological variables. Therefore, this section will describe the responses this system will undertake in the face of dramatic soil warming. To provide context regarding these processes, I will also briefly describe the extent of permafrost affected soils, their depth below the ground surface, and the periglacial processes controlling these soils.

Current estimates suggest 26% of global land area is underlain by permafrost, 23.9% of land area in the Northern Hemisphere is affected by permafrost, and ~50% of Canada underlain by permafrost (Tarnocai et al., 2009). Mean annual air temperatures below -1 ℃ allow for the formation of permafrost. However, spatial variation and soil structure induces variable thermal properties within these regions. Therefore, permafrost distribution varies throughout the Arctic and can be defined as continuous (90-100% of land is underlain by permafrost), discontinuous (50-90% permafrost), sporadic (10-50% permafrost) or isolated (<10% permafrost). This -1 ℃ isotherm is commonly reached at subarctic latitudes or at high altitudes, which allows for alpine permafrost to form. In these regions, slope aspect and soil structure become critical to the formation of discontinuous permafrost, and permafrost is far more common across Northern slopes (Dobinski, 2011). Of course, the extent of altitudinal and latitudinal can overlap in Northern mountain ranges, particularly in the Rocky Mountains (Dobinski, 2011). Continuous permafrost occurs in colder regions with average annual temperatures below -6 ℃, a temperature at which influences of slope aspect cannot alter permafrost distribution. Because permafrost soils are buried, estimating the extent of permafrost soils is difficult, which is further complicated by the irregular existence of

discontinuous and alpine permafrost, and so projections must be interpreted with caution (Dankers et al., 2011).

The thickest permafrost horizons can be found in regions which have undergone freezing temperatures for the longest length of time. Permafrost thickness can vary from a few decimetres to 740 m in Northern Alaska and 1500 m in Siberia (Jansson & Tas, 2014). It has been hypothesized that permafrost can reach up to 3000 m of thickness in the most extreme and mountainous regions of Antarctica (Dobinski, 2011). However, the depth of active layer horizons is far more dependent on external variables. Thickness of the active layer is determined by a multitude of abiotic factors (including temperature of ground surface, thermal properties of the ground and its cover, soil moisture content, altitude and slope position) as well as biotic factors, namely the type and extent of vegetation (Calmels et al., 2012; Kujala et al., 2008). Subarctic active layer depth commonly ranges from 30 cm in densely vegetated peat soils to 1 m in sediment rich areas (Bonnaventure & Lamoureux, 2013; Calmels et al., 2012). Removal of surface vegetation, through anthropogenic disturbance or thaw slump progression, can significantly alter the thermal regime and thicken the active layer into previously frozen permafrost (Lantz et al., 2009). As such, the thickness of active layer and permafrost soils are likely to change with human activity (vehicle ruts, foot tracks, mining, stockpiling, and modern as well as ancient construction), warming surface temperatures, and vegetation succession (Forbes et al., 2001; Lin et al., 2016). Active layer thickening degrades upper layers of permafrost, allowing for the mineralization of frozen organic matter (Jansson & Tas, 2014). Increasing anthropogenic activity in Arctic regions may significantly impact the stability of permafrost soils, and augment greenhouse gas emissions from these soils.

Periglacial soils are characterized by seasonal freeze/thaw processes, the presence of strong winds, and short warm seasons. As such, permafrost and other periglacial soils are linked with a cold climate, where water can exist in either a liquid or solid state for differing amounts of time. Numerous periglacial processes affect these soils, most important of which is cryoturbation. Cryoturbation (*i.e.* the mixing of materials across the active layer due to freeze/thaw cycles) can homogenize chemical and biological materials from thawing permafrost into upper active layer soils and vice versa. Periglacial processes in thawing permafrost may thereby aid in the transport of active layer taxa into newly accessible permafrost matrices. Due to the steady conditions within permafrost soil horizons, the extent of geological processes in permafrost horizons amount to permafrost aggradation or permafrost degradation (Dobinski, 2011). Permafrost aggrades when the winter freeze of overlying active layer exceeds the extent of summer thaw, while permafrost degradation occurs when summer thaw exceeds winter freeze. As the Arctic experiences ever increasing temperatures, permafrost thaw will become more common. Ice-rich permafrost which is subjected to abnormally high temperatures can rapidly thaw, thereby creating thermokarsts (Kokelj & Jorgenson, 2013). Periglacial processes are therefore liable to change in the coming decades. Characterizing how the landscapes of Arctic regions are impacted by climate change is paramount to understanding the microbial ecology of Arctic ecosystems.

1.2.1 Geological response of permafrost and active layer to climate change and anthropogenic disturbance

Active layer soils play important roles in surface energy balance, carbon cycling, hydrology, and landscape processes in cold regions. However, climate warming induces permafrost degradation and thickening of the active layer into permafrost due to warming

temperature regimes, longer summer seasons, changing vegetation composition and density, and thinner snow cover (Vincent et al., 2009). With increasing warming, all near surface permafrost will undergo active layer thickening by 2100 (Lawrence et al., 2008). The rate of active layer thickening is as high as 7.5 cm yr^{-1} in the Qinghai-Tibetan Plateau and 4 cm yr^{-1} in Alaskan active layers, having drastic impacts on surface characteristics such as uplift and subsidence (Jia et al., 2017; Osterkamp, 2003). As well, the southern limit of permafrost extent in Yukon has moved poleward by 100 km in the last 30 years and 2.0 km along the Qinghai-Xizang Highway, dramatically changing the soil processes undertaken in in these regions (Solomon et al., 2007). These rates of active layer thickening and northward migration of the southern limit of permafrost are expected to increase with continued warming, releasing micronutrients, macronutrients, and greenhouse gases from permafrost (Reyes & Lougheed, 2015). These developments are of particular concern because the thaw of ice-rich permafrost can result in dramatic landscape modification, ultimately leading to thermokarst formation (Kokelj & Jorgenson, 2013).

While permafrost thaw is occurring as active layer thickening in the subarctic, abrupt thaw is becoming more common across the Low and High Canadian Arctic particularly in regions with ice-rich permafrost (Streicker, 2016). Rapid permafrost thaw occurs through the abrupt thaw of ice wedges and collapse of sediment-rich soils. Collapse of these permafrost soils forms thermokarst lakes, ponds, bogs, fens, and pits (circular depressions formed by the thaw and settlement of ice-rich sediments which fill with permafrost thaw water); retrogressive thaw slumps (landslips of ice-rich sediment); active-layer detachment slides (detachment and rapid downslope movement of thawed active layer); and thermal erosion gullies (channels of thaw caused by water runoff) (Kokelj & Jorgenson, 2013). This vast

array of possible outcomes from permafrost thaw creates a dynamic landscape. However, of these landforms, thermokarst lakes are the most common and recognizable result of permafrost thaw.

Rapid formation, drainage, and in-filling of thermokarst landscapes creates an extremely dynamic system. Lake areas in Seward peninsula have seen a 10.7% increase in lake abundance from 1950 to 2007 and are becoming increasing common across the Arctic (Kokelj & Jorgenson., 2013). Both natural and artificial disturbances can induce lake formation. Artificial disturbances – namely infrastructure development – are the most common cause of thermokarst lake formation in the Qinghai-Tibetan Plateau (Lin et al., 2016). The construction of oil wells, pipelines, gold mines, and other mineral extractions are also becoming more common in the Arctic (Elias, 2014). These industrial developments are conducive to the formation of thermokarst ponds across the Circumpolar North. Thermokarst lakes can also promote additional permafrost thaw by conducting heat through sediments; promoting colluvial transport down collapsing banks; by increasing thaw settlement beneath the lake; and by eroding shorelines (Kokelj & Jorgenson, 2013). Thermokarst lakes drain at the end of the thermokarst lifecycle, allowing drained basins known as alases to form and fill with sediments, plants, and peat. The termination of thermokarst lakes creates a productive and organic rich environment, which may allow for sequestration of carbon in the future. Drainage in discontinuous permafrost regions may occur through open taliks (*i.e*. ground within permafrost regions that remain unfrozen) and penetrate thin permafrost layers, or through a combination of other thaw events connecting thermokarst lakes to aquatic bodies. Indeed, lake area has decreased 14.9% due to partial drainage from larger lakes, though this variability may be resultant from seasonal changes in precipitation (Kokelj & Jorgenson,

2013). Water which drains from thawing permafrost may significantly impact the biogeochemistry of downstream aquatic systems, changing the ecology and carbon budgeting across larger geographic areas.

1.2.2 The chemical response of permafrost subsidence

Thaw represents a rapid physical change in permafrost soils which opens this system to hydrologic and atmospheric interactions. As permafrost often prevents connectivity between soils and inland waters, abrupt thaw can rapidly introduce permafrost sourced sediments, chemistry, and biology into downstream water systems (Heikoop et al., 2015; Lougheed et al., 2011; Reyes & Lougheed, 2015). Export of dissolved organic carbon and soil ions (Mg, Ca, SO_4, and Na) from permafrost has been linked to ecosystem changes in aquatic systems (Frey & McClelland, 2009; Roberts et al., 2017). Permafrost thaw waters may promote the development of macrophyte and periphytic diatom communities due to greater dissolved ion content, lower DOC concentrations, and increased water transparency in affected lakes (Vonk et al., 2015). Changing regimes in primary productivity may have further impacts on macroinvertebrates and other higher trophic levels, including arctic char (Roberts et al., 2017; Vonk et al., 2015). Regional variation and the source of these thaw waters can be very important for the geochemistry of resultant thermokarst lakes. Aquatic systems downstream from degrading permafrost may undergo increased DOC import from peat soils and decreased metal import, when compared with mineral-rich soils (Olefeldt & Roulet, 2012; Raudina et al., 2017; Walvoord & Striegl, 2007). Likewise, lithalsa soils, raised permafrost-affected landforms with mineral rich soils, have been associated with greater concentrations of phosphorus, suspended sediments, and conductivity (Crevecoeur et al.,

2017). The geochemistry of permafrost may therefore have a significant impact on the aquatic ecosystems in the Arctic.

Worldwide permafrost organic carbon stocks are considerable, with 500 Pg of soil organic carbon residing in the seasonally thawing active layer, while 800 Pg of this carbon is stored within permafrost (Hugelius et al., 2014). Permafrost soils are capable of cryopreserving organic carbon, as indicated by similar or higher quality and biodegradability of permafrost organic carbon compared to active layer organic matter (Ding et al., 2017; Uhlířová et al., 2007). High quality organic stocks are consistent across deeper permafrost horizons, and across both alpine and Arctic permafrost (Chen et al., 2016; Strauss et al., 2015). Permafrost soils will ultimately undergo greater rates of carbon degradation than active layer soils, both due to greater lability of carbon compounds in permafrost as well as the abrupt thermodynamic changes which occur with permafrost thaw (Walz et al., 2017). This data suggests that anthropogenically induced climate change may open vast stores of permafrost soil organic matter to global biogeochemical cycling.

Landform differences can also impact organic carbon content and export, particularly in the subarctic. Northern peatlands contain 277 Pg of organic carbon, or roughly 20% of the total permafrost organic carbon pool (Tarnocai et al., 2009). Peatlands found in discontinuous permafrost regions export DOC which is more reactive than found in continuous or sporadic permafrost regions (Panneer Selvam et al., 2017). Therefore, the input of water from discontinuous permafrost may represent a greater impact on carbon balancing than other permafrost types. Soil type can also have significant impact on carbon flux from periglacial soils. Lee et al. (2012) showed that northern mineral soils had similar or lower CO_2 flux on a per gram basis of carbon mass as organic soils under either aerobic or anaerobic conditions,

suggesting aerobic and anaerobic conditions can alter carbon flux (Lee et al., 2012). Formation of thermokarst lakes can create these anaerobic environments which are amenable to methanogenesis and resulting methane emission from permafrost soils (Walter et al., 2006). While anaerobic environments emit lessCO_2 than well-drained aerobic soils, methane represents a highly potent greenhouse gas which may make anaerobic soils contribute more to climate change than aerobic environments (Schuur et al., 2015). Thaw lakes in the Arctic and subarctic may represent a substantial source of carbon, exporting DOC into aquatic systems and exporting methane and CO_2 into the atmosphere.

Carbon balance in the Arctic is a function of both carbon gains (including escalation of CO_2 uptake by plants due to longer growing seasons in a warmer climate and fertilization from rising atmospheric CO_2 levels) and carbon losses (including flux of methane clathrates from thawing permafrost, increasing rates of microbial degradation of soil organic matter, longer seasons conducive to soil respiration, greater frequency of forest fires, and escalating drought stress on plant photosynthesis) (Mack et al., 2011). Early models during the 1990s suggested northern latitudes were a net carbon sink, sequestering carbon from the atmosphere (Baker et al., 2006; Gurney et al., 2004; Rodenbeck et al., 2003). However, recent research is suggesting that the ability of Arctic landscapes to assimilate CO_2 is rapidly degrading. From 2000 to 2011, Alaskan soils represented a carbon neutral system (Belshe et al., 2013; Chang et al., 2014). However, from 2012-2014, Alaskan tundra represented a net carbon source, with high rates of soil respiration during the early winter months (Commane et al., 2017). With permafrost thaw, increasing carbon availability and microbial activity is liable to induce a positive feedback of warming (Schuur et al., 2015). Over a few decades, the Arctic has rapidly changed from an important consumer of CO_2 to a significant source of atmospheric

carbon. However, regionality across the Arctic is still present as temperate and subarctic boreal forest ecosystems remain as carbon sinks (Commane et al., 2017; B. Zhang et al., 2017), and High Arctic soils are predicted to become an increasingly significant carbon sink with warming (Lupascu et al., 2014). Therefore, accurate carbon flux models require in depth characterization of northern landscapes to account for widespread regional variation. Regardless, permafrost thaw is predicted to become one of the most historically significant biogenic sources of CO_2 with 130-160 Pg of carbon vulnerable to mineralization over the next century (Schuur et al., 2015). Catastrophic release of methane (modeled at an approximate 50 Pg over 10 years) is unlikely to occur as predicted by current methane flux rates (Schuur et al., 2015). Instead, flux of CO_2 and methane is likely to be gradual, occurring over century long timescales. Methane is stored in permafrost, both as free gas and as methane clathrates, but can also be microbially produced under anaerobic conditions in both thermokarst ponds and in soils. The radiative forcing effect of methane is approximately 84 times that of the same mass of carbon dioxide (Allen et al., 2014). However, much of the methane released from permafrost is consumed by methanotrophs prior to flux into the atmosphere (Stackhouse et al., 2015). Methane will therefore contribute between 25% and 45% of the overall potential climate impact over a 100-year timescale, within mineral soils and organic soils respectively (Schuur et al., 2015).

1.3.0 Soil Microbiology

Upwards of 1 billion bacteria can be found within a gram of soil, with a similar worldwide belowground biomass as plants (Bar-On et al., 2018). Soil microbial communities have crucial roles in biogeochemical cycling, soil structuring, and maintain the health and

function of terrestrial plants and animals. Consequently, soil microorganisms contribute to many soil processes, including turnover of organic matter and maintaining soil structure for terrestrial biota. Functionality is linked to microbial biodiversity, and our understanding of how the soil microbiome is impacted by soil quality is still in its infancy. Therefore, I will discuss what the soil environment is, the importance of soil microbial diversity, what factors structure the soil microbiome, and how this microbial community structure impacts ecosystems functioning.

1.3.1 Drivers of the soil microbiome

Globally, there are several key soil parameters that govern the environmental conditions of soils. Soil parameters exhibit large variation and are shaped by climatic variables, organisms (both microorganisms and macroorganisms), the parent material, and time. Microorganisms themselves can be classified as "ecosystem engineers" with the ability to manipulate their environment, thereby iteratively creating new niches within soils (Odling-Smee et al., 2013). As an example, the ability of bacteria to stabilize sediments through the exorbitant secretion of extracellular polysaccharides allows for the establishment and propagation of microbial populations in sediment and soil environments (Gerbersdorf et al., 2009). Extracellular polysaccharide production provides structure for soil aggregation, nutrient entrapment, as well as protection from environmental conditions, antibiotics, and predators (Costa et al., 2018). The establishment of this soil environment allows for the colonization of larger plant species as well. These plant communities may directly impact environmental conditions through the formation of rhizospheres and the production of litterfall. The decomposition of plant litter can alter the physiology of soil microbiota through a "home field advantage", wherein soil microbiota preferentially decompose litter derived

from the plant above it (Ayres et al., 2009). This “home field advantage” promotes the proliferation of soil microbiota which are beneficial to the degradation and reintegration of its own nutrients back into the soil. Spatial variation in soils is even more pronounced between rhizosphere and bulk soils, wherein rhizodeposition (*i.e.* the release of proteins, sugars, and nutrients from plant roots) alters the community composition and diversity of microorganisms in the rhizosphere (Philippot et al., 2013). Furthermore, animal waste products can increase microbial activity, such as denitrification and carbon degradation, by abruptly introducing large amounts of nutrients (Petersen et al., 1996). Together, these examples illustrate that habitats within soils may drastically change across only centimeters of separation. The physical environment of soil is heterogeneous, composed of mineral components held together through organic compounds and microbial exudates. Soils can be extremely porous, holding gasses and liquids with composition which can vary considerably. All of these factors create heterogeneous and discontinuous microenvironments, creating “hot spots” of microbial activity, and resulting in diverse microbial communities.

No single factor can explain the entirety of microbial biodiversity across either local or global scales. This creates incongruencies and complexities within the literature, wherein communities across different sample sites have different hierarchies of important biotic and abiotic drivers. Regardless, recent research is emerging which suggests that microbial diversity and function are largely determined by soil physicochemical parameters most notably pH (Chu et al., 2010), but can also include: organic carbon quality and quantity (Sul et al., 2013); soil oxygen and redox status (Mondav et al., 2017); soil moisture (Shi et al., 2016); nitrogen and phosphorus availability (Cederlund et al., 2014); and soil texture, structure, and temperature (Oliverio et al., 2017). Soil acidity/basicity, expressed as pH, can

have dramatic impacts on nutrient bioavailability as well as microbial function and survival. Most soils range from pH 4 to 8, although higher or lower extremes can be reached because of soil conditions and human impacts (Tecon & Or, 2017). Finer scale variation in pH is controlled by plant communities, microbial communities (due to respiration, nitrification, sulfur oxidation, iron oxidation, or and many other), as well as soil history (Tecon & Or, 2017). While overall microbial richness and evenness is a function of soil fertility (an integrated factor of organic matter, nitrogen and chloride content), soil pH may control phylogenetic structure and diversity (Siciliano et al., 2014). Domain-wide selection occurs with pH variation, as fungi are considerably more tolerant of acidic conditions than bacteria (Rousk et al., 2010). As well, some bacteria are selected upon acidification – such as Acidobacteria group 1, 2 and 3 – while others are predominant under more basic conditions – such as Acidobacteria group 4, 6 and 7 (Kielak et al., 2016). In summation, soil pH is likely to be the strongest predictor of microbial biodiversity in soils.

Beyond the drivers of biodiversity, the large amount of spatial variation can allow for the formation of persistent abiotic stressors (low temperatures and oligotrophic conditions), frequent and repetitive disturbances (such as freezing and thawing), and uneven distribution of nutrients over space and time (as found in brine films within frozen soils) (Schostag et al., 2017). These stressors can limit the growth potential of microorganisms in soil. Numerous lines of evidence suggest that soils represent a harsh environment for microorganisms to grow and persist: (1) inoculation of foreign microbial communities into new soils have shown that few taxa can persist for long periods of time (Acea et al., 1988), (2) only 1% of soil surface area contains microbial cells at any given time (showing that dispersal is non-random by microbes) (Young & Crawford, 2004), (3) Antarctic surface soils are completely lacking

in active microorganisms (identifying environments on the surface of earth which are incapable of life) (Goordial et al., 2016), and (4) less than 5% of microbial biomass is active at any point in time, suggesting that only a fraction of potentially active microorganisms are under optimal conditions for growth (Blagodatskaya & Kuzyakov, 2013).

1.3.2 The role of biodiversity and community composition in soils

Domain-level differences are widely replicable across global scales. Bacteria (10^3 μg biomass C g soil^{-1}) and fungi (10^3 μg biomass C g soil^{-1}) are commonly dominant in soils, exhibiting 10^3 times as much biomass as other soil microorganisms (Fierer, 2017). Archaeal taxa (10^1 μg biomass C g soil^{-1}) are less abundant in soils but exhibit much greater spatial variability than other taxa, with predominance in water logged soils (Fierer, 2017). Representing far less biomass are protists and viruses (10^{-2} μg biomass C g soil^{-1}), but these taxa can still impact overall functioning primarily through predation and viral lysis (Fierer, 2017). Despite this low biomass, the high abundance of viral particles in soil (approximately 10^7-10^9 viruses per gram of soil) can have critical impacts on soil functioning (Emerson et al., 2018).

Taxonomic profiles show only a small number of dominant groups across all soil habitats. Acidobacteria and Proteobacteria are the most abundant bacterial phyla across 66 unique soil samples, collected across multiple ecosystems and latitudes. While Verrucomicrobia, Bacteroidetes, Planctomycetes, and Actinobateria are also present they are in lower abundances than Acidobacteria and Proteobacteria (Fierer, 2017). Likewise, fungal groups generally consist of Agaricomycetes (a class of Basidiomycota) and Archaerhizomycetes (a class of Ascomycota), with lower abundances of Zygomycota, and

other Ascomycota groups. Amongst archaeal groups, Crenarchaeota are the most abundant phyla across worldwide soils with a significant minority of Euryarchaeota (Fierer, 2017). However, the bulk of soil microorganisms remain undescribed and current methods have yet to adequately address this issue. Poor characterization is not unique to the field of soil microbiology, as large numbers of rare and novel taxa are still found within thermokarst waters (Wurzbacher et al., 2017). Adequate predictions regarding the functionality of microbial communities will not be possible without additional characterization of environmental taxa. Thus, while work is ongoing to better describe the entirety of soil microbial diversity, more work is needed to describe the roles of specific microorganisms within soils.

Soil heterogeneity provides a vast array of habitats and niches which structures the ecology and evolution of soil microorganisms. Genomes from soil bacteria routinely show greater genomic complexity than from other environments (Tecon & Or, 2017). This habitat complexity encourages larger genome size, with greater abundance of accessory genes involved in environmental sensing, in substrate transport and degradation, secondary metabolism, stress response, and motility (Guieysse & Wuertz, 2012; Mackelprang et al., 2017). Genomic adaptations to soil conditions are likely resulting from high variability of soil microhabitats. Indeed, larger microbial genomic size has been attributed to fluctuations in moisture content, temperature, and spatial heterogeneity (Tecon & Or, 2017). Community level adaptations may also require intimate interactions between soil microorganisms. Greater GC content has also been attributed to soil environments; indicating a larger contribution of phylogenetically distant DNA to soil genomes (Mackelprang et al., 2016). Therefore, large amounts of metabolic versatility are likely required for the variability of soil habitats.

1.3.3 Ecosystem function of soil microbial communities

Soil formation and structure is important for microbial colonization and carbon turnover. Stabilization of soil structure is required for long-term soil functioning at both the microbiological and macrobiological level, allowing plant colonization, fungal hyphae development, and microbial evolution (Rillig et al., 2017). Soil aggregate life cycles of (1) formation, (2) stabilization, and (3) disintegration can control microbial evolution and microbial community composition within soils (Rillig et al., 2017; Vos et al., 2013). Maintaining structure of aggregates and microscale soil creates strong feedbacks in the spatial organization of niches and microbial population hot-spots (Nunan et al., 2003; Young & Crawford, 2004). These hot-spots are primarily maintained through EPS (extracellular polymeric substances), which bind sessile microorganisms to soil aggregates and contribute to soil strength (Tecon & Or, 2017). Most microbial soil activity is performed within these microbial hot-spots.

Microbial processes undertaken in soils can include the production and consumption of atmospheric gases, the manipulation of soil acidity, and nutrient cycling (particularly carbon, nitrogen, phosphorus, iron, and sulfur cycling) (Fierer, 2017). Microorganisms also have critical indirect impacts on ecosystem functioning through soil-borne plant and animal symbioses (including parasitism, mutualism, and commensalism) (Fierer, 2017). Under these assumptions, characterization of abundant soil taxa is required to understand ecosystem-level processes and provide a better understanding of the biotic and abiotic processes undertaken soils. In the absence of complete characterization, microbial ecologists apply the insurance hypothesis to understand the impact biodiversity has on ecosystem functioning (Yachi & Loreau, 1999). The insurance hypothesis suggests that greater biodiversity confers greater

stability (resilience and resistance) while also providing greater ecosystem functioning by filling a maximum number of niches. How this increasing diversity impacts functioning has several hypotheses, including (1) the complementarity hypothesis, (2) the redundancy hypothesis, and (3) the selection hypothesis. The complementarity mechanism hypothesis suggests that as species richness linearly increases, ecosystem functioning also linearly increases, as each species within a community occupies a different niche (Bell et al., 2005). However, due to the high level of functional redundancy, wherein there is an overlap in function across bacterial species, complementarity is not seen as a driving factor in ecosystem functioning (Bell et al., 2005). The redundancy hypothesis therefore suggests that once an optimal number of species is included within an ecosystem the addition of new species will not contribute to ecosystem function (Bell et al., 2005). Redundancy does allow for additional resilience in the face of perturbation, wherein the loss of a species will not impact ecosystem functioning due to the presence of another species with similar function. Likewise, the selection hypothesis posits that species-rich systems will harbor higher functional potential on average due to the greater likelihood of containing species with a larger effect on ecosystem functioning (Loreau & Hector, 2001). Therefore, the addition of species into low-richness ecosystems will dramatically increase ecosystem services to an asymptote as species function becomes increasingly redundant. However, analysis of key specialized functions can be negatively impacted by even moderate reductions in diversity (Singh et al., 2014). These hypotheses imply that function and stability are dependent upon the preservation of high biodiversity, but it is uncertain what level of biodiversity is optimal within soils, particularly soils within extreme environments.

1.4.0 Microbial Ecology in Permafrost-Affected Soils

Despite slow growth and low activity rates, Arctic soil microorganisms play important roles in global biogeochemical cycling. These extreme environments require a series of community-wide adaptations to low nutrient conditions, cold temperatures, and freezing/thawing conditions. By adapting to these conditions, in part through community compositional changes, microorganisms are capable of turning-over the vast amounts of carbon found within Arctic soils (Schuur et al., 2015). As a result, these microorganisms are critical to the release of climate active gases in response to permafrost thaw. These gases include methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O), the production of which are dependent upon the diversity and composition of bacterial communities within permafrost. However, dormancy and preservation of deceased microbial biomarkers in these cold environments can become problematic to the assessment of community function and biodiversity. Permafrost represents a natural archive which preserves both living organisms (as shown through revival of viral, bacterial, and plant life) as well as dead biomarkers (including macrofossils from plants and animals, as well as ancient DNA from plants, mammals, insects, fungi and bacteria) for thousands of years (Bellemain et al., 2013; Haile et al., 2009; Johnson et al., 2007; Legendre et al., 2014; Willerslev et al., 2003; Willerslev et al., 2004; Yashina et al., 2012). Therefore, this section will discuss the community-level adaptations to Arctic conditions, the biodiversity of the microbial communities, and how to distinguish viable and non-viable taxa within these soils.

1.4.1 Microbial adaptations to the periglacial environment

Seasonality has drastic effects on the active layer of permafrost-affected soils, driving large temperature and geochemistry fluctuations. Freeze/thaw dynamics result in dramatic changes to water availability, osmolarity of liquid water, plant exudation, photosynthetically active radiation, and UV radiation (Schostag et al., 2015). These factors alter microbial habitats, thereby impacting microbial biomass, function, community structure, and microbial diversity across frozen and thawed states. Freeze/thaw events can kill upwards of 50% of bacteria in soils, suggesting that these events can cause massive die off and release of cellular nutrients (Soulides and Allison, 1961; Skogland et al., 1988). Preferential death of microorganisms in winter months, together with the influx of labile nutrients in the spring, can combine to alter the microbiome of active layer soils. Compositional analyses have shown subtle shifts with freeze/thaw events, where frozen months consist of greater abundances of Actinobacteria, Acidimicrobiaeceae, Solirubrobacterales, Conexibacteraceae, Solirubrobacteraceae, TM7 and Chloroflexi; while thawed summer months show higher abundances of Acidobacteria, Cyanobacteria, and Alphaproteobacteria (Kuffner et al., 2012; Schostag et al., 2015). These subtle shifts are driven by edaphic parameters, including pH and dissolved organic carbon (Schostag et al., 2015). While no differences are seen in α-diversity of bacterial communities between summer and winter months in active layer soils, Lipson & Schmidt (2004) found greater fungal diversity during frozen winter months. No change in RNA:DNA ratios have been found in 16S rRNA gene and transcript copy numbers, suggesting no changes in overall metabolic activity with seasonality (Schostag et al., 2015). However, active layer CO_2 respiration and N mineralization have shown large pulses in response to rapid thaw (Schimel & Clein 1996). These pulses are likely due to metabolism of

cell components released from lysed cells during the freezing period. While C and N mineralization decreases during frozen periods in Arctic soils, these pulses of activity negate any lost respiration, do not change net fluxes of CO_2 or N_2O, and so is unlikely to affect carbon balance (Matzner et al., 2008). Active microorganisms in frozen Arctic soils are likely to be rare members of the overall soil microbial communities, as active community members in the winter have little overlap with total community members (McMahon et al., 2011). These active communities have adapted to oligotrophic and nitrogen poor soils, as indicated by the abundance of nitrogen fixers in surface soils (Malard & Pearce, 2018). As well, the abundance of phototrophs indicates community-level adaptation to higher levels of photoreactive and UV radiation (Malard & Pearce, 2018). Although significant proportions of microorganisms die upon freezing, these communities are resilient to freeze/thaw disturbances: decreased microbial activity and decreased viable microbial biomass during winter months recover throughout the summer, while cold-adapted taxa become more abundant during the winter. These taxonomic shifts suggest microbial survival strategies under freezing conditions are present within select taxa.

1.4.2 Structure and importance of microbial communities in permafrost-affected soils

Biogeography, assembly, and biodiversity of microbial communities in cold soils undergo distinct drivers compared to other soils. The taxonomic distribution of Arctic, alpine, and Antarctic active layer soils differ across both large and small spatial scales. Phylum-level classification has indicated similar relative abundances of most phyla across all Arctic regions (including the Canadian High Arctic, Alaska, Svalbard, Greenland, Finland, Iceland, and Siberia) but are distinguished based on differing abundances of Actinobacteria,

Bacteroides, and Acidobacteria (Malard & Pearce, 2018). Active layer soils harbour low abundances of Verrucomicrobia, Planctomycetes, Firmicutes, Chloroflexi, Gemmatimonadetes, AD3, and Cyanobacteria, all which total to less than 15% of total relative abundance (Malard & Pearce, 2018). Across the aforementioned Arctic regions, Proteobacteria are dominant, particularly Rhizobiales (α-Proteobacteria), Burkholderia (β-Proteobacteria), Xanthomonadales (γ-Proteobacteria), and Myxococcales (δ-Proteobacteria) (Malard & Pearce, 2018). Amongst the more definitive phyla: Acidobacteria was prominent across all Arctic regions except Svalbard and Siberia; Actinobacteria were prominent in all Arctic regions except Greenland and Iceland; and Bacteroidetes were abundant in Alaska, Canada, and Svalbard (Malard & Pearce, 2018). Alpine active layer soils exhibit a much more divergent taxonomic structure, with dominant Acidobacteria, Planctomycetes, and Spartobacteria, while being completely absent in Bacteroidia (Malard & Pearce, 2018). Archaeal taxonomic assessment has identified Euryarchaeota (particularly Methanomicrobia and Methanobacteria) to be dominant in Alaska, Greenland, and the Canadian High Arctic, while Thaumarchaeota and Crenarchaeota are more abundant in other Arctic and Alpine regions (Kao-Kniffin et al., 2015; Wilhelm et al., 2011), as well as Crenarchaeaota in Tibetan active layer samples (Malard & Pearce, 2018). Differences in regional abundances are identifiable across Tibetan, Alpine, Siberian, Scandinavian, and Beringian soils. Antarctic active layer soils have far more isolated from other soils and environmental inputs and, as such, have much different taxonomic profiles than other soils, with much lower abundances of Proteobacteria and Archaea. Taxonomic communities are therefore separated across Arctic, Antarctic, and Alpine active layer soils.

Beneath these active layer soils, permafrost soil horizons are perennially at sub-zero temperatures, thereby impacting microbial community structure and biodiversity. The stresses of sub-zero temperatures (including salt stress and dispersal isolation) lead to microbial communities that are strongly influenced by dispersal limitation, resulting in spatial heterogeneity in taxonomic profiles (Bottos et al., 2018). This creates problems when attempting regional comparisons of permafrost taxonomic profiles, though replicate cores sampled within 1 m of each other have established similar trends in depths in Svalbard (Müller et al., 2018). Unfortunately, a rigorous meta-analysis of permafrost sequence data has yet to be performed. Regardless, permafrost soils appear to be dominated by spore/cyst formers (including numerous Actinobacteria, Firmicutes), as well as methanotrophs, archaeal methanogens, cellulose degraders, aerobic and anaerobic heterotrophs, sulfate and iron reducers, as well as nitrifying and nitrogen fixing bacteria (Hultman et al., 2015; Kirby et al., 2012; Steven et al., 2006; Taş et al., 2014). Together, previous studies have suggested the predominance of taxa adapted to dormancy and oligotrophic conditions (Mackelprang et al., 2017). In Alaskan soils, dominant taxa often include Actinobacteria, Chloroflexi, Proteobacteria, and Acidobacteria (Taş et al., 2014). Canadian High Arctic permafrost is predominated by Actinobacteria, Proteobacteria, and Bacteroidetes (Taş et al., 2014). Scandinavian permafrost is dominated by Acidobateria and Actinobacteria (Taş et al., 2014). Finally, Antarctic permafrost is defined by high abundances of Proteobacteria, Firmicutes, and Actinobacteria (Taş et al., 2014). It appears that regionality similar to that found in the active layer may also be found in permafrost.

Similar community composition is found in Arctic soils as found in lower latitudes, with pH being the primary driving factor of bacterial community composition (Chu et al.,

2010). This stands in stark contrast to the latitudinal gradient observed in plants and animals throughout contemporary and paleobiological paradigms. Permafrost and active layer soils have high levels of microbial diversity in comparison to macroecological expectations, which traditionally suggest decreased species diversity with increasing latitude (Stevens & Tello, 2018). Due to insufficient characterization of microbial diversity on global scales, such latitudinal species diversity gradients have yet to be shown. While genus specific sampling has shown decreases in Streptomyces diversity and higher phylogenetic clustering with latitude, these findings do not reflect community level trends (Andam et al., 2016). As pH is often the primary driving factor of community composition, driving similar community composition between Arctic soils and soils found in lower latitudes, this may represent a better predictor of microbial diversity than latitude (Chu et al., 2010). Research using latitudinal sampling strategies in continuous permafrost regions have found that mid-latitude samples within a latitudinal gradient harbour the lowest bacterial diversity, phylogenetic diversity, and richness in active layer samples (Ren et al., 2018). This diversity was explained by plant-community factors and environmental parameters which covaried with latitude, suggesting that permafrost affected regions may not follow expected trends in diversity on the microbiological level.

1.4.2.1 Factors controlling the Arctic soil microbiome

Drivers of microbial community dynamics are not common between active layer and permafrost soil horizons (Chen et al., 2017). In a study of alpine permafrost, numerous environmental parameters correlated with bacterial and archaeal community composition in the active layer (including spatial distance, mean annual precipitation, soil organic carbon, nitrogen, carbon, carbon:nitrogen ratios, phosphorus, pH, soil moisture content, clay

percentage, silt percentage, sand percentage, dissolved organic content, dissolved nitrogen content), while only a small subset of these parameters correlated with the permafrost bacterial and archaeal community (including mean annual temperature, mean annual precipitation, dissolved organic carbon, active layer thickness, and depth) (Li et al., 2017; Shi et al., 2016; Tripathi et al., 2018a). Significantly, the presence of permafrost alters microbial communities more than would be expected by increases in soil depth (Chen et al., 2017). Microbial communities in permafrost are not subjected to atmospheric inputs and cannot interact with overlying plant communities. However, distinct vegetation types have been shown to have a significant impact upon the fungal and bacterial communities within tundra tussock soils (Wallenstein et al., 2007). These findings suggest that characterization of microbial communities in the active layer cannot be extended to permafrost microbial communities. Therefore, studies must look at both active layer and permafrost diversity to characterize soil microbial community dynamics in periglacial soils.

Soil depositional age effects edaphic parameters of permafrost, which in turn affects the bacterial community composition (Penton et al., 2013; Coolen & Orsi, 2015; Coolen et al., 2011). Indeed, fungal reconstruction of ancient microbial community structure across the Holocene-Pleistocene boundary has been conducted using deep permafrost cores (Bellemain et al., 2013). The source of these fungal communities has been attributed to a fungal "seed bank", as 63% of permafrost taxa were common between prairie soils and deep permafrost cores (Penton et al., 2013). As well, work within the Lanoil laboratory has identified that soil edaphic parameters are significantly correlated with bacterial community composition across the Holocene-Pleistocene boundary (Hammad et al., in prep).

1.4.2.2 Microbial abundance across regions of the cryosphere

Regional cell abundance differences are not present in periglacial soils across the Arctic. However, cell abundances range widely in Arctic, alpine, and Antarctic permafrost, from 10^3-10^8 cells/g, 10^6-10^8 cells/g, and 10^5-10^6 cells/g, respectively (Blanco et al., 2012; Hansen et al., 2007; Liebner et al., 2008; Papale et al., 2018; Steven et al., 2006; Vishnivetskaya et al., 2000; E. Vorobyova et al., 2001). Depth and age of permafrost has little impact on the total microbial abundances past 3 m of depth (Mackelprang et al., 2017). Active layers of permafrost often harbour higher cell counts than found in permafrost and range from 10^7-10^{10} cells/g in Arctic regions and 10^5-10^6 cells/g in Antarctic regions (Wilhelm et al., 2011; Papale et al., 2018). Bacterial abundance exhibit differences in geography and depth, particularly across the active layer/permafrost boundary.

Culturable cell counts are often more technically challenging and are incapable of retrieving equivalent counts but allow for identification and cultivation of functional groups. Culturing has allowed isolation of iron reducers (10^2-10^3 CFU/g), iron oxidizers (10^3 CFU/g), methanogens (0-10^7 CFU/g), sulfate reducers (10-10^4 CFU/g), and aerobic heterotrophic bacteria (10^3-10^8 CFU/g) from Siberian permafrost (Vorobyova et al., 1997). Removal of anabiosis autoinducers (such as alkylhydroxybenzenes) and addition of growth regulators (indole-3-acetic acid and wheat germ agglutinin) to reactivate dormant and non-culturable cells improves recovery rates in permafrost (Kryazhevskikh et al., 2012). Other culturing strategies have employed minimal media at low temperatures and a number of enrichment broths (Hansen et al., 2007; Zhang et al., 2013). These findings suggest, unsurprisingly, that permafrost microbial communities are composed of a myriad of functional groups existing in all states of physiological activity.

Copy number of the 16S rRNA gene has been used to estimate microbial abundance in permafrost and active layer soils as well. Interestingly, this approach has shown higher bacterial abundance in permafrost (7.53×10^7 copies/g) than in active layer (3.81×10^7 copies/g), although similar abundances of Archaea were found across permafrost and active layer soils (3.68×10^4 copies/g in the active layer and 2.83×10^4 copies/g in permafrost) (Wilhelm et al., 2011). These trends are likely due to site specific environmental parameters but could also be attributed to gene copy number differences between the Bacterial and Archaeal domains or preservation of relic DNA from ancient microorganisms.

1.4.2.3 Community assembly in soils

Li et al., (2017) investigated the bacterial, eukaryotic, and archaeal communities across a water content gradient in Tibetan alpine soils in the Qinghai Tibetan Plateau using 16S amplicon analysis. Community assembly in these soils were dependent upon domain-level phylogeny, wherein bacterial and archaeal communities are deterministically constructed, while eukaryotic communities were stochastically constructed. To investigate the effects of thaw in Alaskan soils, Tripathi et al., (2018a) characterized microbial community structure and abundance in Alaskan soil cores in both black spruce and tundra soils. These researchers determined that community assembly was more phylogenetically clustered in upper soils than deeper soils, suggesting deterministic assembly drives upper soil horizons, but becomes less important with depth. However, community turnover was driven by soil properties, including pH, EC (electrical conductivity), TOC (total organic carbon), and gravimetric water content. Climate change resulting in glacial retreat and permafrost thaw can also impact community assembly controlled by changing pH across foreland soils (Tripathi et al., 2018b). Water samples from three thermokarst lakes in Northern Quebec

were collected across the entirety of their depths (Crevecoeur et al., 2015). These waters showed microorganisms emerging from permafrost undergo environmental filtering in thermokarst lakes according to valley location, water depth, pH, DOC, total suspended solids, and oxygen content. Environmental filtering effects may have important implications for carbon cycling in these water bodies, e.g. the transition of oxygenic (Cyanobacteria) to anoxygenic (Chlorobia) phototrophs with lake depth. In studies of glacial foreland soils, Tripathi et al., (2018b) examined community assembly dynamics across six different sites across Norway, Switzerland, Washington, Michigan, and New Zealand. Extreme pH, both acidic and basic, was found to drive phylogenetic clustering and deterministic community assembly regardless of successional age, while neutral pH reduced deterministic community assembly. These studies suggest that community assembly may be dependent on soil depth, particularly in permafrost affect soils, and environmental parameters.

1.4.3 Distinction of live and dead microorganisms

In the assessment of environmental health within microbial ecology, characterization of microbial community composition is of prime concern. Microbial communities often reflect a broad range of activity levels and physiological states, of which only a small portion are capable of significantly participating in biogeochemical cycling processes. Four common overarching physiological states are found within soil microorganisms: (1) active microorganisms performing biogeochemical transformations, (2) potentially active organisms which may rapidly respond to nutrient addition and subsequently partake in transformations, (3) dormant viable but not culturable cells (VBNC) and spores which do not contribute to ongoing transformation in the absence of anabiosis molecules, and (4) dead cells representing a reservoir of potentially degradable organic carbon (Blagodatskaya & Kuzyakov, 2013).

However, the question of whether a microorganism is not only viable, but also capable of activity, is often left unanswered within scientific literature due in part to the challenges in quantitatively distinguishing active and dormant microorganisms (Blagodatskaya & Kuzyakov, 2013). The question of whether a detected organism is viable is essential not only to general environmental microbiology, but also the study of extraterrestrial life (to preclude the possibility of dormant and resilient microorganisms on robotics equipment), medical microbiology, and particularly in low biomass sample types (Mahnert et al., 2015; Zhang et al., 2018). Current trends in microbiology still refer to culture-based techniques as the "Gold Standard" of assessing microbial viability (Emerson et al., 2017). However, these techniques preclude the possibility of assessing non-culturable bacteria and can require long cultivation times. Therefore, recent work has focused on the optimization and diversification of culture-independent techniques to assess the abundance and identity of the viable fraction of microorganisms within a given system.

Rapid degradation of DNA can occur through hydrolysis (leading to strand breakage, depurination, or depyrimidination), oxidation, and crosslinking. When released from lysed or otherwise dead cells, preservation of DNA and other organic molecules is dependent on both biotic and abiotic parameters. DNA degradation is highly temperature dependent, but is also influenced by pH, heavy metal content, pressure, and free water content. Although permafrost often has high amounts of water, 3-8% of which is unfrozen in brine films, low temperatures permit long-term persistence of DNA and other organic material. This persistence has allowed study of ancient fungal, mammalian, bacterial, and plant diversity (Bellemain et al., 2013; Lydolph et al., 2005; Mackelprang et al., 2017). However, presence of relic DNA can introduce uncertainty surrounding which organisms are viable and active under permafrost conditions (Bellemain et al., 2013). Upon thaw, increased water availability and higher free

energy levels allows for higher rates of DNA hydrolysis, affecting DNA based analysis. Numerous techniques outlined in this section have been employed to differentiate live, dead, and active microorganisms within permafrost (*e.g.* Vorobyova et al., 1997; Coolen & Orsi 2015; Hultman et al., 2015; Mackelprang et al., 2017; Papalea et al., 2018…).

Cell membrane integrity has become a vital biomarker to assess microbial viability (Emerson et al., 2017) as it is an essential component of all life, required for cellular respiration, recognition of external cues, and compartmentalization of the cell. Proportions of viable microbial cells have ranges from 18-86% across permafrost samples from the Canadian High Arctic to Antarctica (Hansen et al., 2007; Mackelprang et al., 2017; La Ferla et al., 2017). Rather than assessing viability, some cell counting methods also allow the identification of metabolically active microorganisms. Active cell counting with CTC staining (5-Cyano-2,3-ditolyl tetrazolium chloride) is used to evaluate the activity of microorganisms through the formation of a red formazan product in the presence of an active electron transfer chain. Within the extreme permafrost conditions of Antarctica, metabolically active cell abundances have been similar to, or greater than, viable microbial counts (La Ferla et al., 2017; Papale et al., 2018). Therefore, while microbial communities are under extreme stress in permafrost soils, they are still capable of retaining viability and undergoing cellular respiration. A large population of dwarf cells were also found within these active layer soils (Papale et al., 2018). Within soils, particularly permafrost, dwarf cells and nanoforms (<0.3 μm^3 in cell volume) can comprise upwards of 75% of the microbial abundance which can make accurate counts of microbial cells exceedingly difficult (Bakken & Olsen, 1987; Vorobyova et al., 2001). The abundance of dwarf cells and nanoforms in permafrost indicate the ability of cells to alter their cell morphology in response to extreme conditions, suggesting a decrease in active microbial biomass may not indicate a decrease in the number

of active cells. The inability to adequately enumerate these dwarf cells may therefore contribute to the underestimation of microbial abundance within permafrost soils.

Assessment of DNA from viable organisms in soils has become a continuing topic in recent years. Preservation of relic DNA (*i.e.* preserved extracellular DNA from dead microorganisms) in soils is primarily dependent upon the edaphic parameters of the soil. Relic DNA is best preserved within soils with low exchangeable base cation concentration, lower electrical conductivity, and low to neutral pH, and so soils which harbour these characteristics are more likely to have inaccurate biodiversity assessment (Carini et al., 2017). Subsurface soils protect relic DNA from direct damage caused by UV irradiation, which dimerizes DNA bases, and deeper soils will have higher proportions of relic DNA than surface soils. However, relic DNA also represents a nutrient source rich in carbon, nitrogen, and phosphorus with dramatic impacts on microbial communities and biogeochemical cycling in ocean sediments (Dell'Anno et al., 2005). Microbial degradation of DNA is highly dependent on both temperature and microbial population distributions. Microbial hotspots of activity, which are common in soils, permit greater persistence of large amounts of divergent relic DNA than evenly distributed populations (Lennon et al., 2018). The heterogeneous environment of soils also allows the adsorption of DNA to sediments, preventing enzymatic degradation of relic DNA (Romanowski et al., 1991). Finally, the perennially cold temperatures of permafrost soil are hypothesized to preserve relic DNA for up to 400 kya, as all DNA degradation pathways (including hydrolysis, oxidation, and alkylation) are temperature dependent (Willerslev et al., 2004).

Preservation of relic DNA from dead organisms can obscure biodiversity and abundance measurements. Microbial community analysis using 16S rRNA gene sequencing cannot differentiate between DNA from viable organisms and relic DNA. Biodiversity

assessment therefore includes all physiological states of life. To prevent this, researchers have treated soils with (1) DNase 1, an enzyme which degrades extracellular DNA, or (2) PMA, photoreactive high affinity DNA binding dye which prevent the amplification of extracellular DNA during PCR (Carini et al., 2017; Lennon et al., 2018). These studies have shown greater amounts of preserved relic DNA are associated with overrepresentation of species richness, though any effect that relic DNA may have on observed community composition has yet to be shown (Carini et al., 2017). The obfuscation of other biodiversity metrics (including Faith's phylogenetic diversity, evenness, and diversity) by relic DNA in soils has also not been observed. Due to the enhanced preservative abilities of permafrost soils, it is possible that relic DNA may have a more substantial impact on quantification of both β- and α-diversity in microbial communities. Evaluation of the total viable/relic microbial community composition of permafrost has yet to be performed. However, viability PCR has been conducted within permafrost which indicated no relic DNA present within the tested samples, though these experiments lacked optimization on cell cultures to ensure proper delineation of live and dead organisms (Yergeau et al., 2010). Further work is needed to quantify the impact that relic DNA can have on the biodiversity of microorganisms in ancient permafrost soils.

Dormancy is commonly found amongst organisms which are adapted to extreme environments and starvation stress. Most microorganisms within soils are metabolically dormant, existing as heterocysts, spores, or conidia (Blagodatskaya & Kuzyakov, 2013). While these taxa cannot participate in biogeochemical cycling, they may be poised to become active under optimal conditions, occurring over daily or seasonal intervals. These organisms represent a "seed bank" which can be revived in response to natural variation or anthropogenic disturbance. These operational definitions of life have allowed for better characterization of microbial viability and activity within permafrost soils. Predicting

whether these microorganisms become reactivated upon permafrost thaw is decidedly difficult, as this would require tracking of individual organismal changes with thaw.

1.5.0 Microbial Responses to Climate Change and Disturbance

The Arctic is currently undergoing the most rapid rates of change in the world. The International Panel on Climate Change (IPCC) is predicting increases in global temperature by 2-8.5 °C by the year 2100 (Allen et al., 2014). This warming is driven by increasing greenhouse gas concentrations, produced primarily through human activities but also by soil microbial feedback. Microbial activity is controlled by biodiversity, with greater functional diversity in response to species richness. Therefore, this section will describe how the Arctic is evolving with climate change, how this change is mediated by greenhouse gas concentrations, and how the microbial communities of northern soils are feeding into this cycle of warming.

1.5.1 Climate Change in the Arctic

Climate change is having a pronounced impact on the Northern Canadian landscape. Human induced warming has reached 0.93°C ± 0.13°C above global 1861-80 averages (Otto et al., 2015). Current policy aims to hold this increase well below 2°C pre-industrial levels and limit it to 1.5°C warming (Millar et al., 2017). However, climate change has an exaggerated impact at Northern latitudes – a phenomenon known as polar amplification – which results in higher surface air temperatures than global averages (Masson-Delmotte et al., 2006; Serreze & Francis, 2006; Zachos et al., 2001). As a result, average air temperatures

in Yukon have already increased 2°C and will continue to warm by more than 2°C over the next fifty years (Streicker, 2016). Active layer soil temperatures in Yukon have already increased by 3°C since the 1980s (Allen et al., 2014). As such, moderate global temperature increases are having a substantial impact on Northern ecosystems.

1.5.2 Greenhouse Gas Concentrations

Increases in atmospheric CO_2 concentrations affect global temperatures and climates by absorbing radiant energy from the sun (Allen et al., 2014). Anthropogenic climate change induced by increased atmospheric greenhouse gas concentrations is poised to alter Arctic climate by increasing air temperatures and precipitation. Current CO_2 concentrations began their increase during the industrial era, eventually reaching 390 ppm (Allen et al., 2014). Projections suggest CO_2 concentrations may increase up to 500-900 ppm by the end of the century. Alternatively, the potent greenhouse gas, methane, has 84 times the capability to warm the atmosphere than carbon dioxide over twenty years. For the last 650,000 years, the atmosphere has had methane concentrations of 320-790 ppb (Allen et al., 2014). By the year 2011, global atmospheric concentrations of methane had increased to 1803 ppb, primarily through wetland biogenesis and fossil fuel emissions (Allen et al., 2014). Outside of anthropogenic greenhouse gas production, microbial activity within thawing permafrost will become increasingly important with heightened global temperatures as methane and carbon dioxide atmospheric concentrations increase.

1.5.3 Microbial community responses to permafrost thaw

Permafrost thaw experiments are commonly composed of either field warming experiments or laboratory mesocosm experiments. Field warming studies use a diversity of

approaches to warm soils, including open top chambers, overhead infrared lights, and soil warming cables. Environmental manipulations can also encompass moisture treatments (including irrigation to increase water input, and roof installation to decrease water input), and snow cover manipulation (including snow fences to accumulate snow or warming and snow removal to reduce snow coverage) (Donhauser & Frey, 2018). These approaches best reflect natural conditions but can allow for natural fluctuations and co-varying parameters to obscure real trends. In contrast, laboratory experiments allow for precise manipulation of environmental conditions but are incapable of recreating natural stochasticity. Common manipulation in laboratory mesocosm experiments include temperature manipulation (warming, temperature cycling, and freeze/thaw treatments), moisture manipulation, nutrient manipulation, and introduction of functional microbial groups (methanogens) (Chen et al., 2016; Donhauser & Frey, 2018; Knoblauch et al., 2018; Mackelprang et al., 2017; Vorobyova et al., 1997). Natural thaw gradients are becoming more commonly used to determine the microbial ecology in response to permafrost thaw (Chen et al., 2016; Crevecoeur et al., 2015; Mondav et al., 2017). However, these gradients come with limited site history and natural landscape variation may induce microbial community shifts independent of thaw effects.

1.5.3.1 Soil Warming Experiments

In response to *in situ* warming, active layer soil microbial communities have shown no shift, shifts in phylogenetic profiles without taxonomic changes, or delayed shifts after years of artificial warming (Penton et al., 2013). Manipulation of nutrient and moisture levels have also shown no shift in microbial communities or bulk soil respiration of active layer soils (Lamb et al., 2011). *In situ* permafrost thaw has shown contradictory results with microbial community changes in some studies (Mondav et al., 2017; Monteux et al., 2018;

Woodcroft et al., 2018), or no changes in others (Lamb et al., 2011). While seasonal greenhouse gas flux from these warming soils is likely to become more extreme, experimental warming does not appear to affect microbial community structure in the presence of freeze/thaw cycles in forest soils (Ernakovich et al., 2014; Kuffner et al., 2012). While investigating active layer warming in Alaska, Xue et al., (2016) found increases in carbon degradation genetic potential. These soil warming experiments predominantly assess microbial community shifts only within the active layer. However, Monteux et al., (2018) performed a long term *in situ* thaw experiment in the Storflaket palsa in Northern Sweden, assessing microbial community structure and soil respiration rates after ten years of treatment. Researchers identified a shift in the microbial community of permafrost away from the active layer, however, transition layer soils became like active layer soils. Thaw increased the abundance of Acidobacteria, Firmicutes, Proteobacteria, and Verrucomicrobia within transition layer soils, becoming more similar to active layer phyla. In the absence of edaphic parameter and vegetation changes, community shifts are likely due to invasion of microorganisms from the active layer. Warming experiments therefore suggests that while changes at the genomic level may be rapid in the active layer, other community changes may require longer periods of time for succession to occur.

1.5.3.2 Laboratory Thaw Experiments

Unlike *in situ* experiments, laboratory thaw experiments have identified significant changes in microbial community structure, transcriptional activity, respiration, and genetic potential (Coolen & Orsi, 2015; Coolen et al., 2011; Oelbermann et al., 2008; Mackelprang et al., 2011). Early incubation experiments identified microbial community changes in both active layer and permafrost soils with rapid thaw and suggested that the rapid succession of

microbial communities was related to carbon recalcitrance (Coolen et al., 2011). “First responders” to permafrost thaw are capable of recalcitrant carbon degradation (Basidiomycetes and candidate subdivision OP5), while subsequent taxa were composed of labile carbon degraders (β-Proteobacteria, Actinobacteria, and Firmicutes) (Coolen et al., 2011). While successional patterns were observed, interpretations of function from taxonomic assignment must be made with caution, requiring further study into genetic potential and activity of the community. Short term incubation of permafrost soils at 5°C has shown both microbial community shifts and shifts in genetic potential, the latter of which shifted to become more similar to the active layer (Mackelprang et al., 2011). Genes involved in nitrate reduction, denitrification, and ammonification and in central metabolism all increased in permafrost soils with thaw (Mackelprang et al., 2011). Another study investigating transcriptional responses to short term thaw at 4°C identified Bacteroidetes, Firmicutes, ascomycete fungi, and methanogens as having the largest transcriptional response with a predominance in transcripts associated with soil organic matter decomposition (Coolen & Orsi et al., 2015). The irreproducibility of these results may be due to free water increases decreasing water levels resulting from thaw, which otherwise would drain *in situ*.

1.5.3.3 Environmental Thaw Gradients

To characterize actual natural variation in thaw sites, many studies have investigated the microbiology of natural thaw gradients, primarily in active layer soils impacted by permafrost collapse (Chen et al., 2016; Crevecoeur et al., 2015; Hultman et al., 2015; Mondav et al., 2017; Taş et al., 2018; Yuan et al., 2018). The end-states of thaw (be it upland oxic soils, or lowland anoxic waterlogged soils) have shown significantly higher activity measurements than permafrost soils (Hultman et al., 2015). The type of activity is primarily

driven by water content, with higher rates of fermentation and methanogenesis in wetter soils, and higher rates of carbon mineralization, methane oxidation and nitrogen assimilation in drier soils (Hultman et al., 2015; Taş et al., 2018). Community composition shifts, both at phylogenetic and at OTU levels, have been shown across thaw gradients in both the Qinghai-Tibet Plateau and Sweden (Mondav et al., 2017; Wu et al., 2018a). Thawed soils in Sweden showed lower Acidobacteria and Actinobacteria relative abundances while having increased abundances of Bacteroidetes, Chloroflexi, and Euryarchaeota in thawed sites (Singleton et al., 2018). The microbial diversity of soils affected by permafrost thaw are also much higher than those which have not undergone disturbance (Mondav et al., 2017). Dramatic changes in genetic potential with permafrost subsidence have also been observed, particularly regarding carbon metabolism across pathways for xylose degradation, propionate fermentation, and hydrogenotrophic methanogenesis (Singleton et al., 2018). The underlying drivers of these microbial community changes are commonly by pH, water content, carbon recalcitrance, electrical conductivity, organic carbon, methane, carbon dioxide, as well as pore water chemistry (Mondav et al., 2017; Chen et al., 2016; Tripathi et al., 2018a). These community shifts were also associated with phylotype trophism, including autotrophy, methanogenesis, and methanotrophy, and may be driven by keystone species including Methanomassiliicoccus, Bathyarchaeota, Methanobacterium, Nitrospiraceae, and Gaiellales (Mondav et al., 2017). Landform level changes, though difficult to monitor for long periods of time, deliver more accurate data regarding the fate of soils following permafrost subsidence than microcosm experiments. Environmental gradients provide natural conditions that would not otherwise be replicable in an artificial environment. Therefore, these sites may be more reliable than soil warming or microcosm experiments. These studies suggest

microbial interactions in addition to edaphic parameters may contribute to microbial community structure and function.

Water bodies originating from permafrost thaw can have dramatically different ecosystem function than other lakes, and the microbial study of these landscapes can illuminate the range of community endpoints resulting from permafrost thaw. Early culture dependent studies of thermokarst lake water identified the dominance of Gammaproteobacteria (Boyd & Boyd, 1964), while high-throughput studies have identified the dominance of proteobacterial phyla within thermokarst lakes (Comte et al., 2016; Crevecoeur et al., 2015). Photosynthetic bacterial surveys of lakes in Ellesmere Island have identified picocyanobacteria within the oxygenated upper waters, while green sulfur bacteria were dominated in oxygen depleted depths (Antoniades et al., 2009). These findings were supported in more recent studies across environmental gradients in Northern Quebec (Crevecouer et al., 2015). As well, thermokarst lakes resulting from permafrost thaw and subsidence have been shown to create distinct microbial communities and assembly processes compared to natural lakes (Comte et al., 2016). Thermokarst lakes are significantly impacted by invasion of permafrost taxa, and these microorganisms persist after thaw and inoculation into these waters. However, characterization of these communities is still in its infancy, as demonstrated by the predominance of poorly understood taxa in Arctic thaw ponds (Wurzbacher et al., 2017). These rare taxa and complex community dynamics are due to the unique source from which these waters originate.

1.5.4 Permafrost Carbon Feedback and Microbial Activity

Thermokarst lakes and wet tundra soils represent key methane sources within Northern latitudes, representing ⅓ of the world's soil methane emissions (Bartlett & Harriss, 1993; Mackelprang et al., 2011). Methane fluxes from thawing permafrost may be sourced from either ancient frozen methane clathrates or from anaerobic decomposition of carbon (Wadham et al., 2012). However, the predominant source of released greenhouse gases in thermokarst bogs is from methanogenesis of recently deposited carbon rather than methane clathrates (Cooper et al., 2017). Microbial degradation of carbon, through methanogenesis, is intimately tied to community composition of both methanotrophic and methanogenic microorganisms (McCalley et al., 2014). Together, this evidence suggests that methanogenic community composition is critical to the production and release of methane from thawing permafrost soils. Degradation of methane is also controlled by microbial communities, through methane oxidation. Approximately 88% of methane produced in boreal lakes is removed by these methanotrophic communities (Kankaala et al., 2006; Kankaala et al., 2007), but Northern lakes still represent methane sources (Northington & Saros, 2016; Wik et al., 2016). Therefore, filtering of methane in the Arctic is limited to aerobic well-drained High Arctic soils (Stackhouse et al., 2015). External factors can also mitigate methane release through metabolic inhibition of methanogenesis by phenolic compounds (Freeman et al., 2001), or stimulated through the presence of sedge communities (Knoblauch et al., 2015). Overall, the Arctic is still a net source of methane, representing a key component of climate feedback from thawing permafrost (Denman et al., 2007).

Microbially mediated decomposition of stored organic matter leads to the efflux of CO_2 (carbon dioxide) and CH_4 (methane) from thawing permafrost (Davidson & Janssens,

2006). However, *in situ* and *in vitro* thaw experiments have revealed opposing trends. Long-term *in* situ experiments have shown an initial burst of CO_2 flux with SOM loss, which then decrease in following years (Luo et al., 2001; Oechel et al., 2000. Across circumpolar geography, decade long carbon losses can range from 1-76% of total soil carbon as indicated by microcosm warming experiments (Schuur et al., 2015). Work is needed to replicate the conditions found in laboratory experiments to identify landscape conditions which these experiments replicate. These incongruencies may be due to geographic variability, edaphic parameters (primarily C:N ratios), as well as varying experimental conditions across laboratories (particularly oxygen content) (Schuur et al., 2015). These conditions may result in inconsistent oxygen content across soils and laboratories. Differing oxygen concentrations within a soil sample can dramatically impact soil function, as carbon degradation rates are intimately controlled by oxygen content, with anaerobic soils fluxing 78-85% less carbon than aerobic soils after one year of warming (Schuur et al., 2015). Therefore, heterogeneity of *in situ* oxygen levels make extrapolations from microcosm experiments across landscapes problematic. In addition, year-long incubation experiments do not allow sufficient time for critical microbial succession to take place. For example, methanogenic communities have low dispersal range and succession occurs over much longer time scales than other microbial guilds. As a result, while short-term studies indicate low methanogenic activity, multi-year *in* situ experiments allow for the development of stable and active communities with higher CO_2-C flux than would be predicted from microcosm experiments (Knoblauch et al., 2018). Furthermore, decadal long soil warming experiments have shown non-linear trends in CO_2 flux rates, which are instead driven by microbial community succession in response to external forcing factors (such as litter deposition, changing plant communities, and shifting edaphic parameters) (Melillo et al., 2017). Current research therefore requires validation of

short-term experiments in the field, as well as continued research into the succession and activity of microbial communities over decadal time scales.

1.6.0 Research Project

1.6.1 The Changing Permafrost Microbial Community

Increased human presence in the Arctic and subarctic is predicted to increase industrial disturbance over the next 100 years. There is limited knowledge on the impact that anthropogenic disturbance has on the microbial communities of subarctic soils, particularly those found in permafrost. Excavation of the active layer and upper permafrost horizons may dramatically alter northern landscapes, thereby opening permafrost to rapid thaw. However, it is unknown how these disturbances impact the permafrost microbial community. This study will determine the source of the viable and active microbial community in the face of abrupt anthropogenic thaw, which results in the formation of a thermokarst pond.

1.6.2 Research Question and Hypothesis

Understanding the microbial community of thawing permafrost can aid in quantifying the effect that the changing microbial community can have on permafrost carbon feedback. Therefore, I am asking: How does the microbial community of active layer and permafrost soils respond to anthropogenic disturbance and thaw? Landscape altering permafrost thaw restructures the microbial function, composition, and biodiversity of soils, but these impacts cannot distinguish the possible driving factors of microbial compositional changes due to concomitant changes in soil chemistry, vegetation, space, and time. Other more direct studies into soil warming cannot preclude the ability of active layer microbiota to invade lower soil

horizons. I hypothesize that if the active layer were excavated from this system, the increase in surface energy balance would result in permafrost thaw, and thereby induce a community shift in the permafrost microbiome to become more similar to the active layer.

1.6.3 Aims and Objectives

I aim to assess the effect that the active layer has on permafrost microbial community composition and permafrost carbon dynamics in response to anthropogenic disturbance. I will determine (1) the microbial community composition and biodiversity across undisturbed and disturbed soils and (2) the microbial viability and abundance across disturbed and undisturbed soils. I predict that if permafrost thaw is capable of dramatically impacting microbial community structure, the microbial community of thawed permafrost will shift to resemble that of the active layer. Additionally, I predict that the proportional viability of the permafrost microbial community will not reach similar proportions as found within undisturbed active layer soils.

1.6.4 Experimental Design

Cores from Dominion Creek (NTS:116-B/3; DMS: 60°42”59.5’N,138°33”0.14’W) were used to determine the impact anthropogenic disturbance has on active layer and permafrost soils. The bacterial 16S ribosomal rRNA genes of whole soil community extracts were sequenced on a paired-end MiSeq Illumina platform. Additionally, both permafrost and active layer samples were treated with PMA prior to DNA extraction to evaluate the impact relic DNA has on the assessment of permafrost and active layer microbial biodiversity. To determine the viability and abundance of microbial cells, soils were also subjected to standard

cell extraction protocols and cells were enumerated using the BacLight Live/Dead cell viability staining kit.

Chapter 2: Bacterial community composition changes independently of soil edaphic parameters with anthropogenic permafrost thaw

Neuberger, P., A. Saidi-Mehrabad, D. Froese, and B. Lanoil. Bacterial community composition changes independently of soil edaphic parameters with anthropogenic permafrost thaw. *In preparation for submission to Environmental Microbiology*.

2.1 Originality-Significance Statement

The effect of permafrost thaw on soil microbial communities due to anthropogenic disturbance has yet to be studied in the subarctic of Canada, limiting our ability to predict future microbial community shifts with increased human activity in circumpolar regions. In this study, I examined the viability and composition of the microbial community in an anthropogenically-induced permafrost thaw gradient in subarctic Yukon. Sites were sampled six weeks after initial disturbance. Microbial community membership and composition in the disturbed sites more closely resembled those of active layer soils than those of the permafrost that they originated from, independent of edaphic parameters. Communities from disturbed sites closely resembled each other, regardless of the level of disturbance. Although permafrost microbial indicator species persisted in the disturbed samples, overall these communities more closely resembled active layer samples. Community assembly was deterministic across the active layer, permafrost, and disturbed soils, with increasingly negative interactions with increasing disturbance. This study demonstrates the importance of assessing microbial ecology of permafrost and has identified microbial community shifts for the first time in response to anthropogenic disturbance in the field.

2.2 Abstract

As the Arctic warms, permafrost thaw can lead to dramatic ecosystem changes, including the formation of thermokarst ponds. The potential for microbial degradation of organic carbon increases with thermokarst formation, resulting in flux of the greenhouse gases CO_2 and methane. In laboratory experiments, permafrost microbial community composition and gene expression shift rapidly upon thaw; however, there have been few studies examining the effect of thaw on resident microbial communities under field conditions. To examine the effect of anthropogenically-induced permafrost thaw on living microbial communities, I surveyed a site where thaw was induced by stripping of the area's vegetation and one meter of topsoil in preparation for gold mining at a site near Dominion Creek, Yukon, Canada. I analysed three cores across a permafrost thaw gradient from undisturbed forest active layer to disturbed soils composed of recently thawed permafrost to a thermokarst pond. Subsamples were taken from each core at depths of 15, 30, 45, 75, and 95 cm including the active layer and permafrost where applicable. An additional eight surface soil samples were collected along the same gradient. Soil physicochemical parameters and microbial community composition, using sequencing of the V4 region of the 16S rRNA gene, was profiled across these soils. The soil edaphic parameters did not change significantly six weeks after disturbance, however, microbial communities showed significant changes. Cluster analysis of the total bacterial assemblage identified three distinct groups within the dataset: (1) undisturbed active layer, (2) lower active layer, disturbed active layer, and disturbed permafrost, and (3) intact permafrost, indicating that soil disturbance alters the microbial community of surface soils. Community shifts were controlled by pH and Zn, in addition to community interaction metrics, suggesting that these shifting communities are

primarily controlled by internal forcing factors. This study suggests a strong microbial community response to permafrost disturbance under field conditions and that this response occurs prior to shifts in the measured soil edaphic parameters. Anthropogenic disturbances may significantly change microbial biodiversity in permafrost-affected soils, thereby impacting ecosystem functioning and stability.

2.3 Introduction

Permafrost, *i.e.* soil below 0°C for at least two consecutive years, contains a disproportionately large amount of soil organic carbon. While 24% of terrestrial soils are underlain by permafrost, approximately 25-50% of all global soil organic carbon is harboured within these perennially frozen materials (Schuur et al., 2009). Roughly 1,700 Gt of carbon is present in the top three metres of permafrost affected soils, more than twice as much as currently in the atmosphere (Schuur et al., 2015; van Huissteden & Dolman, 2012). A large portion of this permafrost carbon is vulnerable to release over the next century as surface temperatures increase due to climate change (Cooper et al., 2017). Alaskan tundra ecosystems have already switched from net carbon sinks to net carbon sources suggesting this process of carbon release from the Arctic is already ongoing (Belshe et al., 2013; Commane et al., 2017; Deluca & Boisvenue, 2012). All permafrost sediments combined may be responsible for the release of ~130-160 Pg of carbon in the form of greenhouse gases by the year 2100, similar to or greater than other substantial biospheric carbon sources (Schuur et al., 2015; Le Quéré et al., 2015).

Permafrost thaw is a dynamic process which can result in active layer thickening or abrupt permafrost subsidence and landform changes (van Huissteden & Dolman, 2012). Laboratory permafrost thaw experiments have shown carbon fluxes are affected by soil water content, but how this relates to abrupt forms of permafrost thaw has not been well characterized (Schuur et al., 2015). Additionally, anthropogenic disturbances are known to create soil environments similar to those created by thermokarst, including active layer detachment slides and retrogressive thaw slumps (Forbes et al., 2001; Kokelj & Jorgenson,

2013). How the permafrost microbial community, which drives this carbon flux, changes in response to direct-anthropogenic disturbance has not been extensively studied.

The microbial diversity of permafrost is substantial despite extreme environmental conditions, though this diversity is often lower than observed in active layer soils or thermokarst pond sediments (Hultman et al., 2015). These diverse microbial communities are capable of microbial activity, growth, and division at sub-zero temperatures, possibly having important implications for carbon turnover in permafrost, both before and after permafrost thaw (Demkina et al., 2008; Kryazhevskikh et al., 2012; Tuorto et al., 2014). With laboratory thaw, permafrost microbial communities respond with increased metabolic activity (Mackelprang et al., 2011), increased diversity (Mackelprang et al., 2011), increased transcriptional activity (Coolen & Orsi, 2015), increased genetic potential for nutrient cycling (Mackelprang et al., 2011; Xue et al., 2016), and a shift in the microbial community to resemble those of active layer soils (Mackelprang et al., 2011). Dynamics between anoxic/oxic environments are likely to replace the frozen permafrost/active layer dynamic, impacting landscape of soil physicochemistry, with distinct microbial communities between oxic and anoxic environments (Singleton et al., 2018). Within the last decade, an abundance of information has been collected regarding *in situ* permafrost thaw in response to landscape changes, suggesting that shifts in water regimes may have critical impacts on thawing permafrost regions (Taş et al., 2014; Taş et al., 2018; Singleton et al., 2018; Woodcroft et al., 2018; Emerson et al., 2018; Hultman et al., 2015). As such, spatio-temporal community and environmental dynamics of soils undergoing permafrost thaw may not be replicated in small-scale discrete and controlled laboratory experiments.

The observation of shifts in microbial community composition and genetic potential has primarily been performed in laboratory incubations and has seldom been corroborated in

field experiments. Warming experiments of permafrost-affected soils in the field have produced contradictory data, showing no community shift (Lamb et al., 2011), community shifts (Monteux et al., 2018), or shifts in genetic potential but not community composition (Yuan et al., 2018). Field studies using permafrost thaw gradients have shown that permafrost subsidence creates distinct microbial communities, but often lack sufficient site history to delineate the effects of permafrost thaw from other forms of disturbance (Hultman et al., 2015; Mondav et al., 2017; Taş et al., 2014). Differences between field and laboratory experiments may arise due to the absence of critical environmental factors in laboratories such as: a sizeable plant community and associated rhizosphere, complex hydrological systems, and microbial dispersal (Andam et al., 2016; Monteux et al., 2018). However, little is known concerning the impact that direct-anthropogenic-disturbance has had on the microbial communities of permafrost-affected soils. Plant communities undergo dramatic long-lasting changes in response to low levels of disturbance in the High and Low Arctic, as shown through altered plant community composition and physiognomy as well as altered soil chemistry in both modern and historic sites (Forbes et al., 2001). The greatest of these disturbances follows soil horizon removal, requiring 20-75 years to recover function, composition, and physiognomy of plant communities (Forbes et al., 2001). These effects are accompanied by chemical changes (pH increasing with disturbance) and vegetation changes (decreased plant abundance and biomass) analogous to those found in active-layer detachment slides and retrogressive thaw slumps (Kokelj & Jorgenson, 2013; Forbes et al., 2001). Changes in edaphic parameters have been identified in some soils across permafrost thaw gradients, including increasing water content, dissolved methane, dissolved CO_2, and pH, thereby driving microbial community composition (Mondav et al., 2017). However, no

work has been conducted to determine the impact of anthropogenic disturbances can induce in active layer and permafrost soil microbial communities.

Thawing permafrost may undergo increases in activity and carbon flux. Field studies have identified mineralization of young recently deposited and fixed carbon occurs at greater rates than the release of old stored methane and recalcitrant carbon (Cooper et al., 2017). Carbon dynamics of active microbial communities may change in response to permafrost disturbance as deposition of young carbon in excavated plots may be lacking with the absence of a sizeable plant community. Permafrost thaw enhances microbial activity as observed through increases in respiration, methanogenesis, and denitrification (Hultman et al., 2015; Palmer et al., 2012). It is unknown whether this increase in microbial activity is accompanied by a corresponding increase in microbial viability with permafrost thaw, or if a small fraction of live microbes is responsible for permafrost carbon cycling feedback. While specific taxa become more transcriptionally active with permafrost thaw, only a fraction of the total community can participate in biogeochemical cycling (Coolen & Orsi, 2015). Despite low levels of activity under frozen conditions, the viability of Arctic permafrost does not differ from temperate soils and is composed of viable – either dormant or active – microorganisms (Hansen et al., 2007; La Ferla et al., 2017; Mackelprang et al., 2017). Reactivation of eukaryotic and bacterial taxa after permafrost thaw has been observed, but how viability and abundance of bacteria at the community level responds to abrupt permafrost thaw has yet to be discerned.

Edaphic parameter changes as well as carbon and nitrogen dynamics of abrupt thaw sites have been well characterized across retrogressive thaw slumps, active layer detachment slides, thermal erosion gullies, and thermokarst water bodies (Abbott & Jones., 2015; Becker et al., 2016; Lantz et al., 2009; Vogel et al., 2009). However, the microbial community of

rapidly thawing soils in the microtopographies resulting from these abrupt thaw processes are seldom characterized (Taş et al., 2018; Wu et al., 2018b), while no previous studies have examined the effect of direct anthropogenic disturbance on permafrost microbial community structure. I therefore attempted to determine how microbial community structure changes in response to thaw in the field. As microbial communities have been closely linked to edaphic parameters, I hypothesized that microbial community shifts are dependent on edaphic parameter changes in response to disturbance. Soils and vegetation were stripped in preparation for mining, thereby exposing permafrost to atmospheric temperatures for six weeks prior to sampling at a site in Dominion Creek, Yukon, Canada. I characterized the edaphic parameters, microbial community composition, microbial cell abundance and viability of this thawing permafrost disturbance gradient from undisturbed active layer soils to disturbed active layer and permafrost soils, ultimately culminating in a thermokarst pond. Additionally, viable and total assemblages of permafrost, undisturbed active layer, and disturbed permafrost soils were compared and described in the Appendix. These disturbed soils were compared to reference samples from beneath and adjacent to the disturbance gradient. My results suggest that six weeks of thaw shifted the bacterial community of permafrost to resemble that of the active layer and that this occurred without a significant shift in edaphic parameters.

2.4 Methods

Field Site and Sampling Procedure

Surface samples and soil cores were collected in May 2016 at a gold mining site near Dominion Creek, Yukon Territory, Canada (NTS:116-B/3; DMS:

60°42”59.5’N,138°33”0.14’W), approximately 50 km southwest of Dawson City, Yukon, Canada (Figure 1). One metre of soil was excavated, and all surface vegetation was removed by the miners, exposing permafrost to atmospheric temperatures and inducing permafrost thaw as well as thermokarst pond formation. Field work was conducted six weeks following this disturbance.

Quadruplicate surface samples (unfrozen soils) were taken at 6 locations across the disturbance gradient with a gradual progression to the thermokarst pond edge: in undisturbed permafrost-affected soils (site A; 474 cm from the thermokarst pond); disturbed active layer soils (sites B-D; 363 cm, 288 cm, and 213 cm from the thermokarst pond, respectively); and putatively disturbed permafrost sediments (sites E-F; 109 cm and 0 cm from the thermokarst pond, respectively). Surface samples were collected after the removal of the organic layer and any remaining surface vegetation with a spade. Samples were collected with a separate spade below the O-layer at approximately 5 cm depth and stored in sterile Whirlpak® bags and homogenized both in the field and prior to chemical and biological analyses.

Soil cores 1-3 metres in depth were collected at three sites along the same thaw gradient (from Core 1 – Core 3). Core 1 was sampled in a nearby clearing analogous to the conditions at sample A (the “undisturbed” site). Core 2 was sampled 2.6 m from the thermokarst pond between surface sample site C and D. The Core 2 site had all vegetation, the organic soil horizon, and the surface soil horizon of active layer removed; however, active layer subsoils were still present in Core 2. Core 3 was sampled directly adjacent to the thermokarst pond, with all soil layers above the frozen soil removed. For all three cores, coring was conducted below a thaw depth of 15 cm and all thawed active layer soil above this was removed prior to sampling. Cores were taken with an EDR-260 (ECHO Inc., Lake Zurich, IL, USA), a light portable gas-powered permafrost drill based on Calmels & Allard

(2004). Each core had a 10 cm diameter and ranged in length from 14 – 36 cm long and reached a depth of at least 1 m. Loose material on the surface of soil cores was removed by scraping with razor blades and discarded. Frozen soil cores, including core from both active layer and permafrost horizons were placed in clear polypropylene bags (Uline, Pleasant Prairie, WI, USA), transferred to coolers with ice-packs in the field, and stored at -20°C until further analysis. Active layer depth was determined using loss on ignition and water content of each core profile.

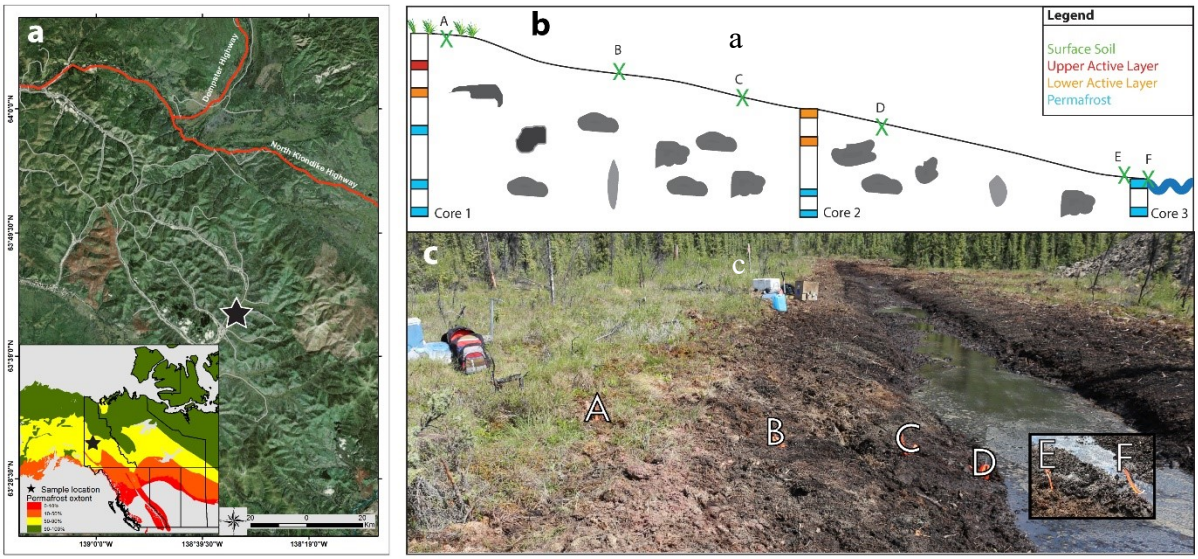


Figure 1. Dominion Creek core and surface sample locations. (a) Map of Dominion Creek area and sampling site within Yukon Territory; (b) cross-section of disturbance gradient soils and permafrost cores; and (c) the disturbance gradient soils including A (least disturbed), B-D (disturbed active layer), and E-F (disturbed permafrost). The map of the Dominion Creek region was assembled by Ali Naeimi Nezamabad in ArcGIS 10.6.1. Disturbed permafrost samples are inset and were found 3 metres away at a shallower location of the thermokarst pond.

Soil Chemistry Subsampling and Analysis

Disturbance gradient soils were subsampled as follows: 0.5 g for loss on ignition (LOI) estimation of total organic carbon content, 0.5 g for gravimetric water content; 0.5 g for pH; 5 g for nitrate analysis; 0.5 g for dissolved metal and TN/TC (Total Nitrogen/Total Carbon) analyses.

Cores were sectioned into ~1/3 (3cm length) and ~2/3 (7cm) sections for chemical and biological analyses, respectively. Cutting was performed using a masonry saw. Two subsections were taken from the 1/3 portions (chemical analysis section) of the cores. Prior to subsampling, subsections were scraped with a razor blade to remove all potentially thawing and contaminating materials. Section 1 was used for pH, soil organic matter (SOM), and gravimetric water content analysis and was cut into cubic subsamples (1 cm^3) which were collected throughout the entire depth profile of each core, resulting in 536 subsamples. Soil water content was determined by oven drying the samples at 105°C for 24 hours. Soil pH was determined using a soil to 0.01 M $CaCl_2$ ratio of 1:2 using an AB15 pH meter (Fisher Scientific, Watham, MA, USA). SOM was measured using loss on ignition (LOI) procedures (Lim & Jackson 1982), using a Lindberg SB Muffle Furnace (Thermo Fisher Scientific Inc., USA).

Section 2 was used to analyse nitrate + nitrite (NO_3), ammonia (NH_4), total nitrogen (TN), total carbon (TC) and dissolved metal analyses including; P, K, S, Mg, Ca, Fe; Cu, Mn, Zn, N and was cut into 5 cm × 3 cm × 3 cm subsamples. Section 2 subsamples were homogenized, and 0.5 g of material were taken for TN, TC and dissolved metals while the remainder was used for NO_3 and NH_4 measurements at the Natural Resources Analytical Laboratory (NRAL, Edmonton, AB, Canada). TN and TC were analyzed using standard dry

combustion methods with a Costech Model EA 4010 Elemental Analyzer (Costech International Strumatzione, Florence, Italy, 2003) (Sparks et al., 1996). NO_3 and NH_4 were extracted using a 2M KCl extraction and measured using a Diazo Coupling method with a SmartChem Discrete Wet Chemistry Analyzer, Model 47 200 (Westco Scientific, Brookfield, CT, USA) (Maynard et al., 1993). Trace metal analysis was conducted after an HNO_3/HCl acid digest using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) which measured P, K, S, Mg, Ca, Fe, Cu, Mn, Zn, Na using standard protocols on a Thermo iCAP6300 Duo ICP-OES (ThermoFisher, Cambridge, United Kingdom) (Skoog et al., 2007).

Biological Subsampling

The 2/3 portions of the cores ("biological sections") were cut into 5 cm × 3 cm × 3 cm subsections. Discrete 5 cm long subsamples were taken at 15 cm, 30 cm, 45 cm, 75 cm, and 95 cm below the soil surface: Core 1 was subsampled at all horizons; Core 2 was subsampled at 30 cm, 45 cm, 75 cm, and 95 cm; Core 3 was subsampled at 75 cm, and 95 cm. Not every horizon was present in each core following disturbance: Core 2 did not have a 15 cm horizon, and Core 3 did not have a 15 cm, 30 cm, or a 45 cm horizon. Depths were aligned between cores according to pH, SOM, and water content.

To enable detection of potential contamination, each subsample was painted with 10 mL of 1 × PBS solution containing *E. coli*:DH10B :: pBAD *mNeonGreen* at 8.3×10^9 cells/mL; for a total of 8.3×10^{10} cells/core. Following decontamination (see below), the presence of PCR-amplifiable pBAD vector DNA would be interpreted as potential contamination, and samples discarded.

DNA extraction subsamples were prepared in a class 10,000 clean lab at the University of Alberta which has not been used previously for DNA amplification. To

decontaminate cores, samples were: 1) immersed in 100% bleach solution, 2) washed with DNA free water, and 3) scraped approximately 4-7 g of material using a razor blade; this cycle was repeated twice. Subsamples were then homogenized within a sterile Whirlpak® bag. The resulting decontaminated soils were then stored at -20°C until DNA extraction. Details of this decontamination protocol are provided in Saidi-Mehrabad et al., (in prep).

Each decontaminated DNA extraction subsample was thawed at room temperature and homogenized within the sample bag and triplicate 0.5 g samples were removed using a sterile spatula. Genomic DNA was extracted using the MOBio PowerSoil kit (MOBio. Carlsbad, CA) according to manufacturer's instructions. Triplicate blank extractions (consisting of sterile water) were performed alongside soil DNA extractions. Replicate extractions were pooled prior to contamination checks and sequencing.

Contamination Detection

PCR amplification with primers pBAD-forward (5′-ATGCCATAGCATTTTTATCC-3′) and pBAD-reverse (5′-GATTTAATCTGTATCAGG-3′) (Invitrogen) was used to detect potential contamination. The PCR mix consisted of 1.25 units of Q5 DNA polymerase; 0.5µM of each primer; 10µL of 5X PCR Buffer; 0.2mM dNTPs; 2µL of DNA template; and DNase RNase free H_2O to a total volume of 50µL (New England Biolabs Inc., Ipswich, Massachusetts, USA). A thermocycler protocol was then conducted in a S1000 Thermal Cycler (Bio-Rad Laboratories, Inc., California, USA): a denaturing step at 95°C for 1 min; 30 amplification cycles of 30 sec at 95°C, 45 sec at the annealing temperature of 55°C, and 45 sec at 72°C; and a final extension for 10 minutes at 72°C. PCR product was absent in all DNA extractions, indicating the decontamination protocol worked with high efficiency.

Blank extractions were performed on each DNA extraction kit. To check for DNA contaminants, PCRs of blank exactions were performed using the primers 341F (5′-CCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer et al., 1993). The PCR mix consisted of 1.25 units of Q5 DNA polymerase; 0.25 µM of each primer; 10µL of 5X PCR Buffer; 0.1 mM dNTPs; 1 µL of DNA template; and DNase RNase free H_2O to a total volume of 50µL (New England Biolabs Inc., Ipswich, Massachusetts, USA). The PCR protocol included: a denaturing step at 95°C for 4 min; 10 touchdown amplification cycles of 15 sec at 95°C, 40 sec at 65-55°C, and 30 sec at 72°C; 25 amplification cycles of 15 sec at 95°C, 40 sec at 55°C, and 30 sec at 72°C; and a final extension for 10 minutes at 72°C. No bands were observed following gel electrophoresis of blank extract PCR amplification.

16S rRNA Gene Amplicon Sequencing and Analysis

The microbial communities of soil samples were surveyed by small subunit rRNA gene amplicon sequencing. DNA from the V4 region of the 16S rRNA gene was amplified with the primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) primers (Caporaso et al., 2012). Sequencing was performed commercially by Microbiome Insights (Microbiome Insights, Vancouver, BC, Canada), with an Illumina MiSeq using V3 chemistry (Illumina, San Diego, California, United States). Prior to sequence processing a total of 552,252 reads were recovered from 23 samples including surface samples, core samples, blank extractions, negatives, and a laboratory-constructed mock community. The read count of experimental samples ranged from 10,525 to 47,389 reads per sample. Sequence processing was performed in USEARCH v10 (Edgar, 2010). Overlapping reads were combined from forward and reverse reads with

the following qualifiers: no unknown base pairs allowed (Ns), a maximum number of sequence mismatches of 10 nt in the alignment, a minimum merge length of 230 bp, a maximum merge length of 300 bp, and an ID cut-off of 80%. Of the 552,252 reads recovered, 515,916 reads were aligned into overlapping reads (93.4% of the total), at an average length of 253 bp, with an expected size of 291 bp. Quality filtering with a maximum expected error cut-off of 1.0 resulted in 510,757 (99.0% of the total) of the reads passing (Edgar & Flyvbjerg, 2015). Dereplication identified 148,294 unique sequences and 105,589 singletons (71.2% of the unique reads) for the total dataset. OTU clustering at 97% using UPARSE identified a total of 5,964 OTUs as well as identifying and removing 12508 chimeras, while removing all singletons and doubletons (Edgar, 2013). After sequence processing 386,419 reads remained. Taxonomy was assigned using de novo picking as implemented in SINTAX using the RDP database at a bootstrap cut-off of 80 (Ribosomal Database Project) (Edgar, 2016). Sequences were separated for bacterial (377,578 reads) and archaeal (8,841 reads) analysis. Taxa identified as chloroplast/streptophyta using SINTAX were removed from downstream analysis and no mitochondrial reads were observed, leaving 361,034 reads in the bacterial dataset (Edgar, 2016). A NEWICK formatted 16S rRNA phylogenetic tree was constructed using USEARCH v10. For bacterial analysis, samples were rarefied to 10,000 reads. For Archaeal analysis, samples were rarefied to 100 reads.

Microbiological Diversity, Taxonomy and Assembly

α-diversity metrics were calculated using Mothur v 1.39.5 including species richness, Chao 1 richness estimation, Shannon Diversity, Shannon Evenness, Simpson Diversity, Simpson Evenness, and Heip's Evenness (Schloss et al., 2009). Checking for significant

differences between samples was performed using a Kruskal-Wallis test followed by post-hoc testing with Kruskal-Wallis multiple comparisons as implemented in SigmaPlot v13.0.

β-diversity was calculated USEARCH v10 using Bray-Curtis, Jaccard presence/absence, and weighted UniFrac distance metrics. Visualization of community dissimilarities was carried out using nonmetric multidimensional scaling (NMDS) and significance testing, was carried out using R v3.4.1, in the RStudio IDE with the *vegan* package v2.4-4 (Philip, 2003). Cluster integrity as assessed through silhouette width was carried out using the *fpc* package v2.1-10 (Hennig, 2018). Hierarchical clustering was visualized with the dendextend package v1.5.2 (Galili, 2015). Differences between clusters and correlation of soil physicochemical parameters with community composition were assessed using permutational multivariate analysis of variance in the *vegan* package v2.4-4. Environmental parameters were fit to the NMDS based on significant Spearman correlation ($p< 0.05$) plotted.

Phylum, class, and genus level tables were constructed using SINTAX (Edgar, 2016). A stacked bar chart of relative abundance for class level taxonomic classification was constructed in MS. Assessment and visualization of significant ($p<0.05$) differential abundances was assessed using analysis of composition of microbiomes (ANCOM) (Mandal et al., 2015). Significance testing was followed by post-hoc analysis using false discovery rate to correct for multiple comparisons. Only significantly different groups were visualized.

Indicator species were identified using the *indicspecies* package in R (De Caceres et al., 2016), and presence of indicator species in disturbance gradient soils were assessed using Mothur v 1.39.5. The net relatedness index (NRI) refers to the mean inter-species distance across a phylogenetic tree in a sample, where positive values correspond to an increased level of phylogenetic clustering, while negative values correspond to wider phylogenetic

distribution. The nearest taxon index (NTI) refers to the mean shortest distance between an OTU and all others within the sample (the degree of co-occurrence between closely related taxa); positive NTI values correspond to higher than expected phylogenetic clustering and taxon co-occurrence, while negative values correspond to lower levels of taxon co-occurrence. Assessing the ratio of NRI:NTI allows for determination of where community assembly is likely to occur: at the tree root (greater negative values) or at tree tips (greater positive values). Faith's Phylogenetic Diversity (PD) was used to assess total phylogenetic distance between community members within each sample. Phylogenetic divergence within samples can be calculated through dividing by OTU richness, thereby assessing overall branch lengths within a community. Community assembly metrics including NRI, NTI, and PD were assessed using the *picante* package in R (Faith & Baker, 2006; Kembel et al., 2010). NTI and NRI were inferred from 1 × ses.MNTD.obs.z as well as -1 × ses.MPD.obs.z, respectively. Observed NTI and NRI values were compared to an abundance-weighted null-model to obtain sample deviation from the standardized effect size. Checking for significant differences between samples was performed using a Kruskal-Wallis test followed by post-hoc testing with Kruskal-Wallis multiple comparisons as implemented in SigmaPlot v13.0. Significance testing and image processing was performed using SigmaPlot v13.0.

Mock Community Analysis

An equivalent amount of DNA (0.77 ng) was taken from 13 bacterial cell extracts and pooled into a mock community. The mock community sample had a total of 36,494 reads and 14 OTUs were recovered (Supplementary Figure 1). Two OTUs were assigned to Nitrosomonas, one of which may represent a spurious OTU recovered due to the bioinformatic pipeline used. However, this OTU represented only 0.0036% of the total reads

and was still assigned accurately to Nitrosomonas at the genus level. Abundance of mock strains was very uneven, with the most abundant OTU representing 46% of the total read count, while the least abundant OTU represented only 0.0015% of the total read count. Copy number of the 16S rRNA gene was not corrected for in the mock community. Caution must be taken when assessing relative abundances for both mock community data and sample data.

Cell Enumeration

Permafrost, active layer, and disturbance gradient soils (0.5 g) were diluted 1:100 (w/v) in 10 mM tetrasodium pyrophosphate (Fisher Scientific, Hampton, NH, USA). The resulting soil slurry was hand shaken for 15 minutes with a monolayer of glass beads then sonicated at 42kHz for 2 × 40 s in an ultrasonic bath (Elma, Singen, Germany), and allowed to settle for 5 minutes. Slurry taken from the middle of the sample bottle was further diluted to a final 1:100 (or 1:500 for surface sample A) in 10mM tetrasodium pyrophosphate (v/v) before pre-filtration through a 5 µm pore size polysulfone filter (Burlington, Massachusetts, United States) to remove larger particles. The resulting filtrate was then passed through a black polycarbonate 0.22 µm filter (Burlington, Massachusetts, United States). Bacterial cell counts were determined by epifluorescence microscopy using the LIVE/DEAD® BacLight™ staining solution with 6µM Syto 9 and 30 µM propidium iodide (Carlsbad, California, United States) (Boulos et al., 1999). Filters were stained in a 1:1 mixture (300µL stain solution: 300µL 0.85% NaCl) for 1 h in the dark at 4°C. Fifteen fields of view were counted on a Leica DMRXA fluorescent microscope, and the resulting cell counts, green being live (excited at 470 nm) and red (excited at 530 nm) being dead cells, were averaged for each sample, with the sum of the two as total cell counts.

Extraction efficiency was determined by inoculating 0.5 g of autoclave-sterilized peat and 0.5 g of autoclave-sterilized sand with *Escherichia coli* suspended in 1X PBS. Viability of the culture suspension was determined after filtration and staining on a black 0.22 µm polycarbonate filter. Cells were inoculated into both peat and sand soils to assess extraction efficiency and the impact extraction has on cell viability.

2.5 Results

Edaphic Parameter Analysis

Previous research by Calmels et al., (2012) suggested a thaw depth from 50-75 cm in the Dominion Creek region; however, edaphic analysis suggests a thaw depth of 30 cm in depth in Core 1 and 34 cm in Core 2, while Core 3 showed no evidence of a permafrost table (Supplementary Figure 2). Therefore, Core 1 30cm and Core 2 30cm are denoted as active layer soils, Core 1 45 cm and Core 2 45 cm are transition layer, and all soils deeper than these were permafrost. High permafrost ice content (averaging 66%, 75%, and 51% for Core 1, 2, and 3, respectively), were similar to previously observed permafrost cores in the region (Kanevskiy et al., 2013; Shur & Zhestkova, 2003). Likewise, lower SOM of permafrost samples than active layer samples were also found (25%, 46%, 10% for permafrost, and 60%, 48%, 71% for active layer, Cores 1, 2, and 3, respectively).

Although not separated across Ward's clustering, frozen active layer and permafrost soils were found to be significantly different ($p = 0.035$). Of the disturbance gradient soils, A (the undisturbed site), B, C, D, and E (the intermediate disturbed sites) all clustered with active layer soils, while F (in the thermokarst pond) clustered with permafrost soils ($p = 0.022$). Disturbed soils did not form a separate grouping to the exclusion of permafrost and

active layer core samples ($p = 0.084$), indicating that while A through E samples likely originated from active layer soils, F likely originated as a permafrost soil. The possibility cannot be precluded that disturbed soils (C, D, and E) shifted to become more similar to the active layer following disturbance. Disturbance gradient samples of C and D were outlying from other soil samples (Figure 2); C and D exhibited higher Mn, P, SOM, and water content than other samples (Supplementary Table 2). Disturbance and thaw results in no systematic trend amongst active layer and permafrost soils. Only 55.4% of total variability can be depicted within this PCA, suggesting there may be no systematic grouping between permafrost, active layer, and disturbed surface samples. The physical state of permafrost soils appears to be affecting soil physicochemical parameters and may be impacting viability and composition of the biological system.

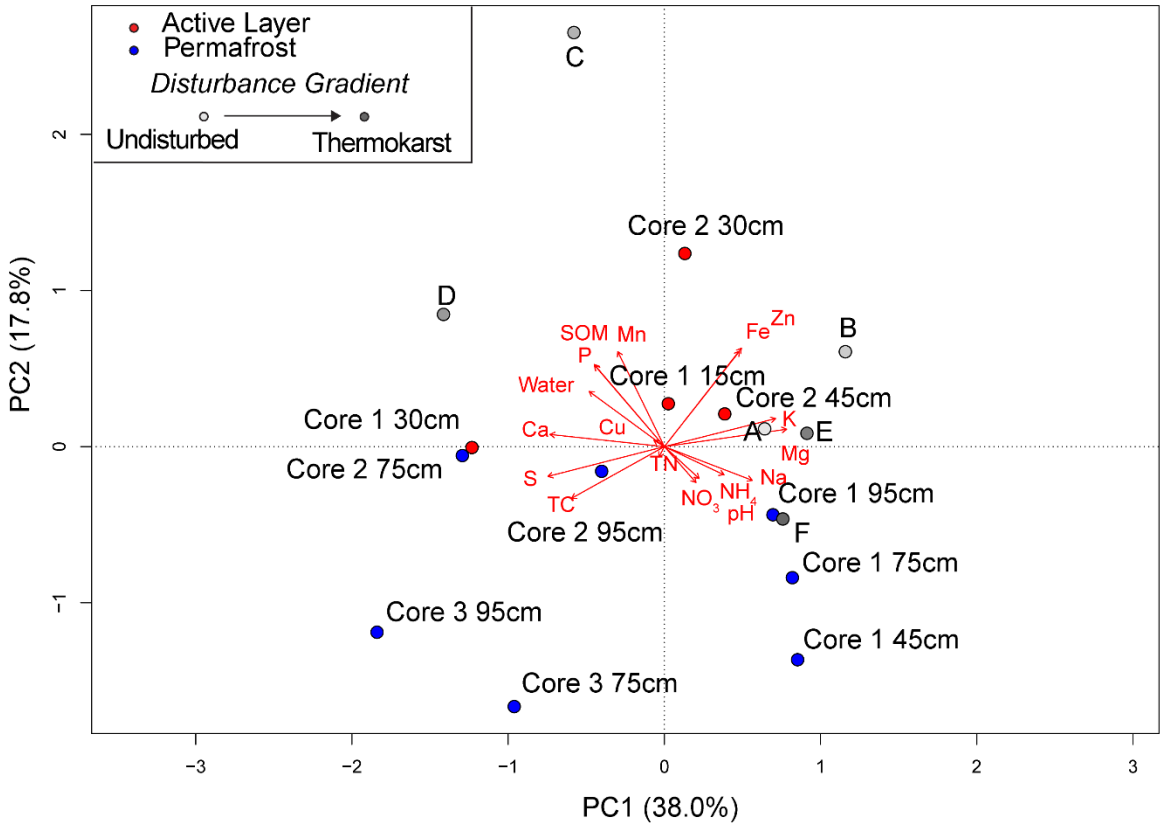


Figure 2. Soil chemistry is similar between active layer and permafrost sourced samples. Principal components analysis of permafrost, active layer, and disturbance gradient soils based on soil physicochemical parameters. Values were normalized to each variable's standard deviation. Arrows depict eigenvectors driving sample location in ordination space. Across the disturbance gradient soils, darker circles represent greater disturbance.

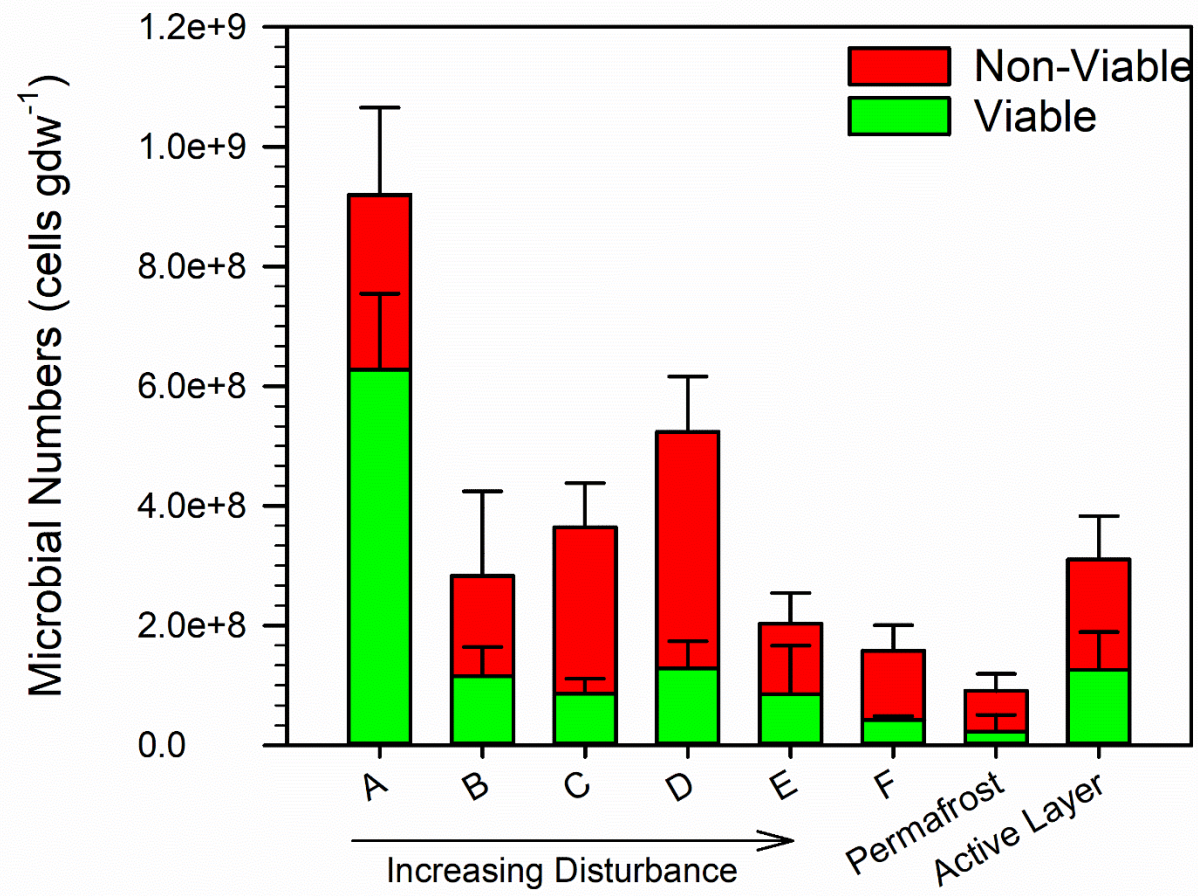
Viability is negatively impacted by disturbance

The impact of cell extraction, as well as cell extraction efficiency, was assessed through extraction and quantification of microbial cells inoculated in sterilized soils prior to analysis on experimental soils. The expected viability of cells in inoculated soils should resemble that of the inoculum. Cell cultures showed 55 % of the suspended *E. coli* cells were viable, and likewise, 62 % and 50 % of the inoculated cells were viable in peat and sand, respectively. Approximately 2.42×10^8 cells of *E. coli* were inoculated into each of the soil types. Peat soils inoculated with *E. coli* recovered 1.37×10^8 cells at 56 % extraction efficiency. The sand extraction retrieved 1.31×10^8 cells at 54 % extraction efficiency. Both extractions were well within the recommended extraction efficiencies of 10 – 60 %. This cell extraction method is adequate for data interpretation, though it is likely that actual cell counts are approximately twice what are currently reported due to the reported 54-56 % recovery.

To assess the impact of disturbance on active layer and permafrost soils, microbial cell abundance and viability was assessed across surface samples and core samples. Surface samples (n=3) were grouped across their placement in the disturbance gradient (A-F). Permafrost samples were grouped across Core 1, Core 2, and Core 3, including depths 75 – 80 cm and 95 – 100 cm as well as 45 – 50 cm in Core 1 (n = 7). Frozen active layer samples were grouped together across Core 1 (15 – 20 cm, 30 – 35 cm) and Core 2 (30 – 35 cm, 45 – 50 cm) (n = 4). Direct cell counts ranged from 1.0×10^8 -1.2×10^9 cells/gdm (grams per dry mass of soil) across permafrost and active layer soils (Figure 3). Permafrost cell counts were consistent with previous Arctic permafrost counts across the circumpolar north, ranging from 1.0×10^7 - 1.7×10^9 cells/gdw (Hansen et al., 2007; Wilhelm et al., 2011). The proportion of viable cells (Figure #) was consistent with previous direct count analyses of permafrost

ranging from 18-86% (La Ferla et al., 2017; Mackelprang et al., 2017). Microbial cell viability in Dominion Creek is greater than culturable viability counts in Arctic samples, which range from 0.1% - 10% (Vorobyova et al., 2001). Microbial abundance in active layer soils was similar to previous studies in Arctic soils, ranging from 4.0×10^7-1.0×10^{10} cells/gdw (Steven et al., 2004). Temperate soils exhibit higher cell abundances than these soils, ranging from 1.3×10^9 - 8×10^{10} cells/gdw (Cavalca et al., 2015; Olsen & Bakken, 1987).

Viability ratios and non-viable cell counts of permafrost soils were not significantly different from frozen active layer soils (Figure 3). Regardless, total and viable cell counts of permafrost were much lower than active layer soils. Non-viable cell counts of active layer soils were similar to permafrost soils. These results suggest a switch, where any disturbance leading to thaw is sufficient to dramatically decrease microbial viability to levels similar to that of the lower active layer and permafrost. While total microbial counts were highest at the undisturbed site (A), low total cell counts in samples E and F, directly adjacent to the thermokarst, suggest a second switch at which point absolute cell counts were negatively impacted by disturbance. Cell abundance was negatively impacted by disturbance in both active layer and permafrost soils. Viability in undisturbed soils (A) was higher than in all frozen active layer soils, including those of the top 15 cm, possibly suggesting an increase in viability with seasonal thaw of the active layer. Disturbed soils had similar viable abundances to both active layer and permafrost soil. Non-viable abundances were greatest in the intermediately disturbed soils C and D. Viability ratios did not change significantly with the degree of disturbance. The abundance and viability of microorganisms within active layer and permafrost soils are impacted by disturbance and may have subsequent impacts on microbial biodiversity.



	A	B	C	D	E	F	Permafrost	Active Layer
Viable	a	bc	bc	bc	bc	bc	b	c
Non-Viable	ab	bc	ab	a	bc	bc	c	bc
Total	a	bcd	bc	b	cd	cd	d	bc
Viability Ratio	69 ± 12	44 ± 9	24 ± 3	24 ± 2	38 ± 25	27 ± 7	40 ± 9	25 ± 14
Viability Ratio Grouping	a	ab	b	b	b	b	b	b

Figure 3. Live-dead cell counts of viable and non-viable microbial cells across the disturbance (A-F) gradient, as compared to undisturbed permafrost and active layer core samples. A table of statistically significant groupings determined using a one-way ANOVA ($P<0.05$) with a post-hoc Tukey HSD calculator. Error bars indicate standard deviation as calculated through propagation of error across each field of view as well as biological replicates.

Diversity of disturbed soil resembles active layer soils

Biodiversity of disturbed soils was observed and compared against frozen permafrost and active layer soils. Coverage was high for all samples, with the lowest coverage at 89% (Supplementary Table 3), which rarefaction analysis confirmed (Supplementary Figure 3). Observed OTU richness across all samples ranged from 387 OTUs in the deepest Core 1 permafrost sample to 1738 OTUs in F, the disturbed permafrost soil. Lower Shannon diversity was observed in permafrost soils; however, Faith's phylogenetic diversity was greatest in permafrost soils (Figure 4; Figure 9). As expected, Shannon diversity suggests that active layer communities harbour a more diverse microbial community at the OTU level than in permafrost. Lower Heip's evenness was also identified in permafrost assemblages in comparison to active layer soils, suggesting the permafrost microbial communities are dominated by a small number of abundant OTUs.

No reliable trend was observed across α-diversity metrics in the disturbance gradient, though low evenness was observed in the disturbed permafrost soil (F) and the highest evenness was observed in the undisturbed surface soil (A). Disturbance gradient soils were not significantly different from active layer soils in any α-diversity metric: having higher richness, diversity, and evenness than permafrost soils (Figure 4). Microbial diversity of disturbance gradient soils most closely resembled undisturbed active layer soils and differed from permafrost.

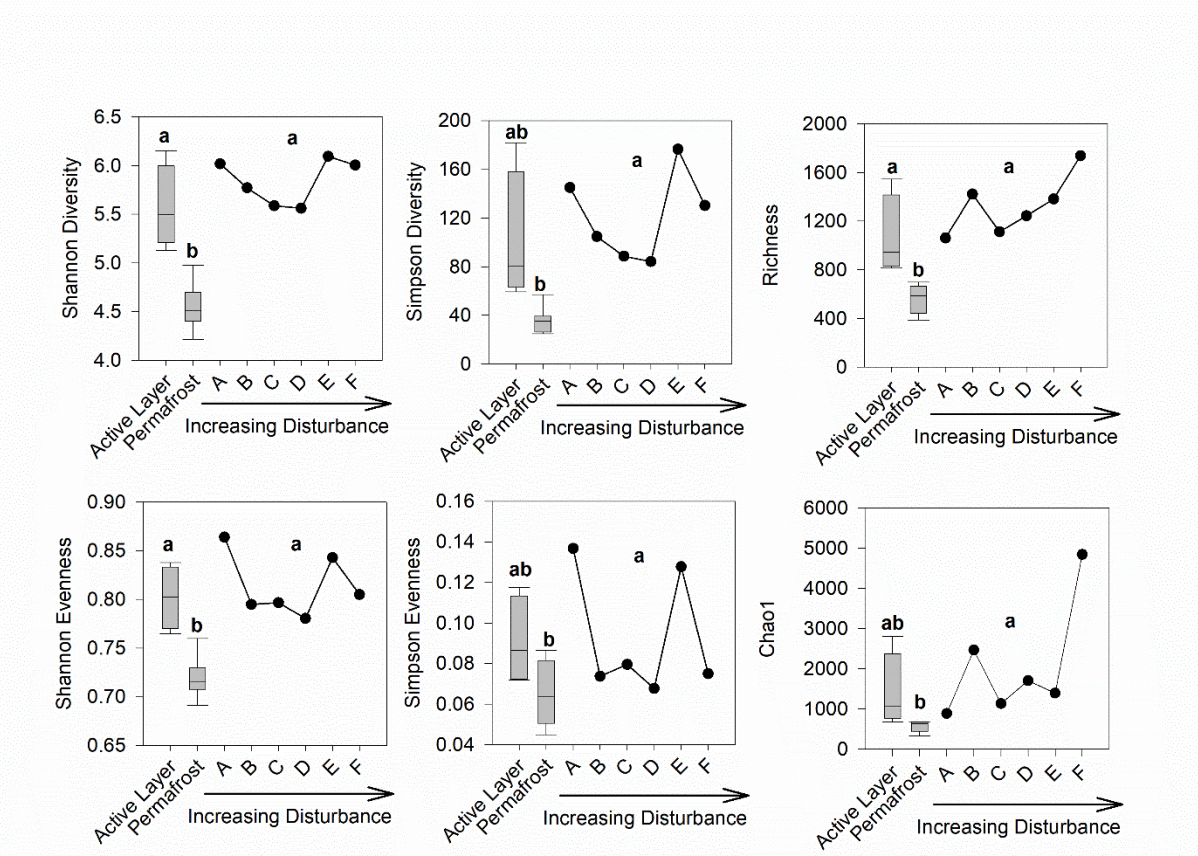


Figure 4. Microbial diversity in permafrost responds to abrupt thaw. Box and whisker plots of diversity metrics across frozen active layer, permafrost, and disturbance gradient soils. Disturbance gradient soils were combined for statistical testing. Letters signify statistically different groupings ($p < 0.05$) as assessed using one-way ANOVA with a post-hoc Tukey test.

Microbial community composition shifts with disturbance

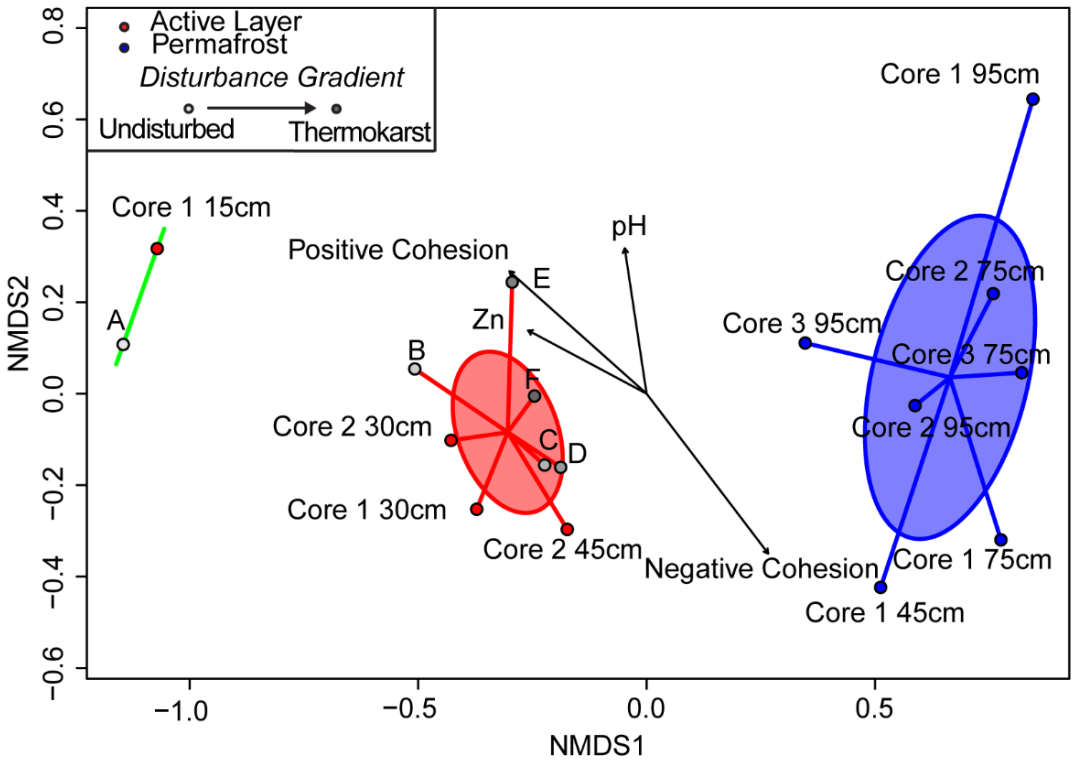
Bacterial community structure was assessed to determine the impact soil disturbance has on community composition and membership of active layer and permafrost soils. There were three bacterial community clusters in the studied soils: Cluster 1 included upper active layer and undisturbed soils; Cluster 2 included lower active layer and disturbed soils (B-F); and Cluster 3 included only permafrost (Figure 5). These clusters were observed with different community distance comparisons, including OTU membership (presence/absence; Jaccard distance), OTU composition (relative abundance; Bray Curtis distance), and phylogenetic dissimilarity (UniFrac, both weighted for relative abundance and unweighted). Optimal cluster number was determined using silhouette width. Highest silhouette width was found at three clusters, recovering a silhouette width at 0.34 and cluster groupings were significantly different ($p < 0.05$) (Rousseeuw et al., 1987). All disturbed soil communities clustered with lower active layer soil communities (including Core 1 30 cm, Core 2 30 cm, and Core 2 45 cm). Samples clustered across NMDS1, while the secondary axis separated within-cluster community differences. Active layer and disturbed soils group together to the exclusion of permafrost soils (Figure 5). It therefore appears that disturbance creates a microbial community not unlike that of the lower active layer.

Community composition within clusters correlated weakly, but significantly, with differences in pH ($r^2 = 0.4316$), while differences across clusters correlated with Zn ($r^2 = 0.3609$) in Bray-Curtis distances; community membership as assessed through Jaccard presence/absence distances pH ($r^2 = 0.5000$) and Zn ($r^2 = 0.3461$) was similar. Similar clusters in community structure for both composition and membership suggest distinct assemblages between each cluster with few OTU shared across clusters. Other edaphic parameters

associated with the thermodynamic differences between the active layer and permafrost likely drove microbial community composition more than edaphic parameters.

In an attempt to describe additional community variation, cohesion metrics were compared against Bray-Curtis dissimilarity (Herren et al., 2017). Positive cohesion (r^2=0.6760) and negative cohesion (r^2=0.8069) explained 29.0% of bacterial community variation and 40.2% of the community variation when combined with pH and Zn (Figure 5). Cohesion not only explained a greater fraction of bacterial community variation, but also explained an additional 20.3% of variation beyond environmental parameter differences as Zn and pH explained only 19.9% of the variation alone, suggesting that microbial interactions are more significantly controlling community composition than edaphic parameters. Greater explanation of variation through cohesion metrics does not preclude the possibility that community cohesion covaries with edaphic parameters which have not been measured.

a


Active Layer
Permafrost
Disturbance Gradient
Undisturbed
Thermokarst
Core 1 95cm
Core 1 15cm
pH
Positive Cohesion
E
Zn
Core 2 75cm
Core 3 95cm
B
F
Core 3 75cm
A
Core 2 30cm
C
D
Core 2 95cm
Core 1 30cm
Core 2 45cm
Negative Cohesion
Core 1 75cm
Core 1 45cm
NMDS2
NMDS1
0.8
0.6
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b

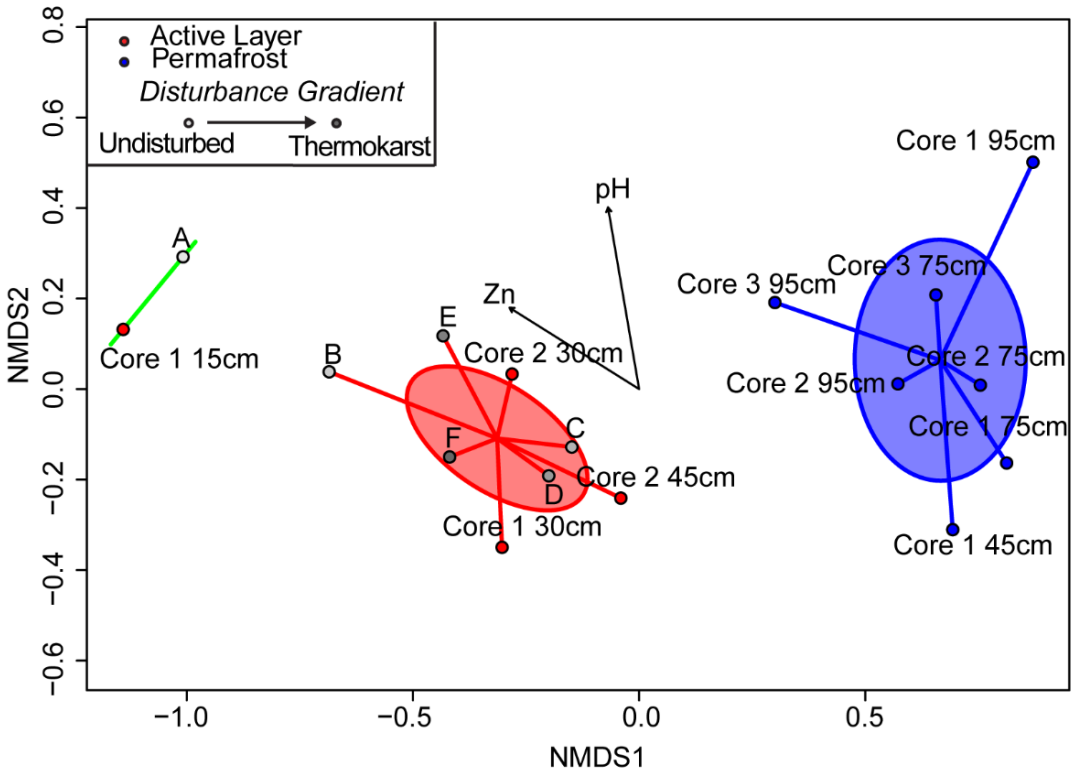
Active Layer
Permafrost
Disturbance Gradient
Undisturbed
Thermokarst
Core 1 95cm
pH
A
Core 3 95cm
Core 3 75cm
Zn
E
Core 1 15cm
B
Core 2 30cm
Core 2 75cm
Core 2 95cm
F
C
Core 1 75cm
Core 2 45cm
D
Core 1 30cm
Core 1 45cm
NMDS2
NMDS1
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
−1.0
−0.5
0.0
0.5

Figure 5. Dissimilarity of microbial communities visualized by nonmetric multidimensional scaling (NMDS) of total microbial assemblages. Clustering of soil samples was based on (a) Bray-Curtis community distance (composition) and (b) Jaccard presence/absence (membership) distance matrices. Ellipses encompass clusters determined by weighted average algorithms: Cluster 1 (upper active layer and undisturbed soils), Cluster 2 (lower active layer and disturbed soils), and Cluster 3 (permafrost). Clusters are significantly different as established by PERMANOVA ($p < 0.05$). Edaphic parameters correlating with these groups at significance ($p < 0.05$) were overlaid onto the ordination using the "envfit" function in *vegan*. The direction of vector indicates the direction of change while the length is proportional to the correlation between the communities and variable.

Persistence of permafrost indicator OTUs in disturbed permafrost soils

Indicator species analysis was conducted to determine if any OTUs were present in surface samples which were indicative of active layer or permafrost microbial communities. The presence of active layer OTUs was predicted to be more common in less disturbed soils, indicating the predominance of active layer inputs. Likewise, permafrost OTUs were predicted to be more common in more disturbed soils due to permafrost soil inputs into these deeper soils. Indicator species analysis calculates the correlative strength between an OTU and its habitat as measured through: OTU prominence within a habitat, its specificity to a habitat, and its mean abundance normalized to the sum of its mean abundance in other habitats (Dufrêne and Legendre, 1997). An OTU is more likely to be an indicator if it is more likely to occur or more abundant in one habitat compared to another. I identified 94 active layer indicator OTUs (Supplementary Table 4) and 60 bacterial permafrost indicator OTUs (Supplementary Table 5). Despite anthropogenic disturbance, this analysis indicates that a proportion of permafrost indicator OTUs can persist with permafrost thaw. The proportion of permafrost indicator OTUs present in surface samples increased across the disturbance gradient, with less than 5% of permafrost indicator OTUs present in the undisturbed surface sample (sample A) increasing to 80% of bacterial permafrost indicator OTUs present in sample F (Figure 6a). By comparison, the presence of permafrost indicator OTUs in permafrost soils ranged from 93-100%.

Shared OTU richness was performed to determine if endemism and/or community similarity to permafrost increases with disturbance. Large proportions of endemic OTUs may indicate introduction of OTUs outside of the sampled system, possibly through aeolian inoculation or surface waters. On the other hand, large proportions of OTUs originating from

permafrost soils would indicate the preservation of permafrost OTUs after disturbance. Across the disturbance gradient, the most disturbed soil (Sample F) shared the highest proportion of OTUs with permafrost soils (9.0%) and the lowest with active layer soils (26.8%) (Figure 6b). Likewise, the undisturbed soil shared the lowest proportion of OTUs with permafrost soils (0.1%), and the highest with frozen active layer soils (45.7%). Endemism was highest in soil F (29.0%), but lowest in intermediately disturbed soils C and D (19.1% and 22.3% respectively). Endemic OTUs were lowest in intermediately disturbed soils but increased with greater degrees of disturbance. OTUs shared with permafrost soil communities increased in prominence with greater levels of disturbance. Thus, although the overall community composition in the most disturbed soils is quite similar to active layer soils, relic bacterial indicator OTUs are still present, suggesting the resident permafrost community has yet to be completely supplanted by an active layer community.

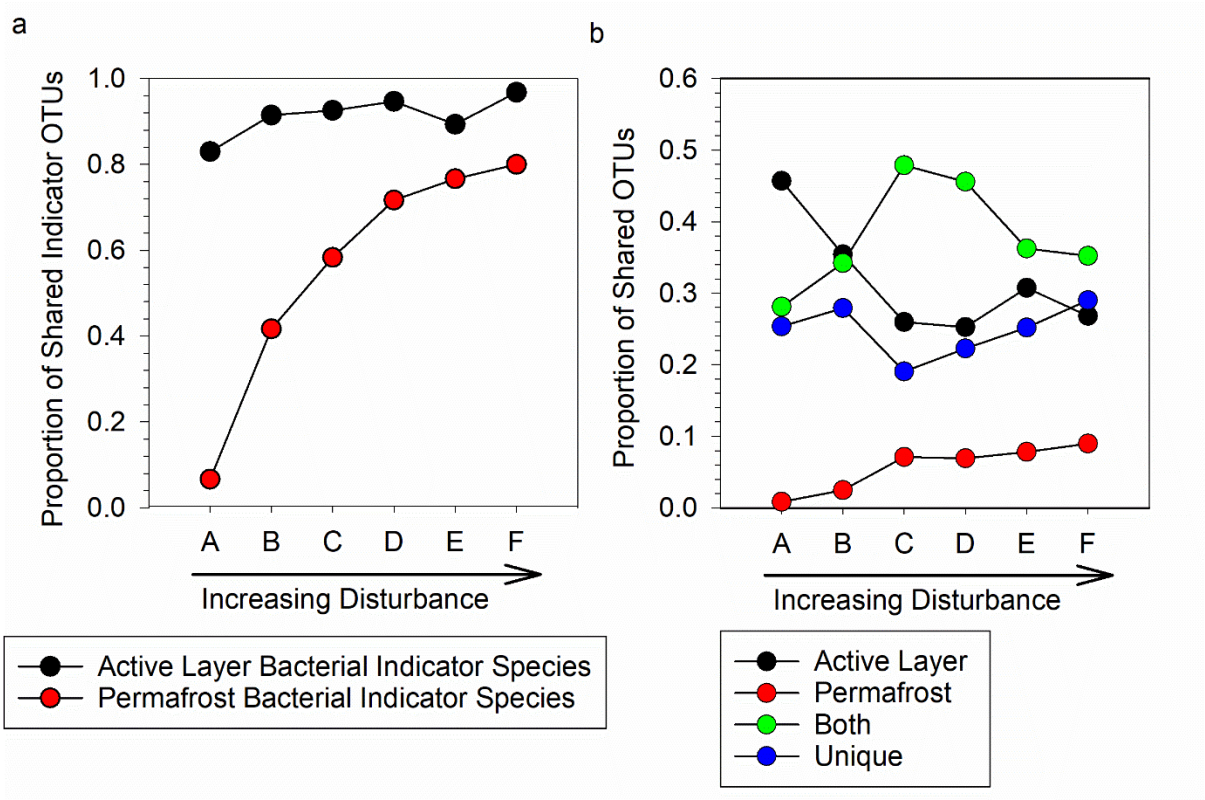

Figure 6. Indicator species analysis and shared richness assessed across the disturbance gradient as compared to combined active layer and permafrost soils. (a) The proportion of indicator OTUs assigned to permafrost or active layer soils that are shared within disturbance gradient soils. Indicator OTUs were defined as having indicator values above 0.80. (b) Shared richness of disturbance gradient soils compared against the combined assemblages of permafrost or active layer. "Uniques" were unique to the disturbance gradient soil, and "Boths" were found in both active layer samples and permafrost samples.

Community membership of disturbed soils resembles active layer soils

Phylogenetic distances of community composition (weighted UniFrac) indicated similar cluster structure as found in community membership and total assemblage (Figure 7). Community dissimilarity was greatest in permafrost soils, indicating that this cluster is more heterogeneous that disturbed soils and undisturbed active layer soils.

A total of 26 phyla, 66 classes, and 235 genera were assigned across all soils with 32.4%, 46.1%, and 62.3% of all sequences unassigned at the phylum, class, and genus level, respectively. At the phylum level, all bacterial communities were dominated by Proteobacteria (21.1%), Actinobacteria (8.6%), Acidobacteria (7.9%), Bacteroidetes (7.9%), Firmicutes (5.5%), with substantial amounts of Verrucomicrobia (4.2%), and Planctomycetes (4.0%). Across class levels, Actinobacteria (8.5%), Alphaproteobacteria (6.6%), Deltaproteobacteria (4.2%), Planctomycetia (4.0%), Gammaproteobacteria (3.7%), Clostridia (3.3%), and Betaproteobacteria (3.2%) were most dominant (Figure 7). Bacilli, Clostridia, Anaerolineae, and Bacteroidia were significantly more abundant in permafrost soils than in frozen active layer soils, while Acidobacteria group 4, Acidobacteria group 17, and Nitrospira were all significantly more abundant in active layer soils than permafrost (Figure 8). Class level taxonomic composition across the disturbance gradient was relatively static with some exceptions: Clostridia (A= 0.04%, B =0.529%, F= 1.96%), Bacteroidia (A=0.03%, B=0.49%, F=2.1%), and Acidobacteria group 7 (A=0.71%, B=1.81%, F= 3.19%) all increased with disturbance, while Planctomycetia decreased (A=2.85%, B=1.63%, F=0.775%). Bacteroidia and Clostridia were more abundant in disturbed and permafrost soils than in active layer soils. With increasing disturbance, taxa which are significantly more abundant in permafrost become more abundant in disturbed soils.

Although primers were not designed for high taxonomic resolution of Archaea, an increasing trend in archaeal sequences was observed with disturbance. Abundances of archaeal sequences were highly variable. Samples from Core 1 15cm, and undisturbed soil (A) had fewer than 100 sequences and were removed from subsequent analysis. Sequencing depth was greatest in Core 3 at 1229 and 2008 reads, at 75 cm and 95 cm respectively. Phylogenetic diversity of Archaea was low, composed of three classifiable phyla, three classes, and seven genera. A total of 29.6%, 31.8%, and 48.8% of sequences were unidentifiable at the phylum, class, and genus level, respectively. At the phylum level, Archaea were dominated by Euryarchaeaota (66.9%), with small abundances of Woesearchaeota (2.5%) and Pacearchaeota (0.9%). At the class level, Archaeal communities were dominated by Methanobacteria (36.3%) and Methanomicrobia (30.5%).The majority of archaeal sequences recovered were methanogenic in origin, increasing in abundance with depth and disturbance. Both archaeal and bacterial abundances differ between active layer and permafrost soils, with disturbed active layer and permafrost soils resembling frozen active layer soils.

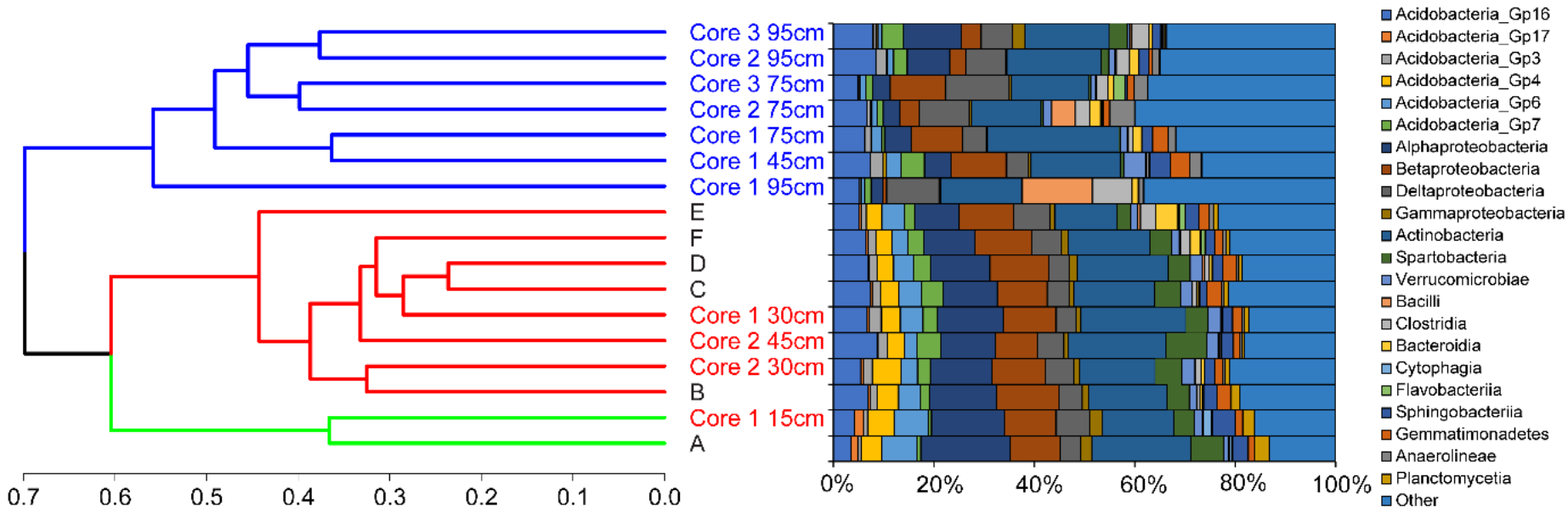

Figure 7. Hierarchical clustering of cores and surface sample communities alongside class level relative abundances. Classes <1% in any sample was grouped into "other" alongside unassigned taxa. Samples which are not significantly different (P<0.05) across weighted UniFrac distances have the same colour. Frozen active layer samples are labeled in red, frozen permafrost is labeled in blue, and disturbance gradient soils are labeled in black.

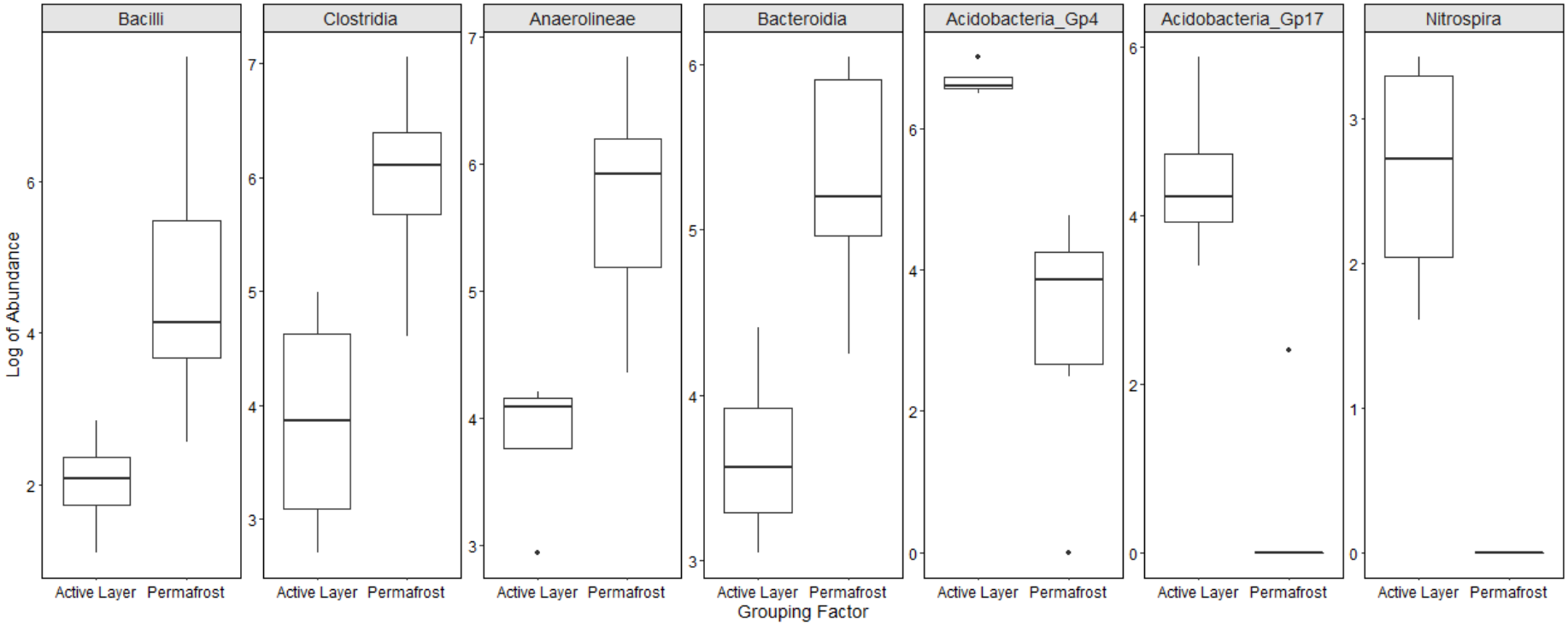

Figure 8. Differential abundance of bacterial classes across frozen active layer and permafrost soils. Using FDR multiple test correction analysis ($p < 0.05$). Only taxonomic groups which were >1% in any sample were included in analysis, and only significantly different groups are shown as assessed using a two-tailed Mann-Whitney U test ($P<0.05$).

Assembly processes change with disturbance

To investigate changes in community assembly processes across states of thaw, net relatedness and nearest taxon indices were calculated. No difference was found in NTI between active layer and permafrost soils, however, NRI and NRI/NTI was higher in active layer soils than in permafrost soils (Figure 9). Faith's phylogenetic diversity was higher in permafrost soils than in active layer soils. Higher NRI in active layer soils than permafrost suggests that deterministic processes including environmental filtering and isolation may play a greater role in community assembly in active layer soils than in permafrost, though both have deterministic assembly processes. No differences were found in NTI between permafrost and active layer soils, though both soils had positive NTI values. Taken together, deterministic processes govern phylogenetic turnover for both soils at both the tree root and tree tips, with a greater role in active layer soils than in permafrost. Community assembly is clustered nearer to the tree tips in all soils with positive NRI/NTI values in all soils, with greater clustering in active layer soils. Based on these data, deterministic processes drive microbial phylogenetic turnover in active layer and permafrost.

Disturbance gradient soils did not exhibit a reliable trend in phylotype distance (net relatedness index; NRI) or phylogenetic distance of closely related taxa (nearest taxa index; NTI) magnitude with disturbance. A trend was observed in NRI:NTI ratios, with community assembly primarily occurring at tree tips. All metrics were within the range of active layer soils, and did not significantly differ from active layers, but were significantly higher than permafrost samples in NTI, PD/OTU, and NRI:NTI. Thus, the disturbed soils, originating from permafrost, has community assembly mechanisms that are more similar to active layer samples than permafrost samples.

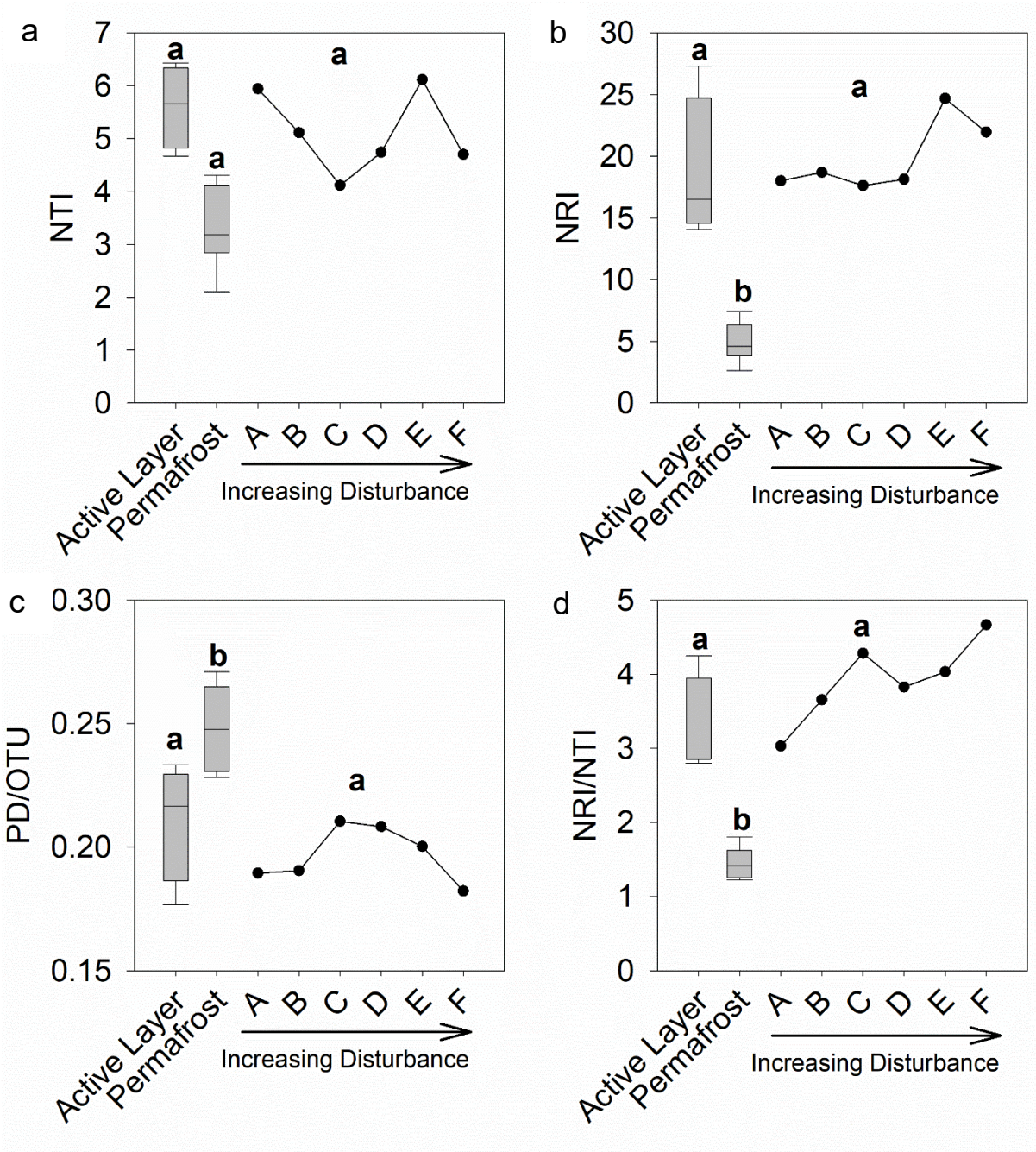

Figure 9. Microbial community assembly of disturbed surface soils resemble active layer soils. (a) nearest taxon index (NTI); (b) nearest relatedness index (NRI); (c) Faith's phylogenetic diversity per OTU (PD/OTU); (d) NRI/NTI ratio are depicted. Letters differentiate significantly different statistical groupings as tested using one-way ANOVA with multiple comparison correction using a post-hoc Tukey test.

2.6 Discussion

Summary

Direct anthropogenic activities, such as road construction and infrastructure construction, as well as indirect anthropogenic activities, including climate change, can lead to permafrost thaw and subsidence (Elias 2014; Lin et al., 2016). The immediate impact of permafrost thaw on the biogeochemistry of Arctic environments can be assessed in thermokarst waters with permafrost inputs, as well as in overlying active layer soils (Reyes et al., 2015). I analysed an anthropogenically induced permafrost thaw gradient culminating in the formation of a thermokarst pond. The soil-physicochemistry of directly disturbed permafrost does not shift with removal of soil horizons, though increases in a subset of macronutrients has occurred. Bacterial community composition of our anthropogenically disturbed soils shift to resemble lower active layer soils, regardless of the level of disturbance as shown through assessment of community level composition, phylogeny, viability, abundance, and diversity. However, remnants of permafrost communities persist in disturbed soils, though at insufficient abundance to influence overall community structure. Together, these findings indicate that anthropogenic disturbance permit the invasion and establishment of a new community, likely sourced from the surrounding active layer, though the original community persists in low abundance. These findings support previous studies showing convergence of permafrost microbial communities with active layer communities upon thaw, both *in situ* and *in vitro* (Monteux et al., 2018: Mackelprang et al., 2011).

Anthropogenic Disturbance does not reliably shift edaphic parameters

The impact of permafrost thaw on Arctic soil edaphic parameters is particularly well studied. Water potential, nitrogen availability, carbon availability, and nutrient availability are amongst the most important drivers of microbial community composition in these soils. Anthropogenic disturbance has been shown to alter soil pH, while thawing permafrost is known to leach micronutrients and macronutrients into downstream thaw waters (Forbes et al., 2011; Lantz et al., 2011). However, in the Dominion Creek system, edaphic parameters did not appear to shift with six weeks of thaw. Active layer and permafrost soils were differentiable, with less disturbed soils clustering amongst active layer soils, and the most disturbed soil resembling permafrost. Organic matter in permafrost is generally more recalcitrant and holds higher concentrations of dissolved N than active layer soils above it (Keuper et al., 2012). The dissolved N preserved within permafrost can be rapidly released following permafrost thaw, releasing nutrients into the active layer and downstream aquatic systems (Reyes & Lougheed, 2015). It is therefore likely that the sulphur and nitrogen concentrations in thawed Dominion Creek soils may increase further as the edge of the thermokarst pond expands and permafrost thaw continues. With time, additional macronutrient release (such as phosphorus) as well as micronutrient leaching (such as manganese) may occur alongside current release of sulphur and nitrogen into the disturbed active layer. Further sampling at a later date may have provided sufficient time for environmental parameters to shift in disturbed soils. Microbial communities of disturbed may therefore undergo subsequent shifts in community composition in response to edaphic parameter changes. However, this study suggests that the microbial communities of permafrost soils shift more rapidly with disturbance than environmental parameters.

The high permafrost ice content and low permafrost SOM content found in Cores 1-3 may predispose this area to permafrost subsidence and thermokarst formation following thaw. These trends have previously been seen in polygonal tundra sites (Wu et al., 2018b), and could indicate sensitivity of this landscape to future permafrost subsidence and rapid landform shifts. Further change could compromise the topography and hydrology of the site, impacting carbon dynamics and community structure in the system. Water logged soils may represent a substantial source of CO_2-carbon equivalents due to increased methane flux from methanogenic archaeal activity (Knoblauch et al., 2018). Water logging induced by direct anthropogenic disturbance is conducive to archaeal proliferation and activity, which is consistent with our observations that higher proportions of methanogenic archaea were observed with disturbance. Additionally, disturbance itself may represent an avenue of dispersal, allowing otherwise dispersal limited archaeal populations to proliferate in nearby soils.

Physicochemical parameters are suggested to control the fate of microbial communities in response to permafrost thaw (Hayden et al, 2012; Rousk et al, 2012); however, Lamb et al. (2011) has suggested that these shifts in microbial biodiversity are directly impacted by thaw, prior to significant changes in chemistry or physical parameters in the system. These opposing hypotheses creates a dichotomy: (1) physicochemical characteristics control microbial communities following permafrost thaw, and so environmental parameter differences should resemble microbial community groupings or (2) permafrost thaw induces microbial community shifts, wherein environmental and microbial groupings do not resemble one another. It is unclear what directly impacts microbial community structure with permafrost thaw. Soil microbial communities of thawing permafrost are driven by environmental factors, most significant of which is pH (Chen et al.,

2017; Tripathi et al., 2018a). However, the compositional, diversity, and assembly changes I observed in sampled soils have little variance explained by physicochemical parameters. Additional measurements of physicochemical parameters may explain more variation within the system, including measurements such as carbon quality (Chen et al., 2017), electrical conductivity (Tripathi et al., 2018a), dissolved organic carbon (Crevecoeur et al., 2015), dissolved organic nitrogen (Comte et al., 2015). Soil pH was a substantial driver of microbial community diversity within active layer and permafrost clusters, respectively. While soil pH does not differentiate these soil types, within habitat diversity is controlled by pH. Thermodynamic differences between active layer and permafrost soil horizons likely superseded the impacts of soil pH and other factors (Coolen et al., 2011). Plant community composition has been shown to alter microbial community composition and root density has been shown to increase with permafrost thaw (Schuur et al., 2007; Monteux et al., 2018). However, in the absence of overlying vegetation, permafrost thaw may instead shift numerous environmental variables which resident microbiota either adapt to or are supplanted by active layer microbiota. As well, permafrost and active layer prokaryotic communities are shown to be controlled by distinct mechanisms. Chen et al., (2017) has previously shown active layer soils to be driven by organic carbon, total nitrogen, total phosphorus, dissolved nitrogen and dissolved organic carbon, while permafrost communities were driven by dissolved organic carbon. Water saturation, CH_4, O_2, and CO_2 content, nutrient bioavailability, DOC, and DON measurements may also better elucidate what parameter are driving the microbial response to thaw in the field (Tripathi et al., 2018a). Microbial community shifts in disturbed permafrost and active layer soils were not explained by edaphic parameter changes; however, small differences in community assemblage within active layer and permafrost community clusters are controlled by these edaphic parameters.

Regardless, the absence of correlation between microbial composition and TC, TN, water content, and C/N was unexpected, as previous work in periglacial soils have indicated these as important explanatory variables (Mu et al., 2018). The lack of correlation between edaphic properties and community measures may reflect the short time between the disturbance and sampling in this study, a period of only six weeks.

Drivers of Microbial Community Composition are biotic

The extreme stressors which microbial communities undergo in permafrost, in addition to low activity and replication rates, suggest that these communities are merely surviving under extreme conditions. I suggest that the indigenous microbial community of permafrost cannot respond to the opportunities presented by permafrost thaw: increased nutrient availability, increased temperatures, and the availability of new niches. Instead of a "blooming" viable permafrost microbial community, active layer taxa appear to invade the disturbed permafrost environment. While taxon membership in disturbed soils appears to be partly of permafrost origin, as indicated by the presence of numerous permafrost indicator taxa in disturbed soils, this signal is not strong enough to affect overall community membership or composition. Increasing abundance of characteristically permafrost taxa (Bacteroidia, Clostridia, and Bacilli) across the disturbance gradient suggests that these taxa may have been more abundant prior to disturbance. These classes are known to predominate in permafrost soils, with lower relative abundances in active layer horizons (Müller et al., 2018). The opposing trend occurred for the abundant active layer class Acidobacteria subdivision 17 suggesting that shifts in microbial community structure, both at the class and OTU levels, are likely due to species replacement rather than reactivation and succession, though permafrost microbial populations may persist in lower abundance. The disturbed

permafrost microbial community converges with the active layer community, driven by biotic processes, and likely occurs through the invasion of active layer taxa.

Dramatic short-term perturbation has been suggested to drive community composition through ecological interactions in deterministically-assembled communities (Stegen et al., 2018). However, discrete disturbance has previously been shown to drive strong stochastic assembly due to overwhelming die-off and proliferation lacking a systematic force (Stegen et al., 2018). NRI and NTI can be used to infer the degree to which communities are controlled by deterministic or stochastic assembly processes. Deterministic assembly corresponds to an increased role of environmental filtering and ecological interactions in determining community structure while stochastic assembly corresponds to an increased role of dispersal, drift, and continued competition in communities. A higher magnitude of clustering is indicative of deterministic community assembly, as niches are filled and retained by specialist taxa through environmental filtering and ecological interactions; communities with lower levels of phylogenetic clustering are controlled by stochastic forces, wherein dispersal, drift, and competition, controls which taxa are the first to colonize and dominate a system. These hypotheses are dependent upon assumptions that closely related taxa are more functionally similar and will occupy similar ecological niches. Within my study, phylogenetic divergence analysis showed deterministic assembly processes across all soils. Communities were phylogenetically clustered across all soil types, although permafrost communities exhibited less phylogenetic clustering. Genomic diversity within phylogenetically clustered microbial communities may be much lower than in evenly distributed communities (Mondav et al., 2017). Phylogenetically clustered microbial communities are often associated with low nutrient availability and other parameters that would otherwise increase selection and promote specialization (Mondav et al., 2017). Cohesion metrics, both positive cohesion

(where both taxa become more abundant with association) and negative cohesion (when one taxon is negatively impacted by the presence of another taxon) support the hypothesis that deterministic biotic filtering was impacting microbial community composition with disturbance.

Taxonomic profiles resemble other regional studies across the Arctic

Taxonomic profiles are similar to other periglacial soils and differential abundance identified numerous taxa that may be ecologically significant across active layer and permafrost habitats region (Malard & Pearce, 2018). Previous studies have indicated that both aerobic and anaerobic degradation of complex hydrocarbons preserved in permafrost are degraded into greenhouse gases, including CO_2 and CH_4, as well as volatile fatty acids including propionic acid. While physiology cannot be directly inferred from OTU analysis, trends in the membership of these communities are consistent with these previous findings. Aerobic heterotrophic bacteria (*i.e.* Bacilli, most Actinobacteria), anaerobic heterotrophic bacteria (*i.e.* Clostridia), methanogenic Archaea (*i.e.* Methanomicrobia, Methanobacteria), and volatile fatty acid oxidizers (*i.e. S. propionica*) were all prominent across Dominion Creek soils and are globally distributed across active layer and permafrost soils (Jansson & Taş, 2014; Malard & Pearce, 2018; Metje et al., 2007). The primers used in this study are not entirely optimized for archaeal analysis but still lack sufficient coverage for detailed analysis and so only taxonomic assessment was performed (Walters et al., 2016). Planctomycetes phylum sequences were shown to be differentially more abundant in active layer than permafrost, and disturbance also decreased the relative abundance of Planctomycetes-related sequences in this disturbance gradient, as has been seen in previous studies (Taş et al., 2014). Relatives of spore forming bacteria, including Clostridia and Bacilli, were more abundant in

permafrost soils than in active layer soils. Recent research suggests that increased abundance of these putative spore formers is due to niche partitioning and survival strategies precluding spore formation, as spore formation is a poor long-term survival strategy (Johnson et al., 2007). The high relative abundance of Acidobacteria classes in active layer soils is similar to worldwide distributions of Acidobacteria in active layer soils (Malard & Pearce, 2018). However, lack of physiological characterization of diverse Acidobacterial taxa limits the inferences that can be made based on taxon abundance. Contrary to previous studies, Acidobacteria subdivision 4 did not correlate with pH, though this may be affected by confounding variables (Kielak et al., 2016). However, Acidobacteria subdivision 17 was positively correlated with soil pH as found previously, as well as Cu concentrations (Kielak et al., 2016). However, previous negative correlations of Acidobacteria subdivision 17 with P, C, and N were not observed. The distinct life strategies between permafrost and active layer classes suggest that community functional differences may also be present across permafrost, active layer, and disturbed soil communities. These results identify distinct microbial taxa across active layer and permafrost, supporting previous suggested differences in relative abundance differences. Disturbance creates a phylogenetic profile more similar to permafrost with class level phylogenetic differences similar to those previously found in permafrost-affected soils.

Viability and survivability in permafrost

The impact of permafrost thaw on microbial survivability is unknown. While microbial communities are known to shift with thaw, it is unknown if this is associated with die-off of indigenous microbes. Should disturbance, and subsequent thaw, provide conditions suitable for microorganisms to proliferate, microbial abundance and viability may rebound to

levels equal to, or greater than, undisturbed active layer. Microbial abundance in Arctic permafrost and active layer soils have been extensively studied and these soils are known to harbour similar abundance, or fewer, cells than temperate soils (Hansen et al., 2007; Mackelprang et al., 2017; Cavalca et al., 2015; Olsen & Bakken, 1987). The viability ratio of microorganisms did not differ between active layer and permafrost soils at Dominion Creek. I posit that this is due to long-term adaptation to both seasonal thaw in the active layer (Schostag et al., 2015), as well as constant and perennial stressor adaptations in the permafrost (Mackelprang et al., 2017). Little research has been done directly assessing the viability of microorganisms across a permafrost thaw gradient. Our data suggest that abrupt thaw does not dramatically increase microbial cell abundance or viability of microorganisms in permafrost soils. However, previous studies have suggested increases in microbial activity, transcription, and genetic potential with permafrost thaw in the laboratory, and in the field (Coolen et al., 2011; Coolen & Orsi et al., 2015; Hultman et al., 2015). Due to these increases in metabolic activity, transcriptional activity, and genetic potential under both rapid and long-term thaw, lower biomass and lower viability with discrete and substantial perturbation was unexpected. Activity changes may result from a small but viable community in thawing permafrost which becomes more abundant with thaw. However, I also predict further microbial community shifts with time as the relict permafrost community decreases in abundance.

Analogues of natural disturbances

Naturally occurring physical disturbances to active layer and permafrost soils, in the forms of active layer detachment slides and retrogressive thaw slumps, are becoming increasingly common. Complete removal of the active layer, as well as subsequent

detachment slides of the newly forming active layer, result in similar geographic formation as anthropogenic excavation. Furthermore, anthropogenic disturbances can also result in active layer detachment slides and thermokarst formations (Lin et al., 2016). Higher dissolved metal concentrations have previously been observed in paleoactive layers produced from active layer detachment slides as well as thaw waters from permafrost thaw. However, I observed no clear trend amongst dissolved metal concentrations with disturbance, though elevated P and S concentrations and declining K, Mg, Zn, Na, and Ca, seem to be associated with intermediate disturbance. It is possible that the relatively shallow disturbance in this study did not reveal larger mineral deposits, which prevented such trace element leaching found in other studies (Kokelj et al., 2002; Reyes & Lougheed, 2015). Unlike other studies, pH decreased with disturbance rather than increased, though this is likely due to the lower pH of buried permafrost soils from which our disturbed soils originated (Forbes et al., 2001; Mesquita et al., 2010). Leaching of nutrients, both carbon and nitrogen, appears to occur in excavated soils, similar to findings in retrogressive thaw slumps (Abbot et al., 2015). Both excavated sites and naturally disturbed sites undergo slow vegetation succession and I predict that although microbial community replacement occurs rapidly, plant community succession may occur over decadal time scales in anthropogenically disturbed sites (Forbes et al., 2001; Lantz et al., 2009).

Conclusions

Overall, our results demonstrate the presence of a shifting bacterial community in response to permafrost disturbance at Dominion Creek. Six weeks of thaw following disturbance was sufficient to create bacterial community composition, membership, and diversity in disturbed active layer and permafrost soils which was similar to lower active

layer soils. These communities are driven by biotic filtering of an invading active layer microbial community in disturbed permafrost communities. Thaw does not impact viability or abundance of bacteria, suggesting increasing activity, transcription, and genetic potential found in other thawing soils may be due to a more active, yet sparse, microbial community. This study may be expanded to determine if anthropogenic disturbance creates similar microbial community shifts in naturally occurring rapid permafrost thaw zones. Interpretation of activity measurements and characterization of important active microbial taxa may suggest future avenues of research to develop our understanding of microbial succession in response permafrost thaw induced by direct anthropogenic disturbance.

2.8 Associated Content

Physical characterization (Supplementary Table 1), edaphic parameter data (Supplementary Table 2), α-diversity data (Supplementary Table 3), taxonomic assignment of active layer indicator OTUs (Supplementary Table 4) and permafrost indicator OTUs (Supplementary Table 5), mock community analysis (Supplementary Figure 1), permafrost core geochemical profiles (Supplementary Figure 2), and rarefaction curves (Supplementary Figure 3), are found in Appendix 1. Detailed analysis and interpretation of PMA treatment is found in Appendix 2.

2.9 Acknowledgements

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fluorescent microscopy of samples. Allan Harms carried out soil chemistry analysis. Ali Naeimi Nezamabad created the site map of Dominion Creek.

Chapter 3: Conclusion

3.1 Contribution to the Field

Polar amplification of climate change has increased annual surface air temperatures by ~2°C in Yukon over the past 50 years, which is already leading to increased rates of permafrost thaw (Streicker, 2016). Modeling of carbon release from Arctic regions is becoming more important as there is significant uncertainty regarding the fate of carbon entrapped in permafrost. Microbial communities regulate the rate of carbon mineralization from the stored organic carbon in permafrost (Monteux et al., 2018). The number of studies examining the effects of permafrost thaw on microbial communities is expanding, due in part to the advent of next-generation sequencing (Hultman et al., 2015; Monteux et al., 2018; Wu et al., 2018b; Yuan et al., 2018). While permafrost microbial communities shift upon thaw in lab experiments, these experiments are not always readily applicable to the field (Federle et al., 1986; Treat et al., 2014). Additionally, while direct anthropogenic disturbance represents some of the most drastic disturbances in Arctic and subarctic landscapes, they remain understudied in microbial ecology. The most severe of these direct-disturbances is the complete removal of soil horizons, including the entirety of the organic-layer (Forbes et al., 2001). While previous studies have noted vegetation recovers within 20-75 years of organic-layer disturbance, the responses of microbial communities to these disturbances has yet to be analysed (Forbes et al., 2001). The purpose of this study was to assess microbial community changes in response to direct anthropogenic disturbance of permafrost, resulting in abrupt thaw at Dominion Creek, Yukon. I hypothesized that the microbial community of disturbed soils, whether permafrost or active layer, would shift in response to anthropogenic

disturbance due in part to edaphic parameter changes. Based upon previous research into the thaw of permafrost soil (Mackelprang et al., 2011; Monteux et al., 2018), I predicted that the microbiome of disturbed soils would resemble active layer soils in community structure, viability, and abundance.

Most previous work has used laboratory microcosm experiments to discern the effect permafrost thaw has on microbial communities. These studies suggest rapid microbial responses to thaw (Coolen et al., 2011; Mackelprang et al., 2011). However, many soil warming experiments in the field have shown limited responses in soil functioning and no reliable changes in microbial community composition or diversity (Lamb et al., 2011). In response to concerns that these experiments are not replicable in the field, more recent studies have begun researching the microbial ecology of thaw gradients across varying levels of permafrost subsidence (Emerson et al., 2018; Mondav et al., 2017; Woodcroft et al., 2018). These studies often have large geographical distances between thaw levels and thus cannot discern the effects of site history. My research uses a small and abrupt thaw gradient which had been directly disturbed six weeks prior to sampling. This is the first study, to my knowledge, of the effect anthropogenic disturbance on microbial community characteristics across a small disturbance gradient which ultimately resulted in permafrost thaw. This site allows analysis on the effects of permafrost thaw in the field without large spatial distribution.

By analysing the effects of abrupt thaw in the field, my work is applicable to other landscapes that are undergoing frequent permafrost subsidence. I suggest that field and laboratory permafrost thaw studies are not directly comparable due to confounding environmental factors and site history in natural field sites. Therefore, more extensive thaw

experiments in the field are necessary to discern whether community changes are similar to those found in the laboratory. Thus, this study allows coupling of microbial community responses directly to soil disturbance processes.

3.2 Primary Findings

My results show that microbial community composition and diversity of both disturbed active layer and disturbed permafrost microbial communities resemble active layer communities while retaining prominent permafrost taxa in low abundances. Other studies have suggested that new and less diverse microbial communities emerge in soils upon thermokarst fen and bog formation (Hultman et al., 2015; Mondav et al., 2017; Woodcroft et al., 2018). However, direct warming experiments, both in the field and in the laboratory, have suggested that the microbial communities of permafrost soils converge with the active layer upon thaw (Mackelprang et al., 2011; Monteux et al., 2018). Changes associated with fen and bog formation may cause additional edaphic parameter changes, such as the formation of anaerobic environments, which create entirely new microbial communities. The microbial communities in anthropogenically disturbed permafrost undergo shifts similar to those of long-term *in situ* warming experiments and boreal forest fire experiments, both of which suggest that the permafrost microbial community converges with the active layer upon thaw (Monteux et al., 2018; Taş et al., 2014).

While microbial community changes in thawing permafrost often appear to be driven by edaphic parameters, including changes in carbon, nitrogen, pH, and moisture content, this was not the case at Dominion Creek (Taş et al., 2014). While differences within clusters were well explained by pH changes, biotic interactions appeared to drive differences across

disturbed and undisturbed soils. Keystone microbial species may be controlling how this system changes in response to thaw. Microbial community composition and function in thawing permafrost at other sites have been well predicted by the presence of the keystone microbial species *Methanoflorens stordalenmirensis*: predicting methane flux (McCalley et al., 2014), connecting clusters of microbial OTUs (Mondav et al., 2017), and explaining more variation in community composition than edaphic parameters (Woodcroft et al., 2018). Similarly, microbial compositional differences in disturbed soils at Dominion Creek were best explained by community interactions. I suggest that the shift in microbial community structure in permafrost soil is derived from invasion of colonizing active layer microbiota and proliferation of these taxa is primarily driven by biotic filtering, though environmental filtering may explain within cluster differences. Identification of colonizing keystone species may predict long-term functional and compositional changes in directly disturbed soils.

Viability and abundance of microbial cells is commonly assessed in permafrost soils to determine the ability of microbiota to survive and maintain viability under stress. To my knowledge, this assessment has never been performed on actively thawing permafrost. Direct anthropogenic disturbance was found to negatively impact abundance and viability of both active layer and permafrost soils. Our results also corroborate previous findings that permafrost holds fewer cells than active layer soils, though it maintains a similar ratio of viable cells (Steven et al., 2006). However, the majority of microbial cells in soils are not physiologically active, possibly having significant impacts on activity measurements in response to permafrost disturbance (Blagodatskaya & Kuzyakov, 2013). Activity and metabolic potential in disturbed permafrost and active layer soils are highly variable, showing between 1-76% increases in CO_2 flux with permafrost thaw (Schuur et al., 2015).

Thermokarst formation, which increases water content of impacted soils, is often accompanied by decreases in CO_2 flux and increases in anaerobic processes including methanogenesis, anaerobic methane oxidation, fermentation, and anaerobic respiration (Bottos et al., 2018; Hultman et al., 2015; Treat et al., 2014). Physiological adaptations clearly occur in response to disturbance. This may explain high viability in disturbed soils, wherein the inhabiting taxa are capable of retaining viability through physiological acclimation. Coupled with these previous findings, our results suggest that viability and biomass are unlikely to respond to anthropogenic disturbance and that any increased activity of disturbed permafrost soils would be due to a small but viable microbial community.

Permafrost thaw will result predominantly in active layer thickening in some areas, but abrupt permafrost subsidence is becoming more common across the Arctic (Kokelj & Jorgenson, 2013; Streicker, 2016). Subsidence and thermokarst formation result in rapidly changing landforms, impact freshwater chemistry, change biogeochemical and ecological properties of aquatic systems, and liberate large amounts of organic carbon into the atmosphere (Becker et al., 2016; Comte et al., 2016; Wurzbacher et al., 2017). I propose that the impacts of anthropogenic disturbance, such as removal of entire soil horizons, will be analogous to permafrost thaw slumps and active layer detachment slides, all of which remove active layer materials, homogenize soils, remove vegetation, undergo similar vegetation succession, and increase soil temperature drastically (Forbes et al., 2001; Kokelj et al., 2009). Anthropogenic excavation is contrasted by active layer thickening, which does not undergo abrupt reorganization of stratigraphy and thermodynamics. As well, direct anthropogenic disturbance will become more active in a warmer climate by opening up the Northwest Passage for industrial activities and encouraging immigration and tourism in the North

(Similä & Jokinen, 2018). My research provides a first look at how abrupt disturbance may impact microbial communities in a dynamic subarctic landscape, suggesting that abrupt disturbances may induce microbial community changes which may not be found in landscapes undergoing active layer thickening. These communities are driven primarily by biotic interactions, rather than by edaphic parameter differences across thawing soils. However, these compositional and functional shifts have been observed on natural disturbances and so they may not be limited to direct anthropogenic disturbances. Due to the novelty of this research, direct comparisons with previous findings in naturally disturbed sites must be interpreted with caution.

3.3 Improvements to the Study and Future Directions

This study analysed total metal concentrations to determine micronutrient differences between permafrost and active layer soils, for the purposes of discerning if they would significantly affect microbial community structure. These parameters were chosen due to the known release of micronutrients with permafrost thaw (Reyes & Lougheed, 2015). Additionally, I also analysed macronutrient concentrations (total nitrogen and total carbon) as well as soil organic matter, pH, and water content. These parameters are known drivers of microbial community composition in periglacial soils (Bottos et al., 2018; Chu et al., 2010; Tripathi et al., 2018a; Tripathi et al., 2018b). However, recent research indicates that dissolved organic carbon and dissolved organic nitrogen are amongst the most important drivers of microbial community dynamics, likely due to the abundance of microbiota residing within brine films within frozen soils. Thus, I recommend the analysis of dissolved macronutrients in future studies; such analysis may better explain the compositional and

diversity changes I saw across the disturbance gradient as well as between permafrost and active layer soil types.

Archaeal communities are integral to nitrogen and carbon turnover in permafrost soils. I analysed the bacterial community of disturbed periglacial soils using prokaryotic primers which have been optimized for some, but not all archaeal taxa. While this has allowed for broad determination of archaeal taxonomic identification and relative abundance, there is likely a unsampled diversity of archaeal taxa in our samples. Archaea are central to methane dynamics in permafrost microbial communities and are responsible for a large proportion of CO_2-carbon equivalent flux from thawing permafrost soils. As well, recent studies have shown the composition of bacterial and archaeal communities may have a significant effect on carbon flux from permafrost soils (Monteux et al., 2018; McCalley et al., 2014). Therefore, deep sequencing of archaeal diversity could improve prediction of compositional shifts of ecologically important taxa in response to permafrost thaw.

While I determined viability of microbiota across my samples, in abundance and diversity, I did not directly assess activity. Field fresh soils are most appropriate for activity measurements, however, the capacity of periglacial soil communities to sustain freeze/thaw cycles and persistently low temperature may allow for the use of previously stored and frozen soil samples (Juan et al., 2018). Community DNA-stable-isotope probing (SIP) allows for identification of actively replicating organisms that consume a specific substrate (Tuorto et al., 2014). SIP is a particularly powerful method in which environmental samples are incubated with stable isotope (13C, 2H, 15N)-labelled substrates; those community members that consume the labeled substrate subsequently incorporate the label into their DNA. Density-gradient centrifugation separates labelled DNA (from replicating organisms) from

unlabelled DNA (from non-replicating organisms). The resulting DNA fractions can be characterized through 16S rRNA gene sequencing or metagenomics. The application of this method could be used to identify metabolically active microorganisms across the disturbance gradient for any number of functional groups. This approach would allow, for example, characterization of methanotrophic taxa and their potential to mediate methane fluxes from thawing permafrost soils.

Samples were taken in May of 2016 before maximal thaw depth was reached in these samples. Both frozen and thawed active layers were sampled, and no significant community differences were found between thawed and frozen active layer samples at similar soil depth. Robust temporal sampling is required for accurate modelling of microbial community composition and function in soils (Männistö et al., 2018; Schostag et al., 2015; Suseela et al., 2012). Seasonal sampling may reveal seasonal variation in community dynamics and subtle shifts community composition, viability, and function that differ with thaw. Ecological recovery after disturbance can often occur over decadal time-scales (Forbes et al., 2001; Melillo et al., 2017). Additional time may be required for ecological recovery and reconstruction of microbial communities in disturbed soils, resulting in either in a fully restored microbial community or a new ecosystem equilibrium with altered composition, structure, and function. Therefore, multi-year sample collection and analysis would allow for determination of additional community changes in samples, as well as changes in viability and biomass.

Thaw slumps and active layer detachment slides represent responses to rapid thaw of ice-rich transient layers and usually develop in ice-rich glaciogenic deposits (Kokelj & Jorgenson, 2013). These events rapidly mobilize carbon, elevate soluble ion concentrations,

change pH, and are known to adversely affect vegetation, similar to anthropogenic thaw (Lantz et al., 2009). As more attention is drawn to the biogeochemical ramifications of thaw slumps and detachment slides, understanding how the microbial community shifts with, and impacts, these cycles may explain how future fluxes may change. This can also have downstream effects on downstream water ecology and biogeochemistry (Tananaev et al., 2018). Therefore, future analysis of soils exposed by retrogressive thaw slumps and active layer detachment slides may directly assess the effects of rapid permafrost thaw forms on microbial community dynamics. Characterizing the microbial communities of active and stable thaw slumps will allow me to assess the vulnerability of these soils and waterways to future changes in microbial function.

Additional surveying of different direct anthropogenic disturbances is required for extrapolation onto the entirety of the Arctic. Direct disturbances in the Arctic have been catalogued according to their type and severity (Forbes et al., 2001). However, these studies have not included the assessment of microbial responses to disturbance. A number of samples within the Dominion Creek area have well documented site history of anthropogenic disturbances: three road construction disturbances (both within and adjacent to road ruts), one fire disturbance, and a wooded control core (Calmels et al., 2012). Characterizing the microbial communities of these sites would allow for comparative analysis of historical, as well as contemporary, permafrost disturbance. As categorized in Forbes et al., (2001), additional microbial sampling should also include soils disturbed by modern and ancient housing, stockpiling of soil and organic matter, and pedestrian trampling. Expanding the sampling area across the Arctic and subarctic should be made with caution, as distinct microbial assemblages have been recovered between Beringian and Canadian High Arctic

permafrost and active layer soils (Malard & Pearce, 2018; Jansson, & Taş, 2014). Therefore, to prevent the confounding effects introduced by biogeographical variation, future work should be conducted within Beringia: both Alaska and Yukon territory. With careful addition of historical and contemporary disturbances to this sample we may better predict long-term trends in microbial ecological recovery in periglacial soils.

3.4 Concluding remarks

Permafrost carbon across the circumpolar region is extremely vulnerable to microbial mineralization in response to thaw. My research identifies microbial community changes in rapidly thawing, anthropogenically-disturbed permafrost. I have identified taxa characteristic of permafrost, active layer, and disturbance gradient soils, suggesting that permafrost taxa are capable of persisting in disturbed soils even after thaw. I have also shown that bacterial community assemblage and membership responds within six weeks of permafrost thaw, which has not previously been shown. Therefore, I suggest that abrupt permafrost thaw is likely to shift microbial community composition drastically, though the same shifts may not be extendable to sites undergoing active layer thickening under a similar timescale. My findings may be applied not only to human disturbances but also to natural thaw slumps, suggesting that these disturbances may have a significant impact on the microbial abundance and diversity across Arctic soils.

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Appendix 1: Supporting Information for Chapter 2

Supplementary Table 1. Physical description of frozen permafrost, active layer, and disturbance gradient soils.

	Series	Material Type	pH (aq)	% SOM (w/w)	Water Content (w/w)	DNA Concentration (ug/mg)
Core 1	Core 1 15	Peat	6.42 0.03	0.667 0.038	82% 3%	106.31 7.18
	Core 1 30	Peat	5.187 0.079	0.638 0.068	77% 2%	148.93 16.74
	Core 1 45	Peat	5.473 0.106	0.088 0.02	59% 10%	29.04 0.50
	Core 1 75	Silt	5.627 0.085	0.365 0.073	64% 8%	25.19 2.41
	Core 1 95	Silt	6.173 0.032	0.382 0.085	62% 3%	2.77 0.36
Core 2	Core 2 30	Peat	5.553 0.237	0.804 0.009	83% 1%	14.99 3.74
	Core 2 45	Peat	5.933 0.059	0.722 0.105	85% 4%	9.46 0.61
	Core 2 75	Peat	5.733 0.003	0.703 0.097	73% 5%	17.94 2.73
	Core 2 95	Peat	5.703 0.022	0.837 0.006	79% 2%	13.73 1.44
Core 3	Core 3 80	Silt	6.13 0.06	0.173 0.054	59% 2%	102.84 3.20
	Core 3 95	Silt	5.84 0.075	0.615 0.047	88% 1%	37.75 9.35
Disturbance Gradient	A	Peat	6.25 0.03	57% 6%	76% 3%	374.62 50.58
	B	Peat	6.04 0.84	44% 21%	67% 10%	140.36 26.01
	C	Peat	5.49 0.18	73% 5%	77% 3%	140.76 9.20
	D	Peat	5.53 0.09	85% 6%	89% 1%	78.13 25.64
	E	Silt	5.76 0.07	33% 6%	62% 4%	36.52 24.52
	F	Silt	6.33 0.26	32% 10%	70% 6%	39.05 22.96

Supplementary Table 2. Soil chemistry including dissolved metals and nutrient levels in frozen active layer samples, permafrost samples, and the surface soil disturbance gradient.

Treatment	P	K	S	Mg	Ca	Fe	Cu	Mn	Zn	Na	NO3-N	NH4-N	TN	TC
	(%)						(mg/kg)				(mg/kg)		(w/w%)	
DC1 AL_1	0.10	0.18	0.18	0.57	2.10	2.13	760.61	1913.42	88.14	260.00	0.35	0.73	0.95	29.66
DC1 AL_2	0.10	0.09	0.53	0.23	2.57	1.71	249.87	2161.04	71.75	190.78	0.13	0.58	1.66	35.53
DC1 AL_3	0.08	0.16	0.13	0.43	0.93	2.06	87.29	645.10	66.28	234.79	5.85	10.97	0.59	9.93
DC1 P_1	0.07	0.12	0.12	0.47	0.84	2.34	42.64	344.53	72.83	220.38	0.09	39.26	0.53	9.01
DC1 P_2	0.09	0.14	0.19	0.47	1.31	2.59	44.99	501.93	89.99	212.79	0.24	27.05	0.75	13.52
DC4 AL_2	0.12	0.16	0.20	0.43	1.28	3.05	99.49	1000.20	116.73	187.93	0.44	12.74	0.92	19.93
DC4 AL_3	0.12	0.20	0.22	0.48	1.24	2.30	67.59	786.22	88.33	266.63	0.41	23.14	1.18	19.84
DC4 P_1	0.12	0.06	0.51	0.20	2.40	1.99	45.15	1693.89	66.17	150.24	0.37	1.14	1.90	37.75
DC4 P_2	0.07	0.18	0.25	0.38	1.91	1.60	61.74	677.83	80.87	176.52	0.28	0.95	1.07	27.26
DC3 P_1	0.10	0.04	0.57	0.12	2.48	1.53	62.05	897.00	49.05	195.00	0.17	6.83	2.04	37.23
DC3 P_2	0.08	0.02	1.12	0.10	2.87	1.50	44.97	858.00	54.11	172.16	0.29	12.92	2.43	41.56
A	0.08	0.23	0.16	0.56	2.43	2.30	33.91	769.77	108.20	214.24	0.82	19.23	6.12	1.01
B	0.07	0.24	0.10	0.63	1.31	2.91	36.18	1487.96	118.79	196.60	0.76	15.23	1.54	0.56
C	0.15	0.10	0.35	0.25	2.35	3.38	32.50	4533.75	128.38	169.81	0.25	4.78	1.05	1.76
D	0.16	0.04	0.49	0.15	2.50	2.16	39.00	1248.00	65.00	136.81	1.55	1.35	0.14	2.70
E	0.07	0.17	0.19	0.53	1.24	2.80	48.13	816.61	111.49	188.01	0.72	13.95	1.40	0.79
F	0.08	0.14	0.17	0.44	1.11	2.36	33.05	639.07	89.42	251.25	1.27	1.28	0.31	0.75
Error	±0.02	±0.03	±0.03	±0.05	±0.03	±0.0025	±1	±7	±6	±0.1	±0.01	±0.20	±1.19	±0.1

Supplementary Table 3. α-diversity metrics of frozen active layer, permafrost, and disturbance gradient soils.

Series	Coverage	Diversity		Richness		Evenness		DNA Content	
		Invsimpson	Shannon	Chao1	Richness	Simpson	Shannon	Concentration (ng/uL)	Standard Deviation
Core 1 15	0.924412	189.61	6.20	2884.41	1552.00	0.12	0.84	26.58	1.80
Core 1 30	0.930006	76.86	5.51	1232.44	1039.00	0.07	0.79	37.23	4.19
Core 1 45	0.979709	36.68	4.55	1269.60	570.00	0.06	0.72	7.26	0.12
Core 1 75	0.979167	27.42	4.46	1008.00	600.00	0.05	0.70	6.30	0.60
Core 1 95	0.978103	26.80	4.26	430.58	393.00	0.07	0.71	0.69	0.09
Core 3 80	0.967037	41.82	4.48	571.28	456.00	0.09	0.73	3.75	0.93
Core 3 95	0.969964	35.32	4.63	1064.23	682.00	0.05	0.71	2.36	0.15
Core 2 30	0.965359	84.04	5.47	1215.38	871.00	0.10	0.81	4.48	0.68
Core 2 45	0.967889	63.00	5.19	1261.78	829.00	0.08	0.77	3.43	0.36
Core 2 80	0.959523	60.05	5.00	1110.76	714.00	0.08	0.76	25.71	0.80
Core 2 95	0.961462	42.39	4.79	738.61	636.00	0.07	0.74	9.44	2.34
A	0.958626	153.05	6.07	1064.00	1064.00	0.14	0.87	93.66	12.65
B	0.9238	110.12	5.84	2686.24	1433.00	0.08	0.80	35.09	6.50
C	0.946705	87.23	5.56	1947.19	1127.00	0.08	0.79	35.19	2.30
D	0.94213	86.87	5.60	2722.53	1263.00	0.07	0.78	19.53	6.41
E	0.89999	186.53	6.15	1649.87	1400.00	0.13	0.85	9.13	6.13
F	0.89436	138.37	6.08	4494.70	1773.00	0.08	0.81	9.76	5.74

Supplementary Table 4. The taxonomic assignment of indicator OTUs associated with the frozen active layer.

OTU ID	Phylum	Class	Order	Family	Genus	Species
OTU5	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis			
OTU29	Proteobacteria	Betaproteobacteria				
OTU31	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	
OTU33	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	Nakamurella	Nakamurella_flavida
OTU36	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU39	Proteobacteria	Betaproteobacteria				
OTU42	Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	Solirubrobacter_taibaiensis
OTU44	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	Ilumatobacter	
OTU48	Proteobacteria	Alphaproteobacteria	Rhizobiales			
OTU63	Proteobacteria	Betaproteobacteria				
OTU64	Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae		
OTU70	Proteobacteria	Betaproteobacteria	Burkholderiales			
OTU76	Proteobacteria	Betaproteobacteria				
OTU78	Acidobacteria	Acidobacteria_Gp4	Gp4			
OTU101	Acidobacteria	Acidobacteria_Gp4	Gp4			
OTU109	Actinobacteria	Actinobacteria	Gaiellales	Gaiellaceae	Gaiella	Gaiella_occulta
OTU132	Acidobacteria	Acidobacteria_Gp4	Gp4			
OTU133	Acidobacteria	Acidobacteria_Gp4				
OTU144						
OTU152	Actinobacteria	Actinobacteria	Actinomycetales			
OTU153	Acidobacteria	Acidobacteria_Gp3	Gp3			
OTU158						
OTU159	Proteobacteria	Betaproteobacteria				
OTU167	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU168	Actinobacteria	Actinobacteria				
OTU171	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae		
OTU181	Acidobacteria	Acidobacteria_Gp4	Aridibacter			
OTU190	Actinobacteria	Actinobacteria	Solirubrobacterales			
OTU197						
OTU203	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis			
OTU208	Proteobacteria	Deltaproteobacteria	Myxococcales			
OTU221	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU222	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis			
OTU224	Acidobacteria	Acidobacteria_Gp3				

OTU237	Proteobacteria	Betaproteobacteria				
OTU240	Acidobacteria	Acidobacteria_Gp3	Gp3			
OTU256	Acidobacteria	Acidobacteria_Gp16	Gp16			
OTU273	Actinobacteria	Actinobacteria	Acidimicrobiales	Iamiaceae	Aquihabitans	Aquihabitans_daechungensis
OTU279	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	
OTU288	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis			
OTU301	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae		
OTU310	Actinobacteria	Actinobacteria				
OTU321						
OTU322	Proteobacteria	Deltaproteobacteria	Myxococcales			
OTU327	Proteobacteria	Betaproteobacteria				
OTU331	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	Opitutus_terrae
OTU353	Actinobacteria	Actinobacteria	Solirubrobacterales			
OTU361	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae		
OTU367	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU369						
OTU383	Actinobacteria	Actinobacteria	Actinomycetales			
OTU390	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU430	Actinobacteria	Actinobacteria				
OTU444	Actinobacteria	Actinobacteria				
OTU459	Proteobacteria	Deltaproteobacteria	Myxococcales			
OTU471	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae		
OTU481	Proteobacteria	Betaproteobacteria				
OTU497						
OTU514						
OTU569	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Alterococcus	Alterococcus_agarolyticus
OTU600	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae		
OTU614						
OTU636	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae		
OTU659	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis			
OTU682						
OTU686	candidate_division_WPS-1					
OTU692						
OTU724	Actinobacteria	Actinobacteria				
OTU745						
OTU772	Proteobacteria	Gammaproteobacteria				
OTU868	Actinobacteria	Actinobacteria	Gaiellales	Gaiellaceae	Gaiella	Gaiella_occulta
OTU885	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU943	Proteobacteria	Betaproteobacteria				

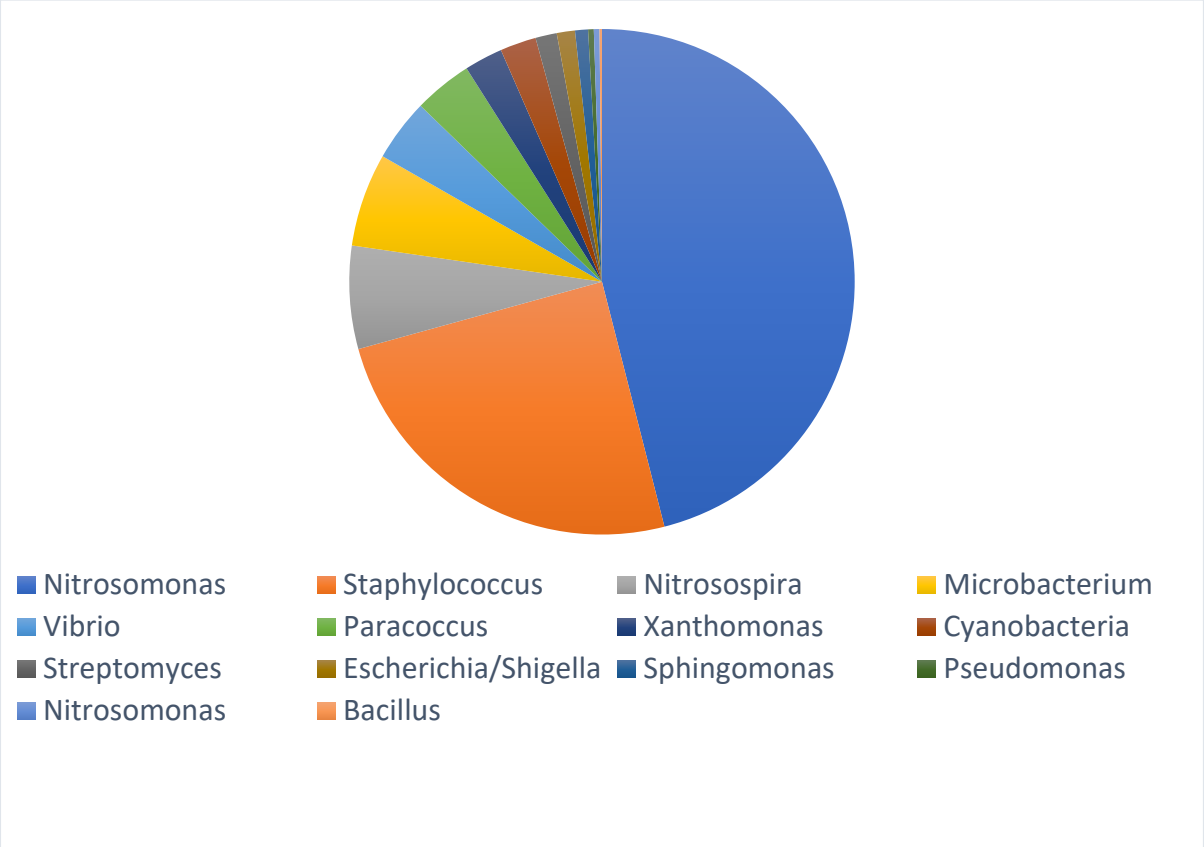
OTU964	Acidobacteria	Acidobacteria_Gp16				
OTU987						
OTU1031	Actinobacteria	Actinobacteria	Acidimicrobiales	Iamiaceae	Aquihabitans	Aquihabitans_daechungensis
OTU1180						
OTU1227						
OTU1291						
OTU1301	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU1390	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU1740	Acidobacteria	Acidobacteria_Gp3	Candidatus_Solibacter			
OTU1744	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU1997	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae		
OTU2159	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU2283	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU2393	Acidobacteria	Acidobacteria_Gp6	Gp6			
OTU2972	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_incertae_sedis			
OTU4020	Proteobacteria	Alphaproteobacteria	Rhizobiales			
OTU4207	Acidobacteria	Acidobacteria_Gp4	Gp4			
OTU4526	Proteobacteria					
OTU4972						
OTU5097	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis			
OTU5141	Acidobacteria	Acidobacteria_Gp6	Gp6			

Supplementary Table 5. The taxonomic assignment of indicator OTUs associated with the frozen permafrost.

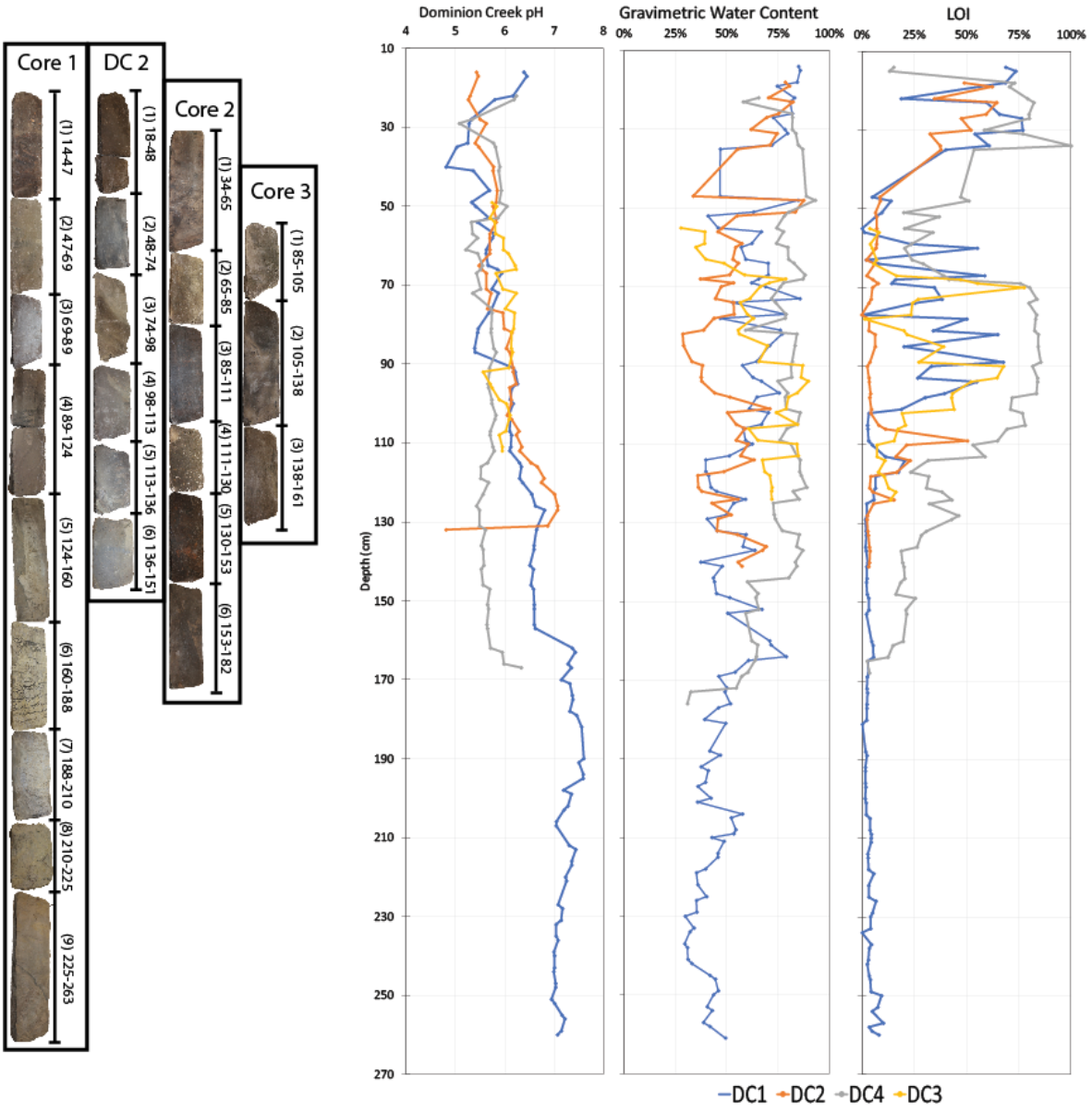
OTU ID	Phylum	Class	Order	Family	Genus	Species
OTU4						
OTU6	Actinobacteria	Actinobacteria				
OTU13	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Smithella	Smithella_propionica
OTU17						
OTU28	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	Nitrosospira	Nitrosospira_multiformis
OTU32	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto	Clostridium_estertheticum_subsp._laramiense
OTU40	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae		
OTU56	Bacteroidetes					
OTU67	Bacteroidetes					
OTU71	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	
OTU80	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU81	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	
OTU82	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae		
OTU84						
OTU87	Firmicutes	Bacilli	Bacillales	Bacillaceae_1		
OTU95						
OTU99	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ercella	Ercella_succinigenes
OTU100	Firmicutes	Clostridia	Clostridiales	Peptococcaceae_1	Desulfosporosinus	
OTU104	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU110	Firmicutes	Clostridia	Clostridiales			
OTU128	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae		
OTU135	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	Saccharofermentans_acetigenes
OTU136	Bacteroidetes					
OTU138	Proteobacteria					
OTU149						
OTU151						
OTU176	Bacteroidetes					
OTU195						
OTU199	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Syntrophus	
OTU202	Actinobacteria	Actinobacteria				
OTU204	Firmicutes	Clostridia	Clostridiales	Peptococcaceae_2		
OTU206						
OTU211	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU233						
OTU269						

OTU313	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Beijerinckia	Beijerinckia_indica_subsp._lacticogenes
OTU328	Armatimonadetes	Armatimonadetes_gp2				
OTU330	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU337	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	Bacillus	Bacillus_psychrosaccharolyticus
OTU366						
OTU370	Firmicutes	Bacilli	Bacillales	Paenibacillaceae_1		
OTU372	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU376	Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina	Sporosarcina_psychrophila
OTU379	Ignavibacteriae	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae		
OTU381						
OTU385	Armatimonadetes	Armatimonadetes_gp2				
OTU407						
OTU414	Firmicutes					
OTU425	Firmicutes	Clostridia	Clostridiales			
OTU491						
OTU564						
OTU771	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ercella	Ercella_succinigenes
OTU857	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Psychrosinus	
OTU896						
OTU1016						
OTU1468						
OTU2041						
OTU2176						
OTU2334	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto	

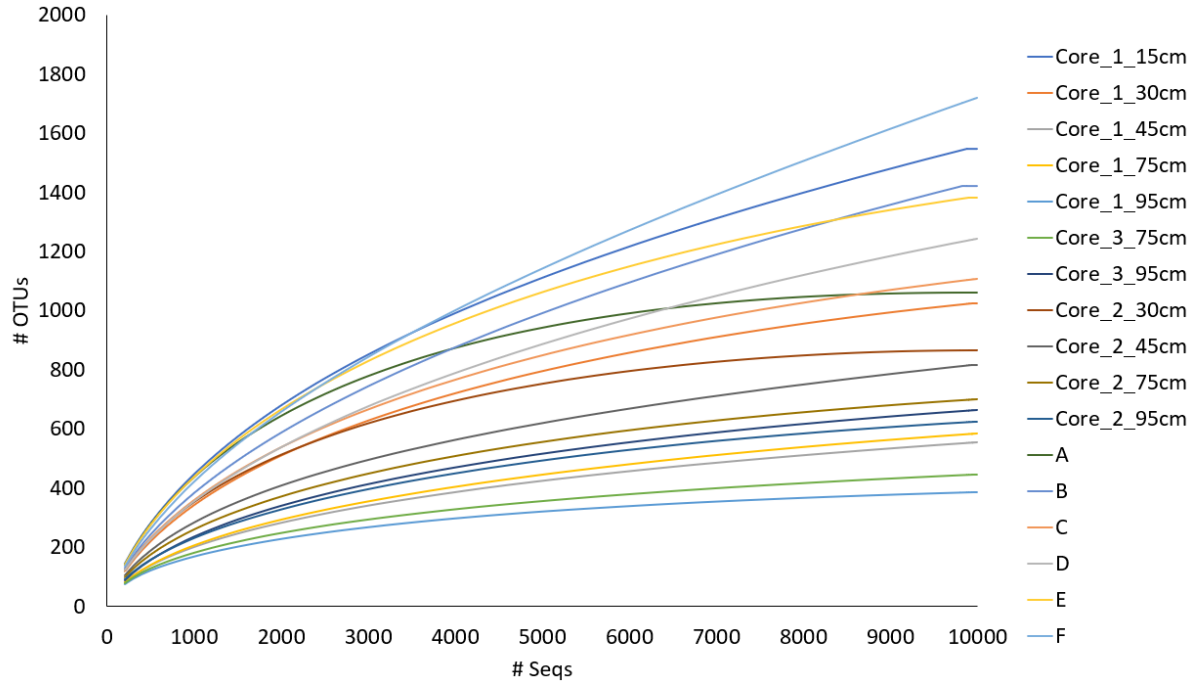
Supplementary Figure 1. Analysis of the presence and relative abundance of mock community members.



Supplementary Figure 2. Geochemical profiles of Core 1, Core 2, Core 3, and DC2. Parameters included pH, gravimetric water content, and LOI. DC2 was not used for later analyses due to the absence of substantial soil organic matter from 70-110 cm in depth.



Supplementary Figure 3. Rarefaction curves present good coverage of most samples with the exception of the most disturbed surface soils. Each curve represents the subsampled richness level at each level of sequencing intensity from 0 to 10,000 sequences.



Appendix 2: Microbial community analysis of thawing permafrost is not impeded by relic DNA

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A limited number of samples were subjected to PMA treatment to delineate between total (non-PMA treated) and viable assemblages (PMA treated) within permafrost samples and disturbance gradient soils. Trends did not differ between the total assemblage and the viable assemblage and so treatments were not extended onto other samples. However, with increasing study into the impact of relic DNA on microbial community analysis in soils, it is prudent to report the results of this analysis in the hopes that this will encourage future work on the subject of relic DNA in permafrost. The aims, methods, results, and discussion of this analysis are described below.

Introduction

Assessing biodiversity in soils is crucial to understanding soil microbial succession, biogeochemical cycling, as well as chemical and biological structures of soils (Malard & Pearce, 2018). Anthropogenic thawing of permafrost, soils which have been frozen for at least 2 consecutive years, may form a feedback loop of warming where microbially mediated carbon release increases global temperatures, which once again speeds permafrost thaw (Walter et al., 2006). Characterization of microbial community diversity and function in these soils would allow for predictive modeling of biogeochemical cycling changes induced by climate change (Schuur et al., 2015). Recent assessment of microbial diversity in Northern soils has identified links between microbial diversity and microbial activity both in field and laboratory permafrost thaw experiments (Monteux et al., 2018; Müller et al., 2018). This is primarily due to the ability of newer high throughput techniques to better assess microbial diversity, whereas earlier studies showed no such relationships (Lamb et al., 2011). Accurate coupling of community function with diversity depends upon continuing advances in assessing microbial diversity.

When organisms die, through a variety of factors including autolysis, senescence, viral infection, or predation, DNA is released into the environment, known as relic DNA. Relic DNA can be well preserved in soil environments, due to the sorption of nucleic acids to soil particles, high electrical conductivity, low to neutral pH, protection from UV associated damage, and high metal concentrations (Lennon et al., 2018). These factors all repress common temperature dependent DNA damaging processes including hydrolysis, oxidation, and alkylation reactions (Willerslev et al., 2004). As DNA represents a macromolecule which is rich in nitrogen, carbon, and phosphate, complex soil environments and cold temperature of permafrost prevents microbial degradation of these molecules (Dell'Anno & Danovaro, 2005). Frozen permafrost is therefore an ideal environment for the long-term preservation of relic DNA for as long as 400 kya due to its low temperature, low pressure, and low levels of microbial activity (Johnson et al., 2007). While relic DNA can serve as a genetic reservoir for permafrost microorganisms, it can also have a significant impact on our understanding of soil microbiology.

Relic DNA (also known as extracellular DNA) is known to interfere with microbial diversity estimations (Carini et al., 2017). Microbial community analysis using 16S rRNA gene sequencing, does not distinguish between intracellular (viable) DNA and relic DNA (Emerson et al., 2017). As a result, current high-throughput techniques simultaneously measure the diversity of all microbial physiological states of life, although only a small proportion of cells can participate in biogeochemical cycling processes (Emerson et al., 2017). Relic DNA is routinely used for reconstruction of paleoenvironments using permafrost soils; however, differentiation of this DNA from viable permafrost microorganisms has yet to be conclusively conducted. Differentiation of viable and relic communities has previously been attempted by distinguishing psychrotolerant/psychrophilic organisms from mesophilic organisms in permafrost (Bellemain et al., 2013). However, selecting viable/non-viable taxa

based upon physiology is open to database bias, improper taxonomic assignment, and is dependent upon the quality of physiological characterization. Characterization of active permafrost microbial communities has also been performed, though this precludes dormant microorganisms which may be capable of reactivation upon thaw (Coolen & Orsi, 2015; Emerson et al., 2018; Hultman et al., 2015; Tuorto et al., 2014). The use of nucleases and photoactivated DNA binding dyes to degrade or inhibit the amplification of relic DNA, respectively, has been integrated into the study other environments including soil, gut, water, sputum, and sediment microbial ecology (Carini et al., 2017; Lennon et al., 2018; Nguyen et al., 2016; Nocker et al., 2007; Nocker et al., 2010). These assessments have shown varying success in identifying rarer taxa, though overestimation of species richness in soil environments has been shown and is dependent upon the proportional abundance of relic DNA in the environment (Carini et al., 2017; Nguyen et al., 2016). While viability qPCR, integration of DNA binding dyes which inhibit the amplification of relic DNA, has been used in permafrost soils previously, it has lacked optimization protocols which may contribute to the production of null results (Yergeau et al., 2010). As a result, viable community analysis of thawing permafrost soils has yet to be conducted.

I intend to determine if the viable microbial community of permafrost is distinct from the total assemblage and if these differences impact microbial community dynamics of thawing permafrost. I hypothesize that (1) permafrost is capable of sustaining relic DNA to extent which is sufficient to create a total microbial community of permafrost which will be more similar to the active layer microbial community than the viable community and (2) that if relic DNA is rapidly degraded during permafrost thaw, then the viable microbial community of disturbed permafrost will resemble the total community of disturbed permafrost due to relic DNA degradation. Permafrost thaw has been hypothesized to reactivate dormant organisms and increase the degradation of DNA (Kryazhevskikh et al.,

2012). I have therefore applied an optimized protocol for propidium monoazide (PMA) treatment of soils for the purposes of removing relic DNA. This procedure defines a viable microbial cell as having an intact cell membrane and removes relic DNA which is extracellular or found in cells with compromised cell membranes. Both PMA treated, and non-PMA treated samples were subjected to 16S rRNA gene sequencing to determine the microbial community diversity of permafrost, active layer, and disturbed permafrost. My findings suggest that relic DNA has limited impacts on diversity analysis in permafrost soils and reduces community differences between permafrost and active layer microbial communities.

Methods

PMA Treatment

Soils A, F, Core 1 95-100, Core 2 95-100, and Core 3 95-100 were subsampled as described in Chapter 2.4. Each sample was weighed to 0.50 g and suspended in 4.5 ml of filter sterilized and autoclaved 1×PBS (pH 7.4; 137 mM NaCl, 2.7 mM KCl, 10mM Na_2HPO_4, and 1.8mM KH_2PO_4). A serial dilution of this 1:10 (w/v) soil:PBS solution was performed to obtain a 1:1000 (w/v) soil:PBS dilution at a final volume of 1 ml. This solution was mixed with 0.04 mM propidium monoazide prepared by dissolving 1 mg PMA in 98 µl dH_2O to create a 20 mM stock solution, of which 2 µl were added to the soil solution (Biotium Inc., CA, USA). The soil solution was then incubated in the dark for 10 min with vigorous hand shaking every 2 min. PMA was cross-linked to DNA with a 500 W halogen lamp (Globe Electric, Quebec, Canada) by placing the samples 20 cm from the light source for 10 min on ice. After light exposure, samples were filtered onto a 47 mm diameter 0.22 pore size polysulfone (Supor) filter (Pall Corporation, NY, USA). Filter papers were cut into

1/3 segments and DNA from each segment was then extracted using a MOBio PowerSoil DNA extraction kit (Qiagen, Germany), following manufacturer's instructions and recommendations as found in Chapter 2 of this thesis. Non-PMA samples were extracted from a 0.5 g solid sample without dilution, PMA treatment, or crosslinking.

16S Gene Amplicon Sequencing and Analysis

The DNA of the V4 region of the 16S rRNA gene was sequenced from both PMA and non-PMA treated samples using the primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) (Caporaso et al., 2012). Sequencing was performed commercially by Microbiome Insights (Microbiome Insights, Vancouver, BC, Canada), with an Illumina MiSeq using V3 chemistry (Illumina, San Diego, California, United States). Prior to sequence processing a total of 190,150 reads were recovered from 10 samples, ranging from 3,475 to 45,100 reads per sample. Sequence processing was performed in USEARCH v10 (Edgar, 2010). Overlapping reads were combined from forward and reverse reads with the following qualifiers: no unknown base pairs allowed (Ns), a maximum number of sequence mismatches of 10 nt in the alignment, a minimum merge length of 230 bp, a maximum merge length of 300 bp, and an ID cut-off of 80%. Of the 190,150 reads recovered 175,940 reads were aligned into overlapping reads (93.4% of the total), at an average length of 253 bp, with an expected size of 291 bp. Quality filtering with a maximum expected error cut-off of 1.0 resulted in 174,181 (99.0% of the total) of the reads passing (Edgar & Flyvbjerg, 2015). Dereplication identified 105,185 unique sequences and 105,589 singletons (71.2% of the unique reads) for the total dataset. OTU clustering at 97% using UPARSE identified a total of 3,760 OTUs as well as identifying and removing 7,557 chimeras, while removing all singletons and doubletons (Edgar, 2013). After sequence processing 132,202 reads remained. Taxonomy was assigned using de novo picking as

implemented in SINTAX using the RDP database at a bootstrap cut-off of 80 (Ribosomal Database Project) (Edgar, 2016). Sequences were separated for bacterial (127,909 reads) and Archaeal (4,293 reads) analysis and Archaeal reads were discarded. Taxa identified as chloroplast/streptophyta using SINTAX were removed from downstream analysis and no mitochondrial reads were observed, leaving 125,397 reads in the bacterial dataset. A NEWICK formatted 16S rRNA phylogenetic tree was constructed using USEARCH v10. Samples were rarefied to 2,000 reads.

Diversity Analysis

α-diversity metrics were calculated using Mothur v 1.39.5 including species richness, Chao 1 richness estimation, Shannon Diversity, Shannon Evenness, Simpson Diversity, Simpson Evenness, and Heip's Evenness (Schloss et al., 2009). Faith's phylogenetic diversity (PD) was assessed using the *picante* package in R (Faith & Baker, 2006). Checking for significant differences between samples was performed using a Kruskal-Wallis test followed by post-hoc testing with Kruskal-Wallis multiple comparisons as implemented in SigmaPlot v13.0 (Systat Software Inc., CA, USA).

β-diversity was calculated USEARCH v10 using Bray-Curtis, Jaccard presence/absence, and weighted UniFrac distance metrics. Visualization of community dissimilarities in nonmetric multidimensional scaling (NMDS) plots was carried out using R v3.4.1, in the RStudio IDE with the vegan package v2.4-4 (Philip, 2003). Cluster integrity as assessed through silhouette width was carried out using the fpc package v2.1-10 (Hennig, 2018). Hierarchical clustering was visualized with the dendextend package v1.5.2 (Galili, 2015). The microbial communities of samples soils were surveyed by small subunit rRNA gene amplicon sequencing

Taxonomic Comparisons

Phylum, class, and genus level tables were constructed using SINTAX. A stacked bar chart of relative abundance for class level taxonomic classification was constructed in MS Excel where phyla which were not present at over 1.0 % relative abundance in at least one sample were combined into “Other” alongside unassigned taxa (Microsoft Corporation, WA, USA). Assessment and visualization of significant ($p<0.05$) differential abundances was assessed using Analysis of Composition of Microbiomes (ANCOM) (Mandal et al., 2015). Significance testing was followed by post-hoc analysis using false discovery rate to correct for multiple comparisons. Only significantly different groups were visualized.

Results

Microbial diversity differs between viable and total assemblages in permafrost soils

The viable assemblage of permafrost was found to be a subset of the total assemblage, where the total assemblage has a large number of OTUs not found in the viable assemblage (Figure 10). A larger proportion of unique OTUs were found in the viable assemblages of the disturbance gradient soils A (25.0%) and F (24.6%), than in permafrost (14.2%). Viable permafrost communities had the shared the most OTUs with its total assemblage (75.5%), compared to A (53.3%) and F (62.4%). Likewise, the permafrost samples harboured the most OTUs unique to the total assemblage (42.1%) compared to disturbance gradient soils A (35.0%) and F (29.0%).

PMA treatment significantly impacted diversity estimations in both permafrost and disturbance gradient soils. Viable microbial richness (both number of observed OTUs and

Chao1 non-parametric richness estimation) of permafrost soils was significantly lower than the total assemblage, while Simpson evenness was significantly higher ($p<0.05$) (Table 1). Richness in permafrost decreased by 36% and 65% in observed OTUs and Chao1 richness estimation, respectively, while Simpson evenness increased by 36% between viable and total assemblages. Diversity levels (richness, evenness, diversity, and phylogenetic diversity) did not significantly change across disturbance gradient soils with PMA treatment. When comparing between surface and permafrost soils, a significant difference was found between surface and permafrost soils in evenness regarding total assemblages, but no significant difference was found in evenness in the viable assemblage ($p<0.05$) (Table 1). Similarly, phylogenetic diversity was significantly different in viable assemblages, but not in the total assemblage ($p<0.05$).

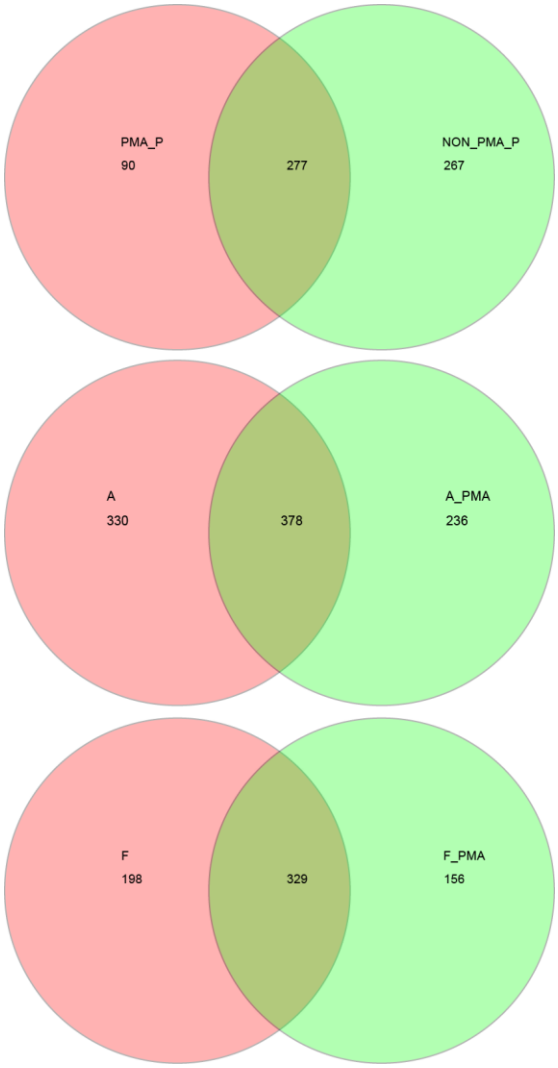

Figure 10. Venn diagram of shared observed richness across viable and total assemblages in permafrost, A, and F samples.

Table 1. α-diversity metrics of bacterial communities in viable and total assemblages of disturbance gradient soils and permafrost soils.

Group	Observed OTUs	Chao1	Inverse Simpson Diversity	Simpson Evenness	Shannon Diversity	Shannon Evenness	Heip's	Faith's Phylogenetic Diversity	PD/OTU	NRI	NTI	NRI:NTI
A	708	1624.019231	144.083243	0.203507	5.837234	0.889491	0.483493	140.08771	0.197863997	19.01354	7.249364	2.62279
A_PMA	614	1475.038462	129.692308	0.211225	5.658511	0.881389	0.466104	123.81563	0.201654121	14.174452	8.404254	1.68658
F	527	1103.111111	106.826001	0.202706	5.436953	0.867525	0.434869	111.76609	0.212079867	17.069337	7.788324	2.19166
F_PMA	485	935.575	75.639784	0.155958	5.212326	0.842853	0.377108	109.5806	0.225939381	13.554406	5.685879	2.38387
Core_1_95cm	247	467.676471	24.726249	0.100106	4.150408	0.753334	0.253902	61.64078	0.249557814	4.85515	4.330423	1.12117
Core_1_PMA	186	209.522727	26.216063	0.140947	4.008001	0.766972	0.292091	48.39722	0.260200108	6.881259	4.773885	1.44144
Core_2_95cm	310	743.15625	36.95431	0.119207	4.486186	0.782032	0.253902	91.27496	0.294435355	4.373524	4.095833	1.0678
Core_2_PMA	172	172	26.721017	0.155355	4.106583	0.797783	0.34935	52.64157	0.30605564	4.119986	3.377273	1.21992
Core_3_95cm	332	609.471698	32.046067	0.096524	4.459681	0.76823	0.258187	82.58873	0.248761235	5.222707	4.569107	1.14305
Core_3_PMA	210	249.487179	27.768285	0.13223	4.149855	0.776093	0.298683	61.26225	0.291725	5.724337	4.178047	1.3701

Viable permafrost assemblages cannot be differentiated from total assemblages

The viable permafrost microbial community shifted away from the disturbance gradient soils communities, though the shift was not significant in assemblage ($p = 0.63$), or membership ($p = 0.30$) (Figure 11). However, this shift did allow for successful differentiation between the viable permafrost and viable disturbance gradient soils assemblages ($p = 0.008333$), which was not possible between the total permafrost and disturbance gradient soil assemblages ($p = 0.6083$). Environmental covariates with ordination space could not be determined with low sample number and redundant groupings. Jaccard dissimilarity between total and viable assemblages was smallest in F (0.52 dissimilarity). Viable and total assemblage distance was higher in surface sample A (0.60), but was more variable in permafrost Core_1, Core_2, and Core_3 samples (at 0.59, 0.58, and 0.65 respectively). Bray Curtis dissimilarity between total and viable assemblages was highest in Core_2 (0.44), and greatest in Core_1 (0.32), while A (0.39), F (0.34) and Core_3 (0.34) had intermediate dissimilarity.

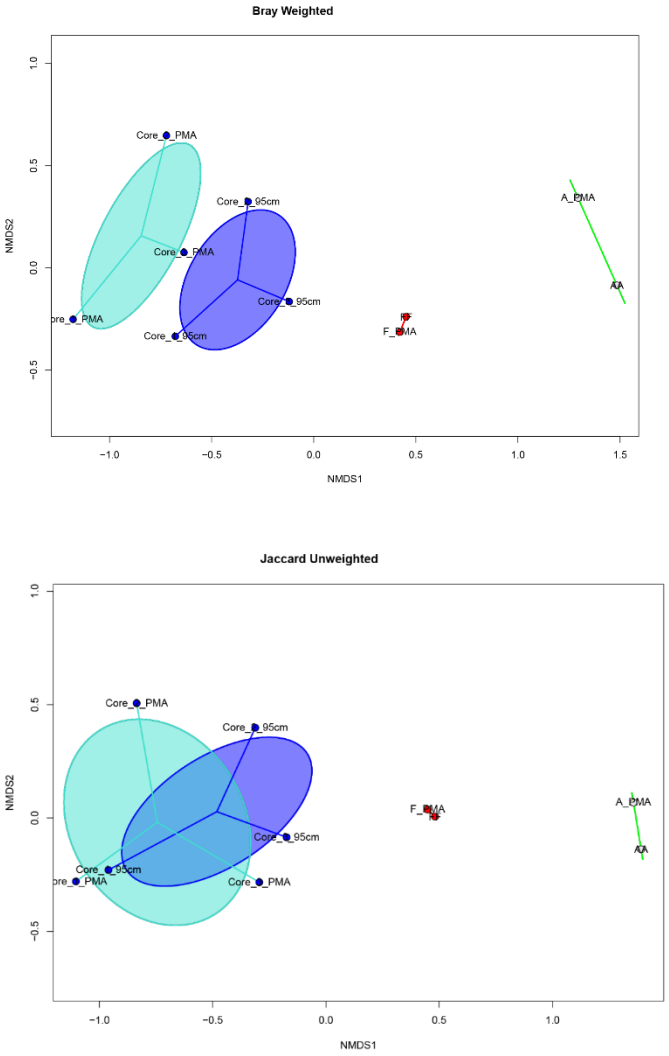

Figure 11. Similarity of viable and total microbial assemblages as visualized by nonmetric multidimensional scaling (NMDS). Ellipses encompass the standard error of sample groups A (green), F (red), viable permafrost (teal) and total permafrost assemblages (blue). Sample points correspond to sample source: A (green), F (red), and permafrost (blue). Ordinations used either (a) Bray Curtis dissimilarity matrices including OTU presence and abundance or (b) a Jaccard dissimilarity matrix assessing the presence/absence of bacterial OTUs.

Relic DNA preservation is not taxonomically or phylogenetically preferential

Ward's hierarchical clustering of weighted UniFrac community distances showed that the closest neighbour to each total assemblage was the viable assemblage (Figure 12). The only exception to this being Core_2_PMA, of which the viable assemblage represents an outgroup to Core_2, Core_3, and Core_3_PMA. Within cluster UniFrac distances was found to be greater in surface samples than in permafrost samples at an average of 0.86 compared to 0.71, respectively. No significant differences at the phylum, class, or genus level were found across viable or total-assemblages in permafrost, disturbance gradient soils, or when all soils were combined (Figure 12). A detailed analysis of taxonomic differences between clusters can be found in Chapter 2. The most abundant classes were Actinobacteria (22.3%), Alphaproteobacteria (9.7%), Deltaproteobacteria (5.8%), Betaproteobacteria (5.3%), Bacilli (4.0%), and Clostridia (3.4%). Similar groups dominated both viable and total permafrost communities at the class level though their ranking changed. Dominant groups in the viable permafrost assemblage were Actinobacteria (27.3%), Bacilli (8.2%), Gammaproteobacteria (5.8%), Clostridia (5.5%), Deltaproteobacteria (5.1%) and Alphaproteobacteria (5%). Dominant groups in the total permafrost assemblage were Actinobacteria (17.4%), Deltaproteobacteria (8.2%), Alphaproteobacteria (7.5%), Bacilli (4.9%), and Clostridia (4.5%). Only Gammaproteobacteria was significantly enriched in the viable assemblage ($p = 0.0076$), while no classes were enriched in the total assemblage ($p > 0.05$).

At the OTU level, OTU71 and OTU92 were both enriched in viable communities, suggesting they may be under-represented without removal of extracellular DNA (Figure 13). No OTUs were significantly enriched in the total community. These two taxa were identified as *Halomonas desiderata* (OTU71; 1.00 confidence value) and *Shewanella haliotis* (OTU92; 0.92 confidence value). OTU 71 represented 2% of total reads in the PMA-treated samples. Nitrate reduction capacity may be underestimated in these soils, due to the prominence of

denitrifying OTU71, although extension of taxonomic assignment of a single OTU to biogeochemical sampling must be interpreted with caution. *Shewanella haliotis* represented 1% of total reads in the PMA treated samples and is a Gram negative, facultative anaerobic bacterium, previously isolated from gut microflora. Differential abundance analysis was performed by combining permafrost and disturbance gradient soils; therefore, these two taxa are underrepresented across multiple sample types.

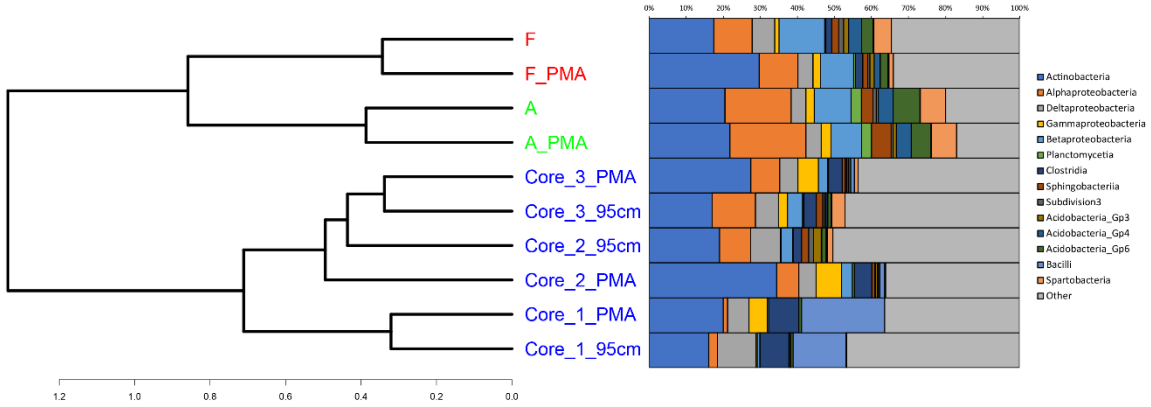

Figure 12. Dissimilarity of taxonomic profiles and relative abundances of viable and total assemblages from permafrost and disturbance gradient soils. Class level relative abundances were shown if taxa comprise >1% of the total number of reads with at least one sample having >1% of its total sample read number. Hierarchical clustering was performed using weighted UniFrac distances.

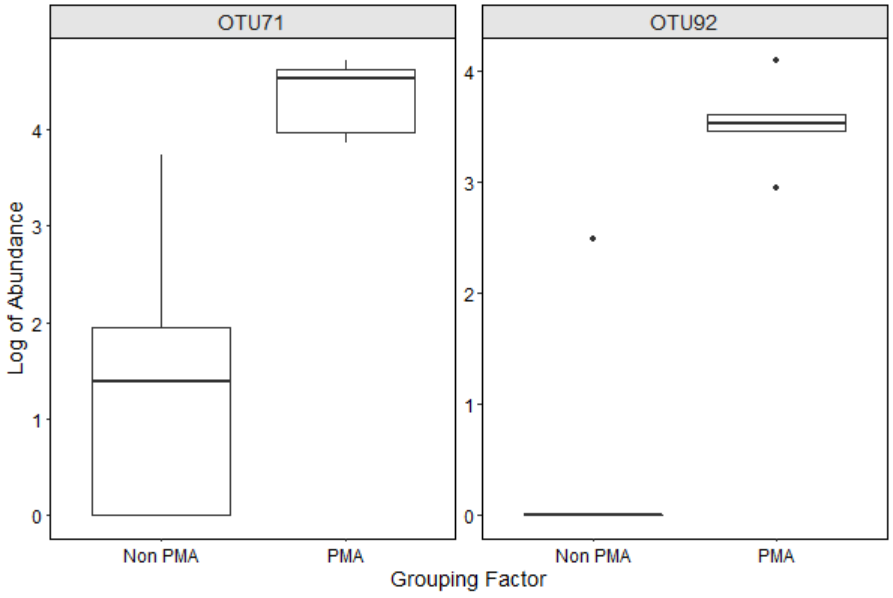

Figure 13. Differential abundance of significantly different log transformed OTUs between Non-PMA and PMA treated samples as assessed using ANCOM ($p<0.05$).

Discussion

Our objective was to delineate the composition of viable and total assemblages in permafrost. Viable and total microbial assemblages of permafrost could not be differentiated at the OTU level through either taxonomy or phylogenetic means with PMA treatment. These results indicate that differences within active layer, disturbed permafrost, and permafrost microbial communities are better described by soil type rather than through assessment of the viable community. More substantial changes may be present in permafrost soils which are more amenable to the preservation of relic DNA, or in older permafrost. These permafrost soils would require higher amounts of silt, lower exchangeable metal concentrations (particularly K^+), lower electrical conductivity, lower pH and lower cation exchange capacity. While previous studies have not integrated these edaphic parameters into β-diversity analysis of viable/total assemblages (Carini et al., 2017); these parameters may have a greater impact within perennially frozen soils. Due to the low number of samples, edaphic parameters could not be reliably used as covariates in compositional analysis, further replication may elucidate whether these parameters have a more pronounced impact in cryosphere materials. However, the viable communities of disturbed soils and permafrost were more dissimilar than would be determined by analyzing their total assemblages. Therefore, relic DNA appears to promote convergence between disturbance gradient soils and recently formed permafrost soil communities. Differentiation of viable microorganism and total assemblages may elucidate important differences between sample types which would otherwise be construed by the preservation of relic DNA. Assessing only the total assemblage of permafrost may underestimate the degree of change with permafrost thaw, though this impact is minimal. Composition has been previously shown to account for differences in soil function, including respiration rates and denitrification rates (Cavigelli & Robertson, 2001; Deng et al., 2015; Monteux et al., 2018). However, *in situ* permafrost thaw experiments have shown varying

degrees of compositional changes ranging from no shift (Lamb et al., 2011), inconsistent shifts (Deng et al., 2015), to microbial community shifts over decadal time periods (Monteux et al., 2018). Viable community analysis may increase the accuracy of functional community prediction by removing OTUs which are not capable of activity. Distinguishing between the viable and total assemblages of permafrost is unlikely to reveal community shifts which supersede those associated with soil type. However, subtle shifts in community assemblages in response to permafrost thaw may be clarified, possibly leading to more accurate prediction of community composition and function with permafrost thaw.

Identifying taxa enriched in total or viable assemblages allows for a preliminary understanding of over- and underestimation of community function. I determined no phylogenetic differences associated with either the viable or non-viable assemblages. The assessment of paleoecological fungal communities has relied upon the selection of taxa from total assemblages which were hypothesized to be viable/non-viable due to their physiological attributes. However, preferential preservation is seldom seen in community analysis of PMA-treated samples, as previous studies have only identified α-Proteobacteria enrichment in bacterial viable bacterial communities, and Agaricomycetes enrichment in fungal non-viable communities (Carini et al., 2017; Nocker et al., 2007; Nocker et al., 2010). Supporting these limited changes in abundances of taxa, I determined only two OTUs were enriched in the viable assemblage with no enrichment in the total assemblage. Limited preservation may be observed at the OTU level, though ecological interpretations from physiology of two OTUs are extremely limited must be made with caution. I suggest that selection of data may not accurately represent past microbial communities as preferential death, degradation, and preservation of relic DNA does not appear to occur in periglacial soils.

Diversity estimation is essential to predict and understand community shifts in response to anthropogenic disturbance. Biodiversity is known to be an indicator of ecosystem

health as the insurance hypothesis states that greater biological diversity results in a system which is more resilient and resistant to disturbance (Yachi & Loreau, 1999). Microbial richness was overestimated in permafrost microbial communities due to the preservation of relic DNA in soil environments. These results support previous findings by Carini et al., (2017), which identified significant differences in richness between viable and total assemblages across a majority of soil types. More recent findings have not replicated these results; though I suggest that cryopreservation of these soils make them exceedingly appropriate for the preservation of relic DNA and formation of spurious OTUs during high-throughput sequencing (Lennon et al., 2018). In comparison to active layer soils, permafrost exhibits lower microbial diversity, evenness, richness, and functional potential (Hultman et al., 2015; Deng et al., 2015). Viable microbial richness and phylogenetic diversity was significantly lower than observed in total assemblage, suggesting that these systems may be more sensitive to disturbance than would otherwise be predicted without assessment of the viable community. Viable microbial communities are therefore likely to have lower overall genetic potential and ecosystem functioning than would be predicted from analysis of the total assemblage. The presence of microbial relic DNA may obscure subtle biodiversity differences between disturbed soils and permafrost soils. Treatment of disturbance gradient soils with PMA may also allow for detection of rare taxa. These interpretations are dependent upon adequate sampling depth. Comparison of poorly sampled communities may instead reveal additional rare taxa, as observed in Dominion Creek disturbance gradient soils. This has previously been seen in PMA treatment of lung sputum, wherein rare taxa were uncovered with additional sampling and sequencing of PMA treated samples. However, the degree of unique OTUs found in the total assemblages of permafrost suggest that these well sampled communities have identified OTUs likely to be products of extracellular DNA preservation, rather than insufficient sampling of the microbial community. Spurious OTUs

introduced through the preservation of dead DNA may therefore be inflating actual richness and skewing evenness measures within permafrost samples. Therefore, viable permafrost microbial communities are more susceptible to disturbance and perturbation due to lower than expected diversity levels. Accurate assessment of permafrost microbial diversity may require delineation of viable and non-viable microorganisms.

This study has several methodological constraints. Current understanding of extracellular DNA has shown the presence of exchangeable ions is integral to assessing the preservative capacity of soils (Willerslev et al., 2004; Carini et al., 2017). Therefore, future work should include the assessment of exchangeable cation concentrations in soils, including exchangeable Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, electrical conductivity, pH, total exchangeable bases, cation exchange capacity, mean weight diameter, potassium, exchangeable NO_3^{-}, and water content in all permafrost soils used for the purposes of microbial community characterization. High throughput sequencing should be preceded by qPCR data to determine the amount of extracellular amplifiable DNA preserved in permafrost, in addition to direct counts of dead of dead microbial cells. Extensive experimental replication is required to accurately assess the effect of extracellular DNA preservation within and between samples, although assessment of viable microbial communities may aid in differentiating communities with a low level of sampling.

Studies of paleoecology and contemporary microbial diversity in permafrost are often blurred, due to the inability of high-throughput sequencing technologies to differentiate between DNA originating from live and dead organisms (Bellemain et al., 2013; Mackelprang et al., 2017). Experimental permafrost warming experiments have shown limited changes to microbial community composition with thaw. This may be due in part to the preservation of relic DNA contributing to the convergence of active layer and permafrost microbial communities, rather than due to rapid degradation of relic DNA in thawing

permafrost. The viable microbial assemblage of permafrost may be more distant from active layer soils than previously realized due in part to relic DNA promoting the convergence of permafrost and active layer communities. However, Dominion Creek permafrost soils appear to preserve sufficient amounts of relic DNA to alter diversity analysis, but this does not drastically alter microbial community composition. The preservation of relic DNA is not phylogenetically or taxonomically preferential in our cryosols and any subtle diversity or compositional differences are likely due to altered abundance across a wide range of taxa. The viable microbiome of cryosols harbours lower microbial diversity than the total assemblage, suggesting future perturbations to the permafrost microbiome are likely to induce community shifts greater than would be predicting from the total assemblage.

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