Investigating Links Between Soil Microbial Structure and Function in Three Major Plant Communities Across Temporal Scales of Arctic Alaska

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INVESTIGATING LINKS BETWEEN SOIL MICROBIAL STRUCTURE AND FUNCTION IN THREE MAJOR PLANT COMMUNITIES ACROSS TEMPORAL SCALES OF ARCTIC ALASKA

BY
KAJ LYNÖE

A thesis submitted in partial fulfillment of the requirements for the Master of Science Major in Biological Sciences Specialization in Natural Resource Management South Dakota State University 2020
This thesis is approved as a creditable and independent investigation by a candidate for the master’s degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

INVESTIGATING LINKS BETWEEN SOIL MICROBIAL STRUCTURE AND FUNCTION IN THREE MAJOR PLANT COMMUNITIES ACROSS TEMPORAL SCALES OF ARCTIC ALASKA

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2020

Arctic microbial systems continue to get attention today as our understanding regarding their structure and function in a changing system is paramount to C feedbacks with warming and changes in precipitation. Plant communities and microbial community processes across the Arctic landscape are central to understanding tundra ecosystem processes because environmental conditions and plant community structure drive microbial cycling of soil organic matter. Here, we want to understand how soil microbial respiration, mineralization, biomass, and community composition are linked to three Alaskan tundra plant communities, namely Shrub, Tussock, and Sedge tundra and the seasonal variability in this system. A total of 64 points were visited between 2018 and 2019 within a spatial extent of ca. 44,800 km². Soils were collected in March, June, July and September, homogenized, and incubated at realistic field temperatures to quantify soil microbial respiration (SMR) and potential N mineralization. Microbial C and N biomass were assessed through fumigation/extraction. PLFA extraction was used to assess microbial community structure (nmol/g) from Gram+ and Gram- bacteria, Actinomycetes and Fungi, among others. We found significant variation in both soil
microbial structure and function across time and among plant communities. In March
SMR rates were low but distinct in all plant communities, and N mineralization rates
were the highest. In July SMR peaked for all plant communities, and for all but Tussock
soil N immobilization rates peaked. Although soil microbial activity was high, overall
microbial biomass as MBC, MBN and PLFA was at the lowest point in July. These
results show the strong influence of seasonality where microbes are mineralizing
inorganic N during winter and immobilizing inorganic N during the growing season.
However, differences in soil microbial community structure among the three plant
communities only accounted for about 10% of total variation which suggests that plant
community drives a change in microbial function, but not a change in community-level
microbial structure. Rather, similar microbial communities display different functions in
terms of C and N cycling.
CHAPTER 1: INTRODUCTION

Soil microbes are fundamental to terrestrial biogeochemical processes such as carbon (C) and nitrogen (N) cycling. Understanding soil microbial community structure and how it correlates with nutrient fluxes, plant community composition and landscape structure are critical to understanding terrestrial ecosystem functioning. Microbial activity is strongly driven by environmental conditions such as changes in soil temperature and moisture as they influence microbial access to soil organic substrates (Brockett et al., 2012; Fierer et al., 2006; Frindte et al., 2019; Mikan et al., 2002; Schimel, 2018). Soil pH is a major driver of soil microbial community structure both on continental (Fierer and Jackson, 2006) and regional scales (Andersonb, 2003; Männistö et al., 2007; Ren et al., 2018; Zhang et al., 2016). Plant litter structures soil organic substrates and therefore strongly regulates soil microbial structure (Eskelinen et al., 2009; Kumar et al., 2016; Zak and Kling, 2006). Landscape structure influences the transportation of soil organic matter and biochemical transformation of organic compounds by soil microbes (Ping et al., 2005; Schmidt and Bölter, 2002; Zak and Kling, 2006). In Arctic surface soils, soil microbes are strongly impacted by the annual environmental and biogeochemical variability and consequently the soil microbial annual variability in community composition and cycling of nutrients such as C and N (Buckeridge et al., 2013; Zak and Kling, 2006).

Arctic ecosystems are strongly influenced by pronounced seasonal variability in weather patterns such as temperature and precipitation with great effects on both abiotic and biotic environments and processes. Most Arctic soils are underlain by permafrost (continuously frozen), where only a shallow fraction (i.e., the active layer) thaws during
the growing season. During this short growing season, there is a burst of activity both by plants and soil microbes. The most pronounced soil biogeochemical transition is from winter to spring with thawing snow and ice coupled with a shallow active layer which causes lateral subsurface flow and transport of leachate that redistributes vegetation matter, nutrients and minerals that in turn influence soil microbial structure and function (Buckeridge et al., 2016; Nikrad et al., 2016). The short growing seasons and cold soils slow microbial activity including decomposition and nutrient cycling resulting in the characteristic nutrient limitations of these ecosystems (Hobbie et al., 2002; Mack et al., 2004). The long winters are a key driver of nutrient limitation and slow nutrient turnover in the Arctic (Brooks et al., 1997).

Despite frozen soils during winter, microbial activity continues albeit at lower rates and microbial activity during winters contributes substantially to annual net mineralization of C and N in the Arctic (Brooks et al., 1998; Schimel et al., 2004; Welker et al., 2000). In Arctic soils, a threshold temperature for microbial activity around -10 and -6 °C has been reported previously with microbial activity decreasing dramatically below these temperatures (Brooks et al., 1997; Clein and Schimel, 1995; Edwards et al., 2006; Mikan et al., 2002; Sturm et al., 2005; Taras et al., 2002). Microbial activity, however, may continue at temperatures as low as -39 °C (Brooks et al., 1997; McMahon et al., 2009; Michaelson and Ping, 2003; Mikan et al., 2002; Nemergut et al., 2005; Nikrad et al., 2016; Panikov et al., 2006). Low microbial activity in frozen soils is partly due to a decrease in soil substrate availability from freezing water in soil pores, which limits nutrient access for soil microbes (Nikrad et al., 2016; Schimel, 2018). However, substantial microbial activity will be sustained throughout the winter if the snow/ground
interface temperatures remain at or above the threshold temperatures (Brooks et al., 1998; Sturm et al., 2005; Taras et al., 2002; Welker et al., 2000).

The snowpack depth and distribution have great importance for winter soil microbial activity and C and N cycling (Brooks et al., 1998; Gavazov et al., 2017; Larsen et al., 2007; Semenchuk et al., 2015; Sturm et al., 2005; Taras et al., 2002). The insulating properties of the snowpack enable soil temperatures to remain warm enough to allow microbial activity including nutrient cycling (Schimel et al., 2004; Sturm et al., 2005; Welker et al., 2000). The lack of competition for nutrients from plants and litter input from dead plants allows soil microbial biomass to reach peak levels during the late winter months. Accumulation of mineralized C and N in soils is also greatest in late winter (Edwards et al., 2006; Nemergut et al., 2005; Schadt et al., 2003).

The transition between winter and summer is coupled to great changes in microbial activity as the physical state of the soil is changing (Brooks et al., 1998; Jefferies et al., 2010; Schimel and Clein, 1996; Schimel and Mikan, 2005). Pulses of microbial respiration have been coupled to freezing and thawing of soils during spring as microbes rapidly metabolize labile compounds released from lysed cells (Schimel and Clein, 1996). Microbial respiration gradually decreases during this transition period as nutrients from lysed microbial cells are incorporated in plant and microbial biomass (Schimel and Clein, 1996). Winter snow accumulation has a large seasonal effect on soil microbial function and structure both through the insulation provided during winter and the meltwater added to the soil in the spring (Buckeridge et al., 2013; Buckeridge and Grogan, 2010).
Like soil microbial function, the seasonal dynamics of Arctic soil microbial community structure and biomass follow patterns driven by environmental conditions and soil organic C and N dynamics (Buckeridge et al., 2013). During the transition to summer, Arctic soil undergoes substantial turnover of microbial biomass and changes in microbial community structure as soils warm, are further wetted due to thawing soil and snowmelt, the depth of the active layer increases and soils experience initial freeze-thaw events (Brooks et al., 1998; Buckeridge et al., 2013; Jefferies et al., 2010; Larsen et al., 2007; Meisner et al., 2017; Schimel and Clein, 1996). During thaw, microbial cells are lysed due to the sudden change in available liquid water and osmotic pressure, leading to flushes of nutrients that are further metabolized by microbes. Microbial growth during summer is limited by competition with plant roots for nutrients, mainly N (Maslov and Makarov, 2016; Nordin et al., 2004). Even if microbes are more capable than plants in soil N acquisition, microbes retain nutrients for less time (i.e. high turnover rates) than plants leading to net plant sequestration of soil N (Nordin et al., 2004; Schmidt et al., 2002). During plant senescence in fall, soil microbial biomass increases as nutrient uptake by plants slows and plant litter adds labile substrates to the soil which are rapidly metabolized. Increased substrate availability enables microbial uptake of labile C and N compounds (Chu and Grogan, 2010; Hobbie et al., 2002; Maslov and Makarov, 2016). Microbial biomass then increases, albeit slowly, throughout the winter. These seasonal changes in nutrient availability and substrate quality subsequently drive changes in soil microbial communities (Buckeridge et al., 2013), and soil microbial function including C and N mineralization rates (Chu and Grogan, 2010; Sistla et al., 2012).
The abundance or accessibility of soil C and N governs microbial metabolism, growth, and respiration (Nordin et al., 2004; Shaver and Chapin, 1991; Weintraub and Schimel, 2005). In Arctic soils, C and N fractionation occurs because of slow decomposition rates and affects the rate and fate of the organic C and N compounds cycled by soil microbes. Easily accessible C compounds are metabolized quickly, leaving Arctic soils with large pools of recalcitrant C and thus high C:N ratios (Weintraub and Schimel, 2003). With limited N, microbes use the available C pool to maintain metabolic activity. This comes at a cost; enzymes needed to metabolize C are rich in N and can therefore dampen microbial cell growth because cell growth requires N. The excess C is then respired instead of incorporated in new biomass (Schimel, 2003; Weintraub and Schimel, 2003). With excess N, lower respiration rates by microbes indicate C is being incorporated into biomass (Schimel, 2003). The plant litter C and N inputs are dependent on the plant community composition and thus drives variation in soil microbial community structure, metabolism, and nutrient cycling rates (Zak and Kling, 2006).

Plant litter inputs drive soil nutrient dynamics and therefore soil microbial community structure and function. Soils with high C:N ratios are mostly related to slower-growing woody plants such as shrubs, lower pH, recalcitrant organic matter, and fungal dominance (Buckeridge et al., 2013; Wallenstein et al., 2007; Zak and Kling, 2006). Low C:N ratios are related to fast-growing plants such as graminoids and forbs, higher pH, labile organic matter, and bacterial dominance. Shrub tundra, Tussock tundra and Wet Sedge tundra have different organic substrate availability, C:N ratios, nutrient uptake rates by plants, as well as soil microbial mineralization potentials (Chu and Grogan, 2010; Eskelinen et al., 2009; McMahon and Schimel, 2017; Weintraub and Schimel,
2003, 2005). Relationships between plant community type, litter input quality and nutrient uptake from plant roots are an established concept, where different growth forms have varying turnover rates both in above and belowground biomass (Shaver and Chapin, 1991). Graminoid litter is associated with more labile C. Shrub litter contains a small pool of labile C in the leaf litter and a large pool of recalcitrant C in woody stems and roots (Chu and Grogan, 2010; Hobbie et al., 2002; Schmidt et al., 2002). During the growing season, plants release labile organic acids and polysaccharides from roots that stimulate soil microbial metabolism of C and more C is lost through soil microbial respiration; simultaneously, N starvation will drive a shift from microbial N mineralization to N immobilization (Chu and Grogan, 2010; Kumar et al., 2016; Weintraub and Schimel, 2005, 2003). This dynamic between plant roots and microbial metabolism is found in Arctic tundra plant communities (Eskelinen et al., 2009; Kotas et al., 2017; Kumar et al., 2016; Zak and Kling, 2006), and nutrient uptake from both plants and microbes varies with varying C and N deposition in the different plant communities (Eskelinen et al., 2009; Shaver and Chapin, 1991). Given the variable composition of soil organic matter (i.e. C:N ratios, recalcitrant/labile) in different plant communities, microbial activity, respiration, and mineralization rates also vary (Eskelinen et al., 2009; Schimel and Schaeffer, 2012; Weintraub and Schimel, 2003).

It is critical to understand the role plant communities play in soil microbial communities and processes as Arctic vegetation structure changes rapidly (van der Kolk et al., 2016). Arctic plants influence nutrient cycling through the uptake of nutrients and release of root exudates and deposition of litter (Dakora and Phillips, 2002; Schmidt et al., 2002). Similarly, Arctic soil microbial function and structure change soil biogeochemistry and
cycling of nutrients such as C and N (Buckeridge et al., 2013; Chu and Grogan, 2010; Edwards and Jefferies, 2013; Larsen et al., 2007; Nordin et al., 2004; Sistla et al., 2012; Wallenstein et al., 2007; Weintraub and Schimel, 2003). Because soil microbial structure and function will be influenced by the variation in plant community structure, plant communities are both regulating and regulated by soil microbial structure and function (Chu and Grogan, 2010; Schimel and Schaeffer, 2012; Shaver and Chapin, 1991; Zak and Kling, 2006). Numerous studies have also demonstrated the strong influence of seasonality where great fluctuations in environmental variables will influence soil nutrient dynamics and soil microbial structure and function in Arctic systems (Buckeridge et al., 2013; Edwards and Jefferies, 2013; Jefferies et al., 2010; Larsen et al., 2007; Lipson and Schmidt, 2004; Schadt et al., 2003; Weintraub and Schimel, 2005). Seasonal (Buckeridge et al., 2013; Edwards and Jefferies, 2013; Schadt et al., 2003; Sistla et al., 2012) and plant community variability (Chu et al., 2011; Chu and Grogan, 2010; Eskelinen et al., 2009; Wallenstein et al., 2007; Zak and Kling, 2006) have been addressed as well as the spatial variability among plant communities and how these variables affect microbial function and structure in a biogeochemical context.

The spatial variability in microbial community structure and function within plant communities has not been thoroughly addressed. In addition to broad spatial variability, extensive sampling is often limited by access in the Arctic. This study incorporates broad spatial sample distribution with multiple samples through time to link seasonal and plant community variability with soil microbial community function and structure in northern Alaska. First, this study aims to quantify the effects of seasonal variation and plant community composition on soil microbial function and structure. Second, this study
quantifies how spatial and environmental variability among existing plant communities drives variability in soil microbial function and structure. Based on previous findings we hypothesize that a strong seasonal and plant community influence will be observed on soil microbial function and structure. More specifically we asked: 1) How is microbial function and structure affected by seasonality? 2) How does microbial function and structure vary among plant communities? We studied response variables including microbial biomass, community composition, nitrogen cycling and respiration in different seasons and among different plant communities. We used samples from Tussock, Sedge, and Shrub communities in multiple locations over a large spatial extent and sampled during four distinct times to account for the variation in plant phenology, abiotic processes, and soil biogeochemistry.
Fig. 1. Map over the study area. The Brooks Range is in the south and the Arctic Ocean is in the north. The red demarcation is the Toolik field station research area. The line on the map is the Dalton Highway. Sample points for the 2018 and 2019 field seasons are presented by plant community type (n = 64).
CHAPTER 2: METHODS

Field site and Climate

Arctic Alaska is delineated by the Brooks Range in the south and the Arctic Ocean to the north leading to a gradual decrease in elevation and net transport of water and material from south to north. Arctic Alaska contains a wide range of topographic features such as rolling hills in the south, braided rivers, water tracks, bluffs, and steep banks, thermokarst topography, pingos and tundra polygons in the coastal plains and lakes and ponds throughout the tundra landscape (Ping et al., 1998). The tundra soils are dominated by mineral soil with different depths of soil organic matter ranging between 0 - 20 cm (Shaver and Chapin, 1991). Old fluvial sediments are dominant towards the coastal plains. A clear pH gradient-based on established soil-forming state factors such as parent material, landform type, vegetation and climate are also present here (Ping et al., 1998, 2005). These soil and landscape features are largely responsible for broadscale differences among plant community species composition observed in Arctic Alaska (Chu and Grogan, 2010; Ping et al., 1998; Raynolds et al., 2005; Walker et al., 2003; Zak and Kling, 2006). The climate in the study area is typical of Arctic region: cold and dry, with mean annual temperatures of -7 °C and annual precipitation of 400 mm where about 45% falls as snow (https://arc-lter.ecosystems.mbl.edu/site-description). There were 258 and 251 days of snow present on the ground in the 2018 and 2019 seasons respectively and 107 and 114 snow-free days were in the 2018 and 2019 seasons, respectively.

The plant communities on the North Slope of Alaska follow a natural gradient from the foothills of the Brooks Range to the coast of the Arctic Ocean (Fig. 1); shrubs typically
dominate the upland portion in well-drained soils, in depressions and along water tracks throughout the landscape. Farther north, the landscape is dominated by tussock-forming sedges. A wet sedge tundra community is predominant near the coastal plain where soils mostly remain wet throughout the summer and the landscape is dominated by polygonal tundra. Shrub tundra is dominated by *Salix pulchra* and *Betula nana*. Shrub communities tend to have a thicker organic horizon and in dense stands the understory is dominated by moss and litter. Shrub tundra also has a forb and graminoid component. Tussock tundra is dominated by the tussock forming sedge *Eriophorum vaginatum* and between tussocks, dwarf shrubs such as *Vaccinium vitis-idea* and *Rhododendron tomentosum* are prevalent along with dense layers of Bryophytes that can form thick acidic peat layers. Tussock tundra is the predominant vegetation type in Arctic Alaska (Shaver and Chapin, 1991). Wet sedge tundra is characterized by a thick organic peat layer above mineral soil and standing water at the soil surface during the growing season and is dominated by *Carex spp.* (Shaver and Chapin, 1991).

**Soil sampling and environmental variable collections**

Samples were collected from an area over 44,800 km² on the North Slope of Alaska during 2018 and 2019 (Fig.1). For each year, sample points were selected from a pool of randomized points that had a 10-km buffer and were representative of the extent of each vegetation community so that the number of points corresponded to the cover of each plant community type. The 2013 NSSI landcover/vegetation classification map (NSSI, UAA-ACCS) was used for assessing points based on the vegetation communities represented in the study area. In addition, nine sample points accessible from the Dalton
Highway located from Toolik field station (TFS) at the foothills of the Brooks Range to Prudhoe Bay near the Arctic ocean were visited each year.

Soil cores were collected in Tussock tundra, Shrub tundra, and Wet Sedge tundra during three sampling bouts in early June, mid-July, and mid-late September. For each sampling bout, approximately 30 sample points were visited during a 7- to 10- day period. In 2019 a subset of sample points was visited for additional sample collection during two weeks in late winter (March/April). Soil cores were sampled in three replicate plots at each sample point during each bout. A 3.5cm diameter soil corer, mounted on a ½ -in drive electric drill, was used to retrieve the sample because most soils were partly frozen until July. Three soil cores at each sample plot were collected to 30- cm depth; vegetation debris, moss and peat were removed following collection. Samples were stored in plastic bags and kept cool in the field and then frozen in the laboratory until further processing. The coring pipe was cleaned, sterilized with ethanol, and wiped with cotton cloths between each sample to avoid cross-contamination. Air, surface, and soil temperature (at 10 - cm depth) were recorded at each plot at the time of soil collections. In winter, snow - soil interface temperatures were recorded with a mercury thermometer. Soil thaw depth was measured by driving a metal rod to the depth where the soil was currently frozen. In the tussock tundra communities thaw, depth was recorded between tussocks.

No formal characterization of soils was done in this study. Tussock soils were predominantly silty mineral soils with a thin top layer of organic matter, except for thick layers of peat. Shrub soils were dominated by a thick (5 – 20cm) layer of organic matter
on top of silty/sandy mineral soil. Wet Sedge soils were dominated by fluvial sediment and peat (Ping et al., 1998).

In addition to *in situ* field environmental parameters, the Winter Biological activity Index (WBI) was calculated as an indicator of soil microbial activity during winter months.

WBI was derived from a spatially explicit climate model predicting snow distribution and snow-ground interface temperatures. WBI is an additive index; it is the sum of the number of days when snow ground interface temperature is predicted to exceed -6 °C at a given location (i.e. a sample point). The WBI index is estimating soil microbial activity based on a -6 °C threshold temperature (Sturm et al., 2005; Taras et al., 2002). WBI data were extracted at each sample point coordinates. WBI was produced by S. Højlund Pedersen from outputs of air temperature and snow depth from MicroMet and SnowModel (Liston and Elder, 2006a, 2006b) and using the snow-ground interface model defined by Taras et al. (2002).

**Lab preparations**

Following thawing, soil samples were sieved through a 2-mm sieve to remove roots and gravel and then homogenized for further subsampling into the different quantitative measurements described below. Soil pH was measured with a pH meter (Accumet AB 150, Thermo Fisher Scientific, Waltham, Massachusetts) using ca. 3 g of oven-dried soil in a 1:5 soil: water suspension. Soil water content was measured by weighing fresh soil and then drying it at 65 °C for 24 hours, then weighing it again and calculating the water content from the difference in weights. All soil sample processing took place at TFS within ten days of sample collection.
Soil Microbial Biomass

Soil microbial biomass was estimated by a standard fumigation – extraction method (Vance et al., 1987). Briefly, two samples of ca. 10 g of fresh soil were placed in 250 ml plastic cups with lids. For one of the samples, organic C and N were extracted immediately by adding 50 ml K$_2$SO$_4$, shaken for 1 h, and then gravity filtered using Whatman 42 ashless filter papers (GE healthcare Life Sciences Solutions USA LLC, Pittsburgh, Pennsylvania, USA). The other sample was placed in a vacuum-sealed glass desiccator with ca. 30 ml ethanol-free chloroform that was set to a boil using a vacuum pump for 5 -7 minutes and then covered with a dark cloth bag to fumigate samples for 24 h in a fume hood. Fumigated samples were then aerated in the fume hood for approximately 30 minutes and extracted as described above. Extracted samples were kept frozen in 20 ml Nalgene bottles until further analysis. The extracts were later analyzed for total organic carbon (TOC) and total nitrogen (TN) with a Shimadzu total C and N analyzer (model TOC- L, Shimadzu Scientific Instruments Inc., Columbia, Maryland, USA). For calculating the microbial biomass C (MBC) and microbial biomass N (MBN) values from the unfumigated soils were subtracted from the fumigated soils, expecting a release of C and N from the fumigation through lysing of the soil microbial cells. The final values for soil MBC and MBN were calculated from the estimated extractable fractions of MBC ($k_c = 0.35$) (Joergensen, 1996) and MBN ($k_n = 0.54$) (Brookes et al., 1985). All values were expressed based on the dry weights of the soil samples.
Soil Microbial Respiration and Potential N Mineralization

For determining potential soil microbial N mineralization, samples of ca. 10 g of fresh soil were extracted with 50 ml KCl, shaken for 1 h and then gravity filtered using Whatman 42 ashless filter papers (GE healthcare Life Sciences Solutions USA LLC, Pittsburgh, Pennsylvania, USA) before the incubation. The same procedure was used with soil samples after the 10-day incubation described below. All samples were kept frozen in 12 ml plastic vials until analysis. NH$_4^+$ concentrations were assessed by Berthelot reaction and the protocol was adapted from Forster (1995). NO$_3^-$ concentrations were assessed by a quantitative reduction of NO$_3^-$ with VCl (Doane and Horwáth, 2003; Miranda et al., 2001). Both protocols use colorimetric reactions to assess NH$_4^+$ and NO$_3^-$ concentrations, respectively. Samples were analyzed by spectrophotometry (Model v - 1200, VWR International, LLC., Radnor, Pennsylvania, USA) and the difference in NH$_4^+$ and NO$_3^-$ concentrations between pre- and post-incubation are considered the mineralization rate. The September samples of 2019 were extracted with KCl contaminated with NO$_3^-$, and meaningful measurements of NO$_3^-$ could not be performed for that sample period.

For soil microbial respiration (SMR) and potential N mineralization rate measurements, fresh soils were weighed, put in mason jars and sealed with polyethylene film with small perforations and placed in a biological incubator (I-36NL, Geneva Scientific, Fontana, Wisconsin, USA). Samples were left for a 10-day incubation period. Mean incubation temperatures for 2018 was 9.3 °C for June, 16.5 °C for July and 0.6 °C for September and 2019 the mean temperatures were 1.9 °C, 5 °C and 2.5 °C, respectively. Incubation
temperatures followed mean surface temperatures measured on a latitudinal gradient spanning the extent of the sample area. Temperature data were extracted from the previous year and subsequently used for the incubations.

An effective and accurate method to measure soil respiration is using an infrared gas analyzer (IRGA) by circulating headspace air from a closed system and continuously measure CO$_2$ accumulation over time. Before the first respiration measurement, samples were left to settle in the incubation chamber for 24 h. Soil microbial respiration was taken using an automated soil gas flux system (model 8100, LI-COR, Lincoln, Nebraska, USA). Each measurement was two minutes and the system were flushed with ambient air to stabilize the CO$_2$ concentration near ambient between each measurement. Each measurement was conducted at incubation temperature. After the 10-day incubation, a second measurement was taken. In late March/ early April of 2019, a subset of soils was sampled for respiration at -10 °C. Respiration was measured over 10 minutes and then incubated in a freezer at a mean temperature of -17 °C for 75 days. These soils were then thawed, and N mineralization was measured as described above.

Soil Microbial Community Structure

Soil microbial community structure was assessed by Phospholipid Fatty Acid (PLFA) analysis. About 10 g of fresh soil was kept in coin envelopes and frozen at -80 °C until freeze-drying using a 4.5 l freeze dryer (Labconco Corp., Kansas City, Missouri, USA) and then stored at -80 °C until extraction using a modified protocol for high throughput PLFA analysis of soils (Buyer and Sasser, 2012). Briefly, 1.5 g of soil was added to 4 ml of Bligh – Dyer extractant (methanol, chloroform and phosphate buffer with pH 7.4) and
2µl of 19:0 phosphatidylcholine internal standard (Avanti Polar Lipids, Alabaster, Alabama, USA), vortexed and placed in a sonicating bath (model 2800, Branson Ultrasonics, Danbury, Connecticut, USA) for 10 minutes and vortexed for an additional 10 seconds. Vortexing and sonication were repeated twice. Extracts were then vortexed again and centrifuged and the upper phase containing the extracted lipids was collected and vacuum dried at room temperature (Savant SPD 2010, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The lipids were separated through solid-phase extraction (SPE) by using an SPE 96 well plate (Phenomenex Inc., Torrance, California). Wells were prewashed with methanol and chloroform and the extract was re-dissolved in 1 ml of chloroform and added to the well, then washed with chloroform and acetone. Lipids were eluted by using 0.5 ml of 5:5:1 methanol, chloroform, H₂O, and drained into 1.5 ml glass vials and redistributed to culture tubes followed by vacuum drying at room temperature. Then, 0.2 ml of transesterification reagent (toluene, KOH) was added to each sample, and samples were incubated in a water bath (type Isotemp GPD 05, Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 15 minutes at 37 °C. Then 0.4 ml of 0.075 M acetic acid and chloroform were added, tubes were vortexed, and the liquid phases were allowed to separate for ca. 15 minutes. The bottom layer was transferred to a 1.5 ml GC vials and vacuum dried at room temperature. Samples were frozen at -20 °C after drying. Before analysis samples were re-dissolved in 0.75 µl of hexane and then transferred into conical glass inserts fitted for the GC vials. The extracted samples were analyzed using a GC (Shimadzu GC 2010 Plus, Shimadzu Scientific Instruments Inc., Columbia, Maryland, USA). The determination of PLFA´s was based on the retention time for each fatty acid compound. Sherlock software (Midi Inc., Newark, Delaware,
USA) was used to identify the different PLFAs in each sample adjusting the output to the molarity of the different fatty acid compounds based on a known amount of the internal standard 19:0 phosphatidylcholine. This gives quick and robust results quantifying and categorizing the different soil microbial functional groups within each sample (Buyer and Sasser, 2012).

Statistical Analysis

All statistical analyses were performed in R (versions 3.5.0 and 3.6.3, R core team, 2020) and results were considered significant when $P < 0.05$. Pearson correlations were performed across all environmental and biogeochemical parameters to infer ties between structure and function at the different sample dates and plant communities (Table 3, 4, 5, 6). Mixed-effects multiple regression models were fitted using package nlme (Pinheiro et al., 2020) treating sample point and year as random effects. First, to test for variables influencing soil microbial function and structure full models with environmental, biogeochemical, sample date, and plant community type were tested and selected using a two-way stepwise selection method, using AIC scores to define the best fit model for each response variable (Table 1). Second, to test for the influence of sample date and plant community type, mixed effect models were run on all sample variables individually with sample date and plant community type as interactive predictors. These tests were used to evaluate the dependence of soil microbial structure and function on soil biogeochemistry and environmental parameters and influence between different plant communities and seasonality. Tukey HSD post hoc tests were used to separate means among the different levels of the predictors.
Nonmetric multidimensional scaling (NMDS) was used for examining differences in soil microbial communities derived from PLFA samples among plant community types and sample periods. The use of NMDS is useful to detect patterns of similarity between input data and no assumptions on linear relationships between data objects are necessary (Paliy and Shankar, 2016). Another advantage with NMDS is that it uses a low number of dimensions, usually just two, which makes interpretation of the ordination space easier. Here, NMDS was also used to infer variability in soil biogeochemistry and environmental variables as drivers of soil microbial function and structure. The relative abundance of PLFAs (mol%) at the different sample sites were run with Bray Curtis distances to create a dissimilarity matrix and transformed with the Wisconsin double standardization using the \textit{metaMDS} function in R package \textit{vegan} (Oksanen et.al., 2019). The \textit{metaMDS} function uses random starts to find a stable solution with the lowest stress; the stress parameter is a measure of the lack of fit between the ordination space and the calculated dissimilarities of the variables. A stable solution is reached when the algorithm succeeds in placing the objects to best fit the ordination space - i.e. global optima. Lower stress indicates a better fit, and all ordinations were considered useful when stress was <0.1 (Paliy and Shankar, 2016). Environmental and biogeochemical variables were also fitted to the ordination space as vectors after 999 permutations, where the projection and reach of the vectors show direction and strength with other corresponding variables in the ordination. This was done to test for correlations between the mol % PLFA as soil microbial community composition defined by NMDS and fitted variables using \textit{envfit} function in \textit{vegan} package. Vectors derived from the \textit{envfit} function were only fitted to the ordination plots if p< 0.05 for any of the fitted variables. Predictability (i.e. R$^2$) was
generally low even for variables with low p values. For all statistical tests, environmental and biogeochemical variables were scaled and centered before analysis. This method was chosen before square root transformations since data contained many zero values. Log transformation was also avoided since the data contained pH values that are already on a logarithmic scale. All PLFA data used in NMDS ordination was converted into relative abundance (mol%).

\[ y \sim \text{Com} + \text{Date} + \text{pH} + \text{Soil T} + \text{Water content} + \text{Thaw depth} + \text{WBI} \]

<table>
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<th>F value</th>
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</table>

Table 1. The reduced, optimal models used for each of the responses analyzed. The full model is expressed on top. Com is plant community type, soil T is soil temperature and WBI is winter biological index.
CHAPTER 3: RESULTS

Effects of seasonality and plant community type on soil environmental variables

Soil temperatures varied between sample dates, but also between plant communities within a given sample date. In March, Shrub soils had the highest mean (-5.50 °C, SD ±0.22)) and Sedge soils the lowest mean temperatures (-11.00 °C, ±0.53)) (Table 2). In March Sedge soils were colder than both Shrub (p< 0.001) and Tussock soils (p< 0.001) by 5.50 °C and 3.76 °C, respectively. In July Sedge soils were warmer than both Shrub (p< 0.001) and Tussock soils (p< 0.001) by 2.56 °C and 3.15 °C, respectively. WBI was higher in Shrub and Tussock soils compared to Sedge soils by about 53 days (p> 0.001) (Table 2).

Water content was strongly dependent on sample dates but did not vary among plant communities. Soil water content in the Tussock plant community decreased between March and June from 65% to 56% (p = 0.021). Water content decreased in Tussock soil, from 56% to 41% (p<0.001) and Sedge soils, from 58% to 44% (p<0.001) between June and July. The soil in the Shrub community continued to dry between July and September decreasing from 48% to 38 % in water content (p = 0.039) (Table 2).

Soil pH showed distinct patterns with increasing pH throughout the season in all plant communities. Overall, Sedge soils had the highest pH with a mean of pH 7.1, and Tussock soils the lowest pH with a mean of pH 5.2 (Table 2). For all sample dates Sedge soils had higher pH than Tussock soils (p < 0.001) (Table 2). In June (p = 0.004) and September (p = 0.001), Sedge soil pH was higher than Shrub soil pH by pH 0.9 and pH
1.3, respectively. Between March and June no change was seen in Sedge soil pH, whereas in July, Sedge soil pH increased, from pH 6.9 to 7.4 (p<0.001). Soil pH in Tussock tundra increased between March and June by pH 4.62 to 5.1 (p<0.001), between June and July by pH 5.1 to 5.4 (p = 0.018). Shrub soils did not show any increases in pH between sample periods; however, an overall increase was found between March and September, from pH 5.7 to 6.1 (p=0.033).

Thaw depth only varied in July and September among plant communities. In March, all measurements were considered zero since the ground was frozen. In June, thaw depth averaged ca. 12 cm with no differences among plant community (Table 2). As expected, thaw depth was greater in July, and also differed among communities (p= 0.004) with the thaw in Sedge soils being 9 cm and 13 cm deeper than Tussock, (p= 0.006) and Shrub (p= 0.001) soils, respectively. In September, all plant communities had their deepest soil thaw depth (Table 2), with Sedge soils being 12 cm and 13 cm deeper than both Tussock (p< 0.001) and Shrub soils (p< 0.001), respectively.
### Table 2: Summary of variables from each plant community and month

<table>
<thead>
<tr>
<th>Month</th>
<th>Plant community</th>
<th>MBC (mg/g)</th>
<th>MBN (ug/g)</th>
<th>SMR (ngCg/h)</th>
<th>10 day N (ug)</th>
<th>ugN/day</th>
<th>Total N (ug)</th>
<th>Soil °C</th>
<th>Thaw Depth (cm)</th>
<th>Water Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Sedge</td>
<td>0.20 (±0.52)</td>
<td>23.87 (±3.59)</td>
<td>25.01 (±5.6)</td>
<td>5.64 (±2.45)</td>
<td>0.08 (±0.03)</td>
<td>3.07 (±1.27)</td>
<td>-</td>
<td>0.00 (±0)</td>
<td>0.56 (±0.03)</td>
</tr>
<tr>
<td>6</td>
<td>Shrub</td>
<td>2.32 (±0.44)</td>
<td>67.38 (±8.78)</td>
<td>78.00 (±14.83)</td>
<td>-</td>
<td>0.35 (±0.33)</td>
<td>-</td>
<td>11.00 (±0.53)</td>
<td>12.47 (±1.52)</td>
<td>0.58 (±0.02)</td>
</tr>
<tr>
<td>7</td>
<td>Shrub</td>
<td>1.11 (±0.23)</td>
<td>24.18 (±2.07)</td>
<td>135.9 (±18.3)</td>
<td>-</td>
<td>0.35 (±0.33)</td>
<td>-</td>
<td>1.81 (±0.17)</td>
<td>38.01 (±1.3)</td>
<td>0.44 (±0.02)</td>
</tr>
<tr>
<td>9</td>
<td>Shrub</td>
<td>1.08 (±0.44)</td>
<td>31.14 (±5.19)</td>
<td>69.94 (±12.25)</td>
<td>-</td>
<td>0.35 (±0.33)</td>
<td>-</td>
<td>6.81 (±0.38)</td>
<td>58.61 (±1.91)</td>
<td>0.47 (±0.02)</td>
</tr>
<tr>
<td>3</td>
<td>Tussock</td>
<td>1.14 (±2.58)</td>
<td>48.50 (±17.15)</td>
<td>59.20 (±31.36)</td>
<td>7.75 (±4.36)</td>
<td>0.10 (±0.06)</td>
<td>8.24 (±3.55)</td>
<td>-</td>
<td>0.00 (±0)</td>
<td>0.70 (±0.06)</td>
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<tr>
<td>6</td>
<td>Tussock</td>
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<td>0.35 (±0.38)</td>
<td>0.04 (±0.07)</td>
<td>2.47 (±2.83)</td>
<td>-</td>
<td>13.47 (±1.52)</td>
<td>0.59 (±0.03)</td>
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<tr>
<td>7</td>
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<td>40.67 (±8.31)</td>
<td>157.88 (±36.17)</td>
<td>-</td>
<td>0.04 (±0.07)</td>
<td>-</td>
<td>4.25 (±0.39)</td>
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</tr>
<tr>
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<td>-</td>
<td>3.39 (±1.58)</td>
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<td>45.20 (±2.2)</td>
<td>0.38 (±0.01)</td>
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<tr>
<td>3</td>
<td>Sedge</td>
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<td>24.95 (±3.94)</td>
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<td>0.13 (±0.08)</td>
<td>7.24 (±2.76)</td>
<td>-</td>
<td>0.00 (±0)</td>
<td>0.34 (±0.16)</td>
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<tr>
<td>6</td>
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<td>2.41 (±0.31)</td>
<td>2.41 (±0.31)</td>
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<td>3.11 (±1.94)</td>
<td>-</td>
<td>9.26 (±0.46)</td>
<td>0.04 (±0.07)</td>
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<tr>
<td>7</td>
<td>Shrub</td>
<td>1.03 (±0.2)</td>
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<td>0.26 (±0.79)</td>
<td>0.05 (±0.04)</td>
<td>0.23 (±0.56)</td>
<td>-</td>
<td>3.66 (±0.19)</td>
<td>0.04 (±0.07)</td>
</tr>
<tr>
<td>9</td>
<td>Shrub</td>
<td>0.21 (±0.18)</td>
<td>31.77 (±3.06)</td>
<td>26.33 (±3.6)</td>
<td>0.49 (±0.35)</td>
<td>0.05 (±0.04)</td>
<td>1.40 (±0.28)</td>
<td>-</td>
<td>2.10 (±0.13)</td>
<td>0.05 (±0.04)</td>
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</table>
Soil Respiration

Soil microbial respiration (SMR) showed distinct variations between both sample period and plant community types (Fig. 2). SMR rates increased between June and July for Shrub (p<0.001), and Sedge (p<0.001) communities (Fig. 2 Table 1). In July, rates of SMR were higher in Shrub (p = 0.022) and Sedge (p = 0.023) soils than Tussock soils. Between July and September, respiration rates decreased for Sedge soils (p = 0.001) (Table 2). SMR was predicted by plant community type (p = 0.010), sample date (p < 0.001), pH (p = 0.010), water content (p < 0.001) and WBI (p<0.001). The correlation between pH and SMR was stronger with increased pH (seasonal effect partly due to an increase in both) (Table A3 - A6). The correlation between water content and SMR was stronger when the water content was higher (Table 2, Table A3 - A6). SMR was also correlated with WBI, with a higher correlation in March and September (Table A3 -A6).

Fig. 2. Mean respiration rates for each sample date and plant community with standard error bars.
Soil microbial N mineralization rates

The strongest drivers of soil N mineralization were sample date (p = 0.012) and plant community type (p = 0.022) (Fig. 3). Overall soil NH$_4^+$ mineralization rates were predicted by plant community type (p = 0.024), sample date (p< 0.001), and the interaction of plant community type and sample date (p = 0.040) (Fig. 3). Between June and July, there was a transition from mineralization to immobilization of soil NH$_4^+$ in Shrub soils (p = 0.033). In the same period Tussock soil NH$_4^+$ mineralization rates decreased (p = 0.005) (Fig. 4). Sample date was the strongest driver for overall soil NO$_3^-$ mineralization rates (p = 0.026) (Fig. 5). Environmental variables that predicted soil N mineralization rates were soil temperature (p = 0.046) and water content (p = 0.039).

![Fig. 3. Mean mineralization rates for each sample date and plant community with standard error bars. The positive values represent net mineralization, and the negative values represent net immobilization.](image-url)
Fig. 4. Mean $\text{NH}_4^+$ rates. The positive values represent net mineralization, and the negative values represent net immobilization.

Fig. 5. Mean $\text{NO}_3^-$ rates. The positive values represent net mineralization, and the negative values represent net immobilization.
Effects of seasonality and plant community type on soil microbial community structure

Microbial biomass expressed as MBC and MBN both differed between sample dates, but not among plant communities (Fig. 6,7). Tussock soil MBC decreased between June and July (p = 0.049). Between June and July MBN decreased in Sedge (p < 0.001), Shrub (p < 0.001) and Tussock (p = 0.002) communities. Overall MBC was predicted by water content (p<0.001) and thaw depth (p = 0.018). The correlation between MBC and water content was high during all sample months (R_{avg} = 0.485). Predictors for overall MBN were pH (p = 0.015), water content (p < 0.001) and sample date (p< 0.001).

![Fig. 6. Soil microbial biomass C for each sample date and plant community with standard error bars.](image)
The mixed-effects regression of the total microbial biomass derived from PLFAs (nmol) showed influence from both plant community type (p = 0.001) and sample date (p < 0.001). Between June and July, the only significant decrease in PLFAs was found in Tussock soils (p< 0.001). There were no differences in total PLFA biomass (nmol) between plant communities during all sample dates. The environmental drivers that predicted overall variation in the total microbial biomass derived from total PLFA (nmol) were pH (p<0.001), water content (p<0.001), and WBI (p = 0.043) (Table 1).

Fungi to bacteria ratios (F:B ratios) derived from PLFAs (nmol) showed little variation among sample dates and plant communities (Fig. 8). The only differences between plant communities were observed in March when F:B ratios differed between Shrub and Tussock soils (p = 0.024) (Fig. 8). The only variation between the sample date was
between June and July where a decrease in F:B ratios in Tussock soils were observed (p<0.001) (Fig. 8).

The NMDS ordination analysis indicated little variation of the microbial community composition between sample date, plant community type, or biogeochemical variables. In March, predictors of variation of soil microbial communities as PLFA (%mol) were: MBN (R² = 0.34, p = 0.029) and plant community type (R²= 0.14, p = 0.036) (Fig. 9). In June both MBC (R²= 0.07, p = 0.011) and MBN (R²= 0.14, p = 0.006) were drivers of soil microbial community composition along with water content (R²= 0.09, p= 0.001). In June plant community type explained part of the variation in microbial communities (R²= 0.14, p = 0.001) (Fig. 10). In July, all explanatory variables had low R². Variables that predicted microbial community composition were; MBC (R²= 0.03, p = 0.037) and MBN (R²= 0.07, p = 0.007), water content (R²= 0.06, p= 0.004), WBI (R²= 0.08, p = 0.001) and
pH ($R^2 = 0.08$, $p = 0.002$) respectively. In July plant community type explained some of the variation of soil microbial community structure ($R^2 = 0.11$, $p = 0.010$) (Fig. 11). For September, predictors were SMR ($R^2 = 0.04$, $p = 0.023$), soil temperature ($R^2 = 0.06$, $p = 0.005$), WBI ($R^2 = 0.09$, $p = 0.001$) and pH ($R^2 = 0.08$, $p = 0.001$). In September plant community type explained a small portion of the variation in soil microbial structure as PLFA (%mol) ($R^2 = 0.08$, $p = 0.001$) (Fig. 12).

Fig. 9. NMDS ordination plot of March with plant communities and microbial communities fitted to the ordination space. For every ordination, biochemical and environmental variables are fitted in the ordination space. Each microbial functional group is fitted as well.
Fig. 10. NMDS ordination plot of June

Fig. 11. NMDS ordination plot of July
Protozoan relative abundance was affected by sample date (p < 0.001) and between plant communities and date (p = 0.001). In June Tussock soils had a significantly higher abundance of protozoa (p = 0.014). Between June and July, there was a significant decline in protozoa in Tussock soils (p < 0.001) (Fig. A1).

For sulfate reducers, plant community type (p < 0.001), sample period (p > 0.001), and the interactive effect of the two (p < 0.001) were all significant. Sedge soils had significantly higher abundances of sulfate reducers than Tussock soils in June (p < 0.001) and September (p < 0.001). For July, soil in the Sedge community had higher abundances of sulfate reducers than both Tussock (p < 0.001) and Shrub (p < 0.001). In Sedge soils, there was a significant increase in sulfate reducers between March and June (p = 0.02) and June and July (p < 0.001). In September there was a significant decline in sulfate reducers in Sedge soils (p < 0.001) (Fig. A1).

Fig. 12. NMDS ordination plot of September
Arbuscular mycorrhizal fungi (AMF) differed among sample dates (p = 0.001) and plant community types and the sample date interaction were significant (p< 0.001). In Shrub soils, there was a significant increase in AMF between March and June (p = 0.044). In Tussock soils there was a significant decrease of AMF between June and July (p = 0.001) (Fig. A1).

Fungi abundances were affected by sampling date (p< 0.001) and the interaction between plant community and sample date (p< 0.001). In March Tussock soils had significantly higher abundances of fungi than Shrub soils (p = 0.004). In Shrub soils, there was a significant increase in fungi between March and June (p = 0.011). Between June and July, Tussock soils had a significant decrease in fungi (p< 0.001) (Fig. A1).

For gram-positive bacteria, plant community type (p = 0.047) and sample date (p <0.001) were significant drivers of variation in abundance. Between June and July, there was a significant increase in gram-positive bacteria in Sedge (p< 0.001) and Tussock (p< 0.001) soils (Fig. A1).

The abundances of gram-negative bacteria differed among plant community types (p = 0.036) and the interaction between plant community type and sample date was significant (p = 0.001). In March Shrub soils had higher abundances of gram-negative bacteria than Tussock soils (p = 0.023). There was a significant decline in gram-negative bacteria in Shrub soils between March and June (p = 0.006) (Fig. A1).

Actinomycete abundances were affected by plant community type (p = 0.040), sample date (p = 0.001) and the interaction between the two (p = 0.002). Between March and
June, there was a significant decrease in actinomycete abundance in Shrub soils \( (p = 0.011) \) (Fig. A1).

Saprophyte relative abundance differed between sample date \( (p < 0.001) \) and interaction between sample date and plant community \( (p < 0.001) \). In March Tussock soils had significantly higher saprophyte abundances than Shrub soils \( (p = 0.006) \). Between March and June, there was a significant increase of saprophytes in Shrub soils \( (p = 0.004) \). Between June and July there were significant decreases in saprophyte abundances in both Shrub \( (p = 0.040) \) and Tussock \( (p < 0.001) \) soils (Fig. A1).

Other eukaryotes showed no differences between sample dates, plant community types or sample date, and plant community type interaction. Other eukaryotes were also the least prevalent functional group among all sample dates and plant community types (Fig. A1).
CHAPTER 4: DISCUSSION

Seasonal patterns among the different plant communities

This study investigated the seasonal differences in soil microbial communities among three dominant plant communities on the North Slope of Alaska. Similar to previous studies, season greatly affected microbial structure and function (Buckeridge et al., 2013; Edwards and Jefferies, 2013; Jefferies et al., 2010). However, vegetation type only accounted for about 10% of the observed variation in soil microbial community structure. Differences among plant community types in microbial function were more prominent, suggesting that influence from plant community-specific biogeochemistry affected microbial activities such as C and N mineralization rates. As the strong seasonal shifts greatly drive plant productivity in Arctic regions a synergetic effect between seasonality, plant community type and local biogeochemistry drive soil microbial structure and function in these ecosystems. This study showed the variation in the function that exists among similar microbial communities and how soil microbes respond to biogeochemical differences among the three different plant communities studied.

Effects of seasonality and plant community type on soil environmental variables

We found clear seasonal patterns regarding temperature, thaw depth, water content, and pH in the different plant communities. WBI is partly explained by the geographic and topographic distribution of vegetation communities and how snow accumulation follows a clear south to north gradient with greater snow accumulates in the foothills where Tussock and Shrub communities are predominant and less snow accumulates on the
coastal plain in the Sedge community. A well-established theory regarding vegetation effects on snow is that shrubs with their intermediate canopy create drifts of snow around them thereby increasing snow depth (Sturm et al., 2005; Welker et al., 2000). In this study, a clear distinction between the effects of snow depth and WBI between Shrub and Tussock communities could not be made. This variation in snow depth could partly be explained by variation in topography which greatly affects local snowdrift patterns on the landscape and thus impacts WBI, particularly in Shrub and Tussock soils. Another possible explanation is that due to the spatial resolution of the WBI predictions (300m), the model fails to capture the effect of shrub cover on the scale the soil sampling was performed.

Thaw depth and soil temperature followed the same patterns, and an explanation for this trend is the physical stature of the vegetation in these different communities. Sedge communities have an open, sparse cover that allows for heating of the surface, but shrubs shade the ground, and the soil remains cool. Tussock communities have a thick layer of bryophytes, often Sphagnum spp., between the tussocks and with the low sun angle in the Arctic even relatively short tussocks can shade intertussock space (Juszak et al., 2014; Walker et al., 2003), which keeps the soil surface cool.

Soil water content varied seasonally but was surprisingly similar between plant communities despite the expectation that Sedge soils would have much higher mean water content because Sedge communities are associated with waterlogged areas (Zak and Kling, 2006). One factor that could play a part is the deeper active layer in Sedge soils that could allow for more drainage (Leffler et al., 2016) or that due to the low
hydraulic conductivity in mineral soil the surface water infiltrates as low rates (Hinzman et al., 1991). During winter, the soil water content does not accurately describe the state of the soil since frozen soils have similar physical attributes as dry soils (Jefferies et al., 2010; Nikrad et al., 2016; Schimel, 2018).

Soil pH gradually increased from winter to autumn and there were also notable differences among Sedge, Shrub, and Tussock communities for each sample period. Soil pH is widely recognized to be a strong driver of soil microbial community structure (Eskelinen et al., 2009; Noah Fierer and Jackson, 2006; Kotas et al., 2017; Männistö et al., 2007) and in this study was a significant predictor of the microbial community in July and September in all vegetation types. Bacteria are more sensitive to change in pH and have a narrower range of optimal growth compared to fungi. Bacterial growth is thus promoted by higher pH and as fungi are less affected by change in pH and tolerant to lower pH, fungal growth is favored in low pH soils (Rousk et al., 2010). The separation among the plant communities regarding pH indicates how the vegetation composition influences pH regimes. For example, the low pH in Tussock soils could be influenced by the abundance of Sphagnum spp. that are known to enhance acidity in soils (Walker et al., 1994). Another driving factor of soil pH in the study area is the downslope transport and accumulation of carbonates that will increase soil pH towards the coastal plain (Ping et al., 2005). The patterns of soil pH in the study area were predictable and expected based on Jenny’s soil-forming factors, namely, parent material, topography climate, time, and vegetation (Jenny, 1946; Ping et al., 2005).
Summer temperature, thaw depth, and water content did not display predicted spatial patterns in terms of a north-south gradient with cooler, wetter soils, and shallower thaw depth further north and warmer, dryer soils with greater thaw depth further south. Instead, the differences among plant communities, indicate that plant community structure influences variation in soil temperature, thaw depth, and water content.

**Soil Respiration**

Respiration rates in Tussock, Shrub, and Sedge soils showed predictable patterns throughout the season. Soil respiration in Tussock tundra was the lowest among plant communities in all sample months (Fig. 2), which contradicts previously reported high respiration rates in this community (Mikan et al., 2002; Weintraub and Schimel, 2003; Zak and Kling, 2006). This finding may result from Tussock soils having a highly recalcitrant soil C pool (Wallenstein et al., 2007; Weintraub and Schimel, 2003). Recalcitrant C substrates not only dampen C mineralization but also stimulate C starved microbes to start mineralizing N (Weintraub and Schimel, 2003). The recalcitrant C pool would also explain the high net N mineralization in Tussock soils during the extent of the study since net N mineralization is only observed when excess N is released during C metabolism of N-rich organic substrates (Weintraub and Schimel, 2003). Another possible explanation for the low respiration rates in the Tussock soils could be the bulk density of the mineral soil creating physical boundaries that reduce CO$_2$ diffusion (Yang et al., 2018).

Soil respiration was highest in July for all plant communities as a result of rapid metabolism promoted by warm temperatures, labile C provided by plant roots, and high
available N (Schimel, 2003). Shrub soils had the highest rate of respiration possibly due to greater labile C from leaf litter used by soil microbes and low quantities of available soil N since shrubs allocate more N to biomass than graminoids (Shaver and Chapin, 1991; Wallenstein et al., 2007; Weintraub and Schimel, 2005). Shrub soils also contained the highest quantities of soil organic matter. In July, when microbial respiration was the highest, there was a negative correlation (R= -0.20) between respiration and N mineralization - i.e. immobilization. This trend of high respiration and net immobilization could be observed in both Sedge and Shrub soils, but not in Tussock soils. These findings contrast previous studies regarding respiration rates in the plant communities studied and could indicate the importance of spatially dispersed samples throughout Arctic Alaska (Weintraub and Schimel, 2003; Zak and Kling, 2006).

**Potential N Mineralization**

Soil N mineralization rates among plant communities and sample dates were found, and distinct trends could be observed both between sample date and within each plant community (Fig. 3, 4, 5). The N mineralization/immobilization trends followed previous studies where not only seasonal shifts have been described, but also how soil microbes differ in mineralization/immobilization rates among plant communities during shorter incubation periods (Chu and Grogan, 2010; Weintraub and Schimel, 2003). It is also important to recognize that the N mineralization incubations were done in soils without influence from plants, consequently, there was no plant uptake to deplete the soil N pool nor were there root exudates to enhance mineralization (Edwards and Jefferies, 2013; Kumar et al., 2016). Mineralization rates in Tussock soils declined from June to
September, but never shifted to immobilization. This lack of immobilization in Tussock soil could partly be explained by the plant community type, where the dominant species *Eriophrum* does not compete well for soil N, but rather partly relies on internal N storage from the previous growing season (Nordin et al., 2004; Weintraub and Schimel, 2005). Additionally, the low quality of available C that will promote N mineralization and thus leaving Tussock soils with more mineralized N as soil microbe N demands are met (Weintraub and Schimel, 2003). All soils showed great variability in June. Both Shrub and Sedge soil microbes were immobilizing N from June to September indicating that higher rates of substrate decomposition as inorganic N forms are used in microbial extracellular enzyme activity and inorganic N is bound in microbial biomass in an initial stage (Schimel, 2003; Sistla et al., 2012). Soil water content and temperature followed a seasonal gradient (Table 1) and are both associated with higher microbial activity at certain threshold levels (Brockett et al., 2012; Fierer et al., 2006; Frindte et al., 2019; Mikan et al., 2002; Nadelhoffer et al., 1991; Schimel, 2018), the interactive effect of soil water content and temperature could, therefore, explain some of the net N immobilization in Sedge and Shrub soils during July (Brockett et al., 2012; Mikan et al., 2002). The influence of both seasonality and plant community type for potential N mineralization illustrates the importance of environmental control as well as plant litter inputs and N sequestration in these systems.

**Effects of seasonality and plant community type on soil microbial community structure**

Microbial biomass can change dramatically during the transition between spring and summer (Buckeridge et al., 2013). Contrary to the increase in Sedge MBC from March to
June reported here, Edwards and Jeffries (2013) found a significant decline in the same period in a Wet Sedge community. This can be an effect of spring to summer transition, and since Sedge communities are located much further north than both Shrub and Tussock, there will be more pronounced differences in these transition periods due to the timing of thaw and plant growth. Moreover, studies have found rapid turnover in soil microbial biomass and drastic changes can occur within a week (Buckeridge et al., 2013; Edwards and Jefferies, 2013; Weintraub and Schimel, 2005). These rapid fluctuations could be due to freeze-thaw cycles, that are occurring in different magnitudes until mid-June (Buckeridge et al., 2013; Jefferies et al., 2010; Koponen and Bååth, 2016; Schimel and Clein, 1996). Thawing soils release nutrients such as N that were previously bound in frozen soils accumulated microbial necromass, and lysed microbial cells.

MBC and the PLFA biomarkers abruptly decreased between June and July for all plant community soils. This decrease in microbial biomass was likely due to nutrient limitation from both increased thaw depth allowing leaching to deeper soils and competition in nutrient uptake from rapidly growing plants (Buckeridge et al., 2013; Sistla et al., 2012). Contrary to this study, Buckeridge et al. (2013) observed an increase in both MBC and MBN after June. As plants become inactive during fall, litter and nutrients become more freely available for soil microbes and regeneration of microbial biomass is therefore likely (Buckeridge et al., 2013; Edwards and Jefferies, 2013). However, a strong increase of either MBC or PLFA biomarkers was not evident in September.

Like the sample date, plant communities did not differ in microbial biomass or community structure despite the considerable difference in function. However, studies
have found variation in soil microbial biomass between different Arctic plant communities, although they do not constitute all the same plant communities as this study (Chu et al., 2011; Chu and Grogan, 2010). Like biomass, distinct microbial community structure has been found among Tussock, Shrub and Sedge communities (Zak and Kling, 2006).

Like soil microbial biomass, F:B ratios did not differ among plant communities, but differences among sample dates were found. Fungal community biomass typically increases during cooler conditions as the availability of labile C compounds decreases (Buckeridge et al., 2013). Consequently, July was the month when F:B ratios were the lowest across all plant communities, which would also be indicative of a plant induced change in substrate availability with more labile compounds from root exudates enhancing bacterial growth (Eskelinen et al., 2009). Warmer temperatures and higher pH observed in July are also consistent with low F:B ratios (Buckeridge et al., 2013; Eskelinen et al., 2009). Between July and September F:B ratios increased likely due to lower temperatures, cessation of plant growth, and the input of plant litter following senescence (Buckeridge et al., 2013; Eskelinen et al., 2009; Nemergut et al., 2005). As expected, Shrub soils had the highest F:B ratios among plant communities since Shrub litter contains large pools of recalcitrant C compounds compared to the small fraction of labile C available which will influence soil C:N and F:B ratios (Buckeridge et al., 2013; Eskelinen et al., 2009). In general, cold Shrub-dominated soils tend to be dominated by fungi, which are more capable than bacteria in breaking down recalcitrant substrates (Eskelinen et al., 2009). Arbuscular mycorrhizal fungi (AMF), fungi, and saprophyte biomass all increased in Shrub soils between March and June (Fig. A1). Tussock soils
typically contain large pools of accumulated recalcitrant C (Wallenstein et al., 2007), and Zak and Kling (2006) found higher F:B ratios in Tussock compared to Shrub soils. Previous studies have displayed similar F:B ratios in Arctic tundra (Buckeridge et al., 2013; Eskelinen et al., 2009; Zak and Kling, 2006). Here, Shrub soils showed consistently higher F:B ratios over Tussock soils.

The environmental and biochemical variables that predicted soil microbial community structure shifted between sample dates. As MBC and MBN also are predictors of microbial biomass, an expected relationship between PLFA derived soil microbial abundances and MBC and MBN was found (Buckeridge et al., 2013). During March MBN was the only variable that significantly influenced soil microbial community composition. This could be explained by the net mineralization rates across communities in March driving net growth of microbial biomass during winter months. But more so, Shrub soil had relatively high respiration rates in March with immobilization of NO$_3^-$ that suggests that Shrub soil microbes either had access to a small fraction of more labile compounds or were more successful in incorporating organic substrates into biomass. Since all soils were incubated at the same temperature during winter measurements, the insulating effect from snow cover did not affect the rates of how soil microbes were metabolizing soil organic matter in the experiment and thus quality, quantity and accessibility of organic compounds would determine the rate and activity of soil microbes.

These results show significant influence from plant community type and sample date for the overall PLFA biomarkers as % mol biomass. However, shifts in soil microbial
communities within plant communities only accounted for about 10% of overall variability. Therefore, distinct soil microbial community types among the plant communities could not be established (Fig. 9, 10, 11, 12). PLFA derived biomarkers typically describe distinct microbial communities associated with both plant community type and season (Buckeridge et al., 2013; Zak and Kling, 2006). Although, there were significant shifts in the abundance of PLFA derived microbial functional groups among sample periods, trends of plant community-specific soil microbial structure were negligible. This suggests that the effects of seasonality have a stronger influence on soil microbial structure and function than plant communities and any plant communities' influence on soil microbial function and structure is indirect.

As a response to snowmelt, a clear shift in microbial community composition and total biomass was expected between March and June (Buckeridge et al., 2013; Edwards and Jefferies, 2013; Jefferies et al., 2010). However, no significant shifts in PLFA derived microbial community composition were found. Previous studies have suggested a decline in soil microbial biomass during spring thaw and it was assumed that this decline should be apparent in June samples (Buckeridge et al., 2013; Edwards and Jefferies, 2013; Jefferies et al., 2010). Instead, PLFA derived biomass did not change and Sedge soil microbial communities even had a net growth in biomass between March and June. An explanation for this discrepancy could be rapid turnover in microbial biomass that our sampling failed to capture between March and June. Fluctuations in soil microbial biomass in this critical period have been observed (Buckeridge et al. 2013). However, the relative abundances (% mol) of soil microbial communities and biomass did not change between March and June samples, which contradicts the general assumption of shifts in
not only soil microbial biomass (Jefferies et al., 2010) but also in soil microbial community composition during spring thaw (Buckeridge et al., 2013).

July was the month with the biggest decline in total PLFA derived biomass following the same trend as MBC and MBN. Although soil microbial community composition did not show distinct differences among plant community types some trends were evident. Sulfate-reducing bacteria, which are associated with anoxic environments, were most pronounced in Sedge soils in July, which would be expected as Sedge soils usually were covered with surface water that will inhibit O₂ entry to the soil. This result again contradicts previous studies that found distinct patterns in microbial community composition between the plant communities described (Buckeridge et al., 2013; Zak and Kling, 2006).

As soil microbial turnover rates are sensitive to changes in soil biogeochemistry on small temporal scales, this study aimed to capture seasonal shifts in soil microbial structure and function. Although soil microbial community structure remained uniform among the different plant communities, soil microbial function varied. In Sedge and Shrub soils, respiration, and N mineralization showed similar trends. However, the Tussock vegetation community not only displayed the most pronounced shifts in microbial structure but also differed with both Sedge and Shrub in terms of function with significantly lower respiration rates and net N mineralization from March throughout September. An explanation could be, that the dominant plant in Tussock communities Eriophrum, is unable to incorporate inorganic N (Nordin et al., 2004; Wallenstein et al., 2007) and therefore microbes are rarely N starved (Weintraub and Schimel, 2003). This
excess of inorganic N in Tussock soils could be especially pronounced in an incubation experiment where there is no competition in N acquisition from plants (Weintraub and Schimel, 2003). Tussock soils are known to have large amounts of recalcitrant C that will slow microbial mineralization and growth and thus reduce the microbial demand for inorganic N (Schimel and Schaeffer, 2012; Weintraub and Schimel, 2003).

These results suggest that soil microbial structure and function are not necessarily correlated in an intuitive way such as greater microbial biomass yielding higher respiration rates or potential N mineralization rates. High respiration rates could, for example, be better explained by temperature and available soil substrates rather than microbial community composition or microbial biomass. This counterintuitive relationship indicates how strongly soil microbial communities rely on resource availability driven by plant inputs and how soil microbial respiration and N mineralization is influenced by the plant community. Here we found that rather than different soil microbial communities having similar respiration and N mineralization rates in the different plant communities, similar microbial communities are functioning differently in terms of respiration and N mineralization rates in the respective plant communities.

Conclusions

This study showed that with greater spatial sample variability and sample size of the Tussock, Shrub, and Sedge plant communities, soil microbial community structure is less well defined with respect to plant community than previously suggested. Although the different plant communities have similar microbial community structures, these microbial
communities vary in function including respiration and N mineralization among plant communities. The strong seasonal patterns in temperatures and water availability between winter and summer drive much of the variability of both microbial structure and function (Buckeridge et al., 2013; Clein and Schimel, 1995; Frindte et al., 2019; Lipson and Schmidt, 2004; Mikan et al., 2002; Nemergut et al., 2005) despite the broad-scale variation in landscape features that drive differences in plant species composition between the Brooks Range and the Arctic Coastal Plain. The extensive sampling and great spatial distribution of similar plant communities therefore suggest that landscape variability affects microbial community structure more than each of the plant communities represented. More studies focusing on broad-scale dynamics in Arctic soil microbial ecosystems are necessary to further understand of the biotic and abiotic perturbations and how these interact with each other in Arctic ecosystems in a spatiotemporal framework. This is necessary since these systems are sensitive and responsive to changes in climate and vegetation, and where future Arctic perturbations may not influence the soil microbial structure as much as function in terms of the rate and the fate of which soil nutrients are cycled in these systems.

Limitations

There were several limitations within this study where new ideas or insights about possible improvements came as the project was progressing. There was no initial consideration of quantifying soil properties or a more detailed classification of the soils in the studied systems. Although the trends in the different vegetation communities were stable, there was, of course, unaccounted for variation. Additionally, the vegetation
classification employed was coarse, and since the extensive Tussock plant community was highly variable among sample locations a finer scale classification for Tussock may improve the results by partitioning the variance among unique Tussock communities. The failure to make distinct divisions of the plant community and soil microbial community structure could be due to more fine-scale variability within each of the soil microbial functional groups. Variation within dominant groups such as gram-positive and gram-negative bacteria and fungal lineages could reveal more of the plant community-specific microbial community structure. Here, a comparison of the within microbial community type between sample dates was not done. Potential changes in within microbial community type between sample dates could help to better explain changes in function. The aim of this study was, however, to describe changes among microbial functional groups. As for the potential N mineralization, longer incubation times improve resolution among samples, since Arctic processes are known to be slow and a ten-day incubation time resulted in minimal change in inorganic N concentrations.
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<th>MBN (ug/g)</th>
<th>SMR (ngC/g/h)</th>
<th>Total N (ug)</th>
<th>Soil C (%)</th>
<th>WBI</th>
<th>Water Depth (cm)</th>
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**Table A1-A4.** Correlation tables for each of the sample months. MBC and MBN are microbial biomass carbon and nitrogen, SMR is soil microbial respiration, WBI is winter biological index.
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Table A5. PLFA biomarkers used to categorize soil microbial communities from the Midi library. (Midi Inc., Newark, Delaware, USA).
Fig. A3. Graphs of individual microbial functional groups by month and plant community type.
LITERATURE CITED


