THESIS

NUTRIENT LIMITATION OF MICROBIAL DECOMPOSITION IN ARCTIC TUSSOCK TUNDRA SOIL

Submitted by

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ABSTRACT

NUTRIENT LIMITATION OF MICROBIAL DECOMPOSITION IN ARCTIC TUSSOCK TUNDRA SOIL

Cold, wet conditions limit microbial activity in many parts of the Arctic tundra, resulting in slow decomposition of soil organic matter, low nitrogen (N) mineralization rates and the accumulation of massive amounts of soil organic carbon (SOC). Climate change is currently reducing these physical environmental constraints, allowing for Arctic SOC to become vulnerable to decomposition. However, historically low decomposition rates due to climatic inhibition have resulted in soils with extremely poor nutrient availability in the active soil layer for much of the year further inhibiting ecosystem productivity and limiting microbial decomposition. N limitation of both primary productivity and microbial activity, in addition to extremely low soil N availability throughout much of the active season, make many Arctic tundra ecosystems among the most N limited in the world. Changing climatic conditions can potentially allow for increased annual N mineralization resulting in greater soil N availability. Enduring increases in soil N availability would alter microbial driven biogeochemical cycles with cascading long-term effects on Arctic tundra ecosystems.

Despite previous experimental findings of N limitation of microbial decomposition in Arctic tundra, seasonal variability in soil N availability in conjunction with the influences of other soil factors indicate that N may not be the primary control of microbial activity in these soils during the entirety of the Arctic active season. The tight coupling of biogeochemical cycles suggests that labile carbon (C) may be co-limiting for portions of the active season when there is greater soil N available. Furthermore, most observations of N stimulation of microbial activities

have originated from relatively few research sites due to the inaccessibility of much of the Arctic, but N limitation of decomposition may be site dependent and vary across small geographic areas. Questions of inter-annual and intersite variability of soil microbial activities within a singular Arctic soil type have never previously been directly addressed.

I conducted laboratory soil incubations to examine intra-seasonal and annual variability of soil microbial N limitation, the potential for co-limitation of labile C and N, and the extent of intersite variability in microbial N limitation across two comparable moist acidic tundra (MAT) sites within close proximity and of similar topography, climate and vegetation.

I found, contrary to previous studies and my hypotheses, that soil microbial biomass growth, C mineralization, and extracellular enzyme activities were not consistently stimulated by N additions, but rather found that N was primarily immobilized in microbial biomass. Stimulation of C mineralization by N addition was short-lived and variable across the course of a single active season. Additionally, there was significant variation in microbial responses to nutrient amendments and temperature across the two consecutive study years; differences in temperature sensitivities of C mineralization and conflicting effects of N amendment on enzyme activities were seen between study years.

Intersite variability was also significant; despite the close physical proximity and similar topography, climate, and vegetation of the sample sites investigated, they differed markedly in their responses to N additions as well indications of labile C co-limitation. The uniquely uniform properties of MAT tussock soils may lead to the presumption of homogeneity of soil microbial activities. However, I found that the significance of microbial N limitation and occurrence of co-limitation by labile C were dependent on the soil sampling site even though soil properties were consistent across sites.

These findings of extensive variability and labile C co-limitation within some MAT tussock soils elucidate some of the current knowledge gaps in Arctic microbial ecology and suggest that the current paradigm of Arctic N limitation as one of the primary active season controls on ecosystem activity needs to be expanded and further refined to better predict the fate of the large amounts of C currently sequestered in Arctic tundra soils.

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CHAPTER 1

INTRODUCTION

High soil moisture and low temperatures limit microbial decomposition in Arctic tundra soils for much of the year, resulting in the accumulation and persistence of large amounts of mineralizable soil organic matter (SOM) (Weintraub & Schimel, 2003; Shaver et al., 2006) and low nutrient availability throughout much of the year (Hole, 2004, Weintraub & Schimel, 2005). Terrestrial Arctic systems currently sequester nearly half of the total global soil organic carbon (SOC) (Tarnocai et al., 2009) and are also among the most nitrogen (N) -limited in the world. Primary productivity (Shaver et al., 2001; Chapin et al., 1995; Gough et al., 2012) as well as microbial decomposition (Mack et al., 2004; Sistla et al., 2012) and extracellular enzyme production (Wallenstein et al. 2009; Sistla et al., 2012; Marklein et al., 2012) have all been shown to be N-limited during the Arctic summer season. Experimental increases in N availability in Arctic tundra systems have altered plant community composition and increased primary productivity (Chapin et al. 1995; Shaver et al., 2001; Jonasson et al., 1999b) while also increasing microbial C mineralization and decomposition (Mack et al., 2004; Sistla et al., 2012). Thus, sustained increases N availability could reduce SOM storage (Mack et al., 2004) and could have dramatic and cascading effects on Arctic tundra ecosystems and their biogeochemical cycles.

Climate change is not only altering physical environmental inhibitions of decomposition in the Arctic, but also potentially changing biogeochemical cycles. High latitude systems are experiencing disproportionately greater effects of climate change compared to lower latitudes (McBean et al., 2005; Anisimov et al., 2007), with the greatest warming occurring in the winter

and spring seasons (Serreze et al., 2000). Nutrient cycles in the Arctic are tightly linked to the physical environmental conditions and exhibit strong seasonal patterns. N cycling in Arctic tundra soils is predominated by N mineralization during the winter; microbial activity is driven by preferential utilization of labile, nutrient rich substrates (Schimel & Mikan, 2005) and there is limited vegetative demand for N during the cold season. Warmer winter soil temperatures can potentially increase cool season N mineralization resulting in increased N availability during the active summer season (Schimel et al., 2004). During the warmer active season, microbial decomposition of recalcitrant SOM and nutrient-poor plant detritus is prevalent (Schimel & Mikan, 2005; Baisi et al., 2005) and both plants and microbes compete for the limited available soil N (Jonasson et al., 1999a; Hole, 2004; Schmidt et al., 1997; Schimel & Chapin 1996). Thus, not only is climate change lessening the environmental inhibitions of decomposition, but also potentially altering N cycling and availability via altered season lengths and soil temperatures.

The large C stocks, temperature sensitive biogeochemical cycles, and disproportionate warming have led to Arctic regions being recognized as the most vulnerable to climate change and possessing the greatest potential for both positive and negative feedbacks (Anisimov et al., 2007). As the physical constraints on biogeochemical cycling in Arctic ecosystems lessen, nutrient limitation of decomposition is becoming increasingly important. However, only a limited number of studies have directly addressed nutrient limitation of microbial activities in Arctic soils, resulting in a knowledge gap of short scale temporal and site-based variability.

The current paradigm of Arctic tundra ecology suggests that N availability is the principal control over both primary productivity and soil microbial activity during the growing season. However, the adverse seasonal N dynamics and variable N availability over the course of the active season suggest that N limitation may not be the primary control of microbial activity

over the entirety of the season. Previous laboratory observations of microbial N limitation (Sistla et al., 2012) may be more reflective of instantaneous soil dynamics, while not necessarily representative of the full dynamics of active season microbial nutrient limitation. A better understanding of the seasonal context and variability of microbial N limitation is needed to refine the paradigm of seasonal ecosystem N limitation and better understand how other Arctic biogeochemical cycles may be impacted by the cascading effects of altered N availability.

Seasonally driven soil N cycling patterns, pulses of nutrient mineralization resulting from freeze/thaw events (Schimel & Clein, 1996), temperature dependent microbial processes and intra-seasonal variation of N availability (Weintraub & Schimel, 2005; Hobbie et al., 2002) all support suggestions of potentially greater N availability early in the growing season following the spring thaw when vegetative demand is also low. These conditions are associated with variable soil N availability, indicating that N is not the primary limiting factor of microbial activity for the entirety of the active season but rather that microbial N limitation varies temporally. Additionally, temperature effects on Arctic soil microbial processes resulting in greater N mineralization at cooler temperatures and immobilization at warmer temperatures suggest that variable active season soil N availability may be partially driven by soil temperature dictating microbial activities. While N has been repeatedly demonstrated to be limiting to primary productivity and microbial activity in Arctic ecosystems, the microbial drivers and implications on microbial activity of intra-seasonal N variability during the active season are largely unknown.

Chapter 2 of my thesis addresses questions of intra-seasonal and annual variability of microbial N limitation in MAT tussock soil. In order to better understand potential seasonal variability in N limitation of microbial activity in Arctic tundra tussock soil, multiple laboratory

soil fertilization incubations were conducted over the course of two years. The experiments were designed with the goals of assessing seasonal variability of microbial N limitation between early and peak active season soils and the role of soil temperature in driving microbial N limitation. Specifically, I addressed the following hypothesis: microbial activity is more N limited at the peak of the growing season and less so shortly after the spring thaw. Additionally, warmer soil temperatures alter microbial activity inducing greater N limitation. Samples of moist acidic tundra (MAT) tussock soils were collected from a site near Toolik Lake Research Station on the north slope of the Brooks Range in Alaska. Soils were sampled shortly after soil thaw and at the peak of the growing season for two consecutive years. Sampled soils were immediately homogenized and received either N or equivalent water amendments and were subsequently incubated in the laboratory for one week at temperatures of 5^{o}C and 15^{o}C. Incubated soils were examined for microbial biomass, soil nutrients, soil respiration/CO_2 mineralization, and potential extracellular enzyme activities. Conducting the N-fertilization laboratory incubation across seasons, years, and temperatures provided insight into temporal variability of and temperature effects upon microbial N limitation.

Limited microbial response to N amendments and significant intra-seasonal and as well as annual differences in microbial activities and response to N additions in the prior laboratory incubation contradicted expectations based on previous studies. These unexpected results and extensive annual variability led to additional questions regarding labile C co-limitation of microbial activity.

The tight coupling of biogeochemical cycles within Arctic ecosystems supports the possibility for co-factors to limit soil microbial activity. Despite large amounts of Arctic SOC, microbial communities may be energetically limited via labile carbon (C) availability in addition

to N. Without sufficient labile C, microbial communities may not be able to fully utilize other soil nutrients for biomass synthesis (Jonasson et al., 1999a; Jonasson et al., 1996), extracellular enzyme production (Weintraub & Schimel, 2005) and C mineralization (Lavoie et al., 2011). Under in situ conditions, regular active season soil inputs of labile C are provided by active plant roots which exude labile, low molecular weight compounds that are easily assimilated by soil microbes without utilization of extracellular enzymes (Bremer and van Kessel, 1990; Bremer and Kuikman, 1994). Root derived carbon inputs constitute the most important carbon source for soil microbes during the Arctic active season (Loya et al., 2004) and preferential use of carbon provided through root exudation may inhibit decomposition of root litter and SOM (Loya et al., 2004) altering nutrient partitioning (Schmidt et al., 1997). Carbon additions provided by root exudates may also induce localized extreme microbial N limitation, prompting increased decomposition of N rich proteins (Weintraub and Schimel, 2005) and greater net N mineralization (Phillips et al., 2011; Drake et al., 2011). This coupling of biogeochemical cycles of N and C make labile C inputs an important controlling factor of biogeochemical cycling and microbial activity within Arctic soils. Knowing how labile C may influence microbial activity and alter utilization of additional available N crucial to fully understanding how increased altered N availability will alter Arctic active season biogeochemical cycles.

The surprising variability and limited response to N amendments in the soil incubation discussed in Chapter 2 may be explained by variable microbial responses to N addition across sites. Questions of variability in biogeochemical cycling across sites with differing soil types (MAT vs. moist non-acidic tundra) have been previously addressed, however little is known about inter-site variability between sites of a single soil and vegetation type as well as exposure to similar climatic conditions.

The vast global expanse of MAT is fairly uniform in regards to climate, topography, vegetation, and nutrient availability. Uniformity in conjunction with the inaccessibility of large portions of the Arctic has led to our understanding of Arctic ecosystem ecology being synthesized from research obtained from only a handful of research sites globally. Additionally, the unique vegetation driven soil formation of MAT tussock soil, which is largely composed of *Eriophorum vaginatum L.* biomass in various stages of decay, have led to questions of how uniform these soils are across the Arctic. The self-forming soil properties of tussock tundra and restrictive physical conditions of low pH and high soil moisture in MAT soils make the possiblity of uniformity seem reasonable. However, other important factors potentially influencing soil properties, such as glacial geology, are extremely variable across small physical areas of the Alaskan Arctic (Hamilton, 2002).

In chapter 3 of my thesis I examine the ability of labile C inputs to influence microbial activity and N cycling and consider the possibility of site-based variability of microbial nutrient limitation. I specifically addressed the hypotheses that labile C will alter microbial utilization of N amendments and allow for greater growth of microbial biomass and that MAT tussock soils will respond similarly to C and N amendments regardless of sample site. In order to address the questions of the interacting influences of N and labile C on microbial mediated biogeochemical cycling and potential site-based variability within MAT tussock soils, I conducted a laboratory soil fertilization incubation utilizing soil from two sampling sites of close proximity. MAT soils were sampled from directly underneath *Eriophorum vaginatum L.* vegetation shortly after snowmelt from sites near the Toolik Late Research Station and Imnavait Creek in northern Alaska. Both sites experience similar climatic conditions and have similar topography, vegetation, and soil properties. However, the sampling sites did differ in regards to their glacial

histories and effective soil age. Soils from both sites were utilized in a factorial soil fertilization laboratory incubation. Soils from each site were amended with treatments of water, or N at the onset of the incubation and also received daily amendments of glucose or water throughout the incubation. Soils were incubated for 2 weeks at 10^{o}C and then subsequently examined for microbial biomass, soil nutrients, soil respiration/CO_2 mineralization, and potential extracellular enzyme activities.

It is becoming clear that the current paradigm of N availability limiting active season Arctic ecosystems needs to be refined. With the potential for climate change to drastically alter decomposition rates of the large amounts of SOM sequestered in Arctic soils as well as N availability, a comprehensive understanding of the controls and variability of active season biogeochemical cycling is of the utmost importance. Current knowledge of microbial mediated decomposition in Arctic tundra soils has been synthesized from a few field and laboratory studies and extrapolated to large geographic areas. However, little consideration has been previous given to potential temporal and intersite variability within a soil type. Evidence of variability in microbial activity and N limitation annually, intra-seasonally, and across sites within MAT tussock soils indicate the need for increased knowledge of what drives active season microbial activity and how much variability may exist within Arctic tundra soils. To better assess the larger questions of the vulnerability of Arctic SOM stores undergoing climate change, a better comprehension of the intricacies affecting Arctic biogeochemical cycles is needed.

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CHAPTER 2

TEMPORAL VARIABILITY OF NITROGEN LIMITATION OF MICROBIAL ACTIVITY IN ARCTIC TUNDRA TUSSOCK SOIL

Introduction

Arctic tundra ecosystems are believed to be among the most nitrogen (N) -limited in the world. Concentrations of available inorganic soil nitrogen are extremely low throughout much of the active growing season (Hole, 2004; Hobbie and Gough, 2002; Weintraub and Schimel, 2005) resulting from low rates of N mineralization (Marion and Miller, 1982). Primary productivity (Shaver et al., 2001; Chapin et al., 1995; Gough et al., 2012), microbial decomposition (Mack et al., 2004; Sistla et al., 2012), growth of microbial biomass (Sistla et al. 2012), and extracellular enzyme production (Wallenstein et al., 2009; Sistla et al., 2012) have all been shown to be N-limited during the Arctic active summer season. These previous studies have led to the construct of the current paradigm of Arctic ecosystem ecology suggesting that low N availability limits ecosystem productivity and decomposition of soil organic matter (SOM) during the active summer season. Biogeochemical cycles in the Arctic are tightly regulated by physical environmental conditions; as climatic conditions change soil N availability may increase through stimulation of N mineralization rates (Elberling, 2007; Schimel et al. 2004). The demonstrated role of N limitation in structuring Arctic tundra ecosystems and constraining ecosystem functioning indicates that increased N availability can have dramatic and cascading effects on these ecosystems.

Arctic soils contain large amounts of C, currently sequestering nearly half of the total global soil organic carbon (SOC) pool (Tarnocai et al., 2009). Decomposition of soil organic matter

(SOM) is inhibited for much of the year by high soil moisture and low temperatures, limiting microbial activity and resulting in the accumulation and persistence of large amounts of mineralizable SOM (Weintraub & Schimel, 2003; Shaver et al., 2006) and low soil nutrient availability (Hole, 2004; Hobbie and Gough, 2002; Weintraub and Schimel, 2005). However, climate change is lessening the strict environmental controls on decomposition in Arctic soils. Terrestrial surface air temperatures in the Arctic have increased at nearly twice the global rate (McBean, 2005; Anisimov et al. 2007) with the greatest air temperature increases in these areas being experienced during the winter and spring seasons (Serreze et al. 2000).

Due to the tight climatic regulations over biogeochemical cycles in the Arctic, climate change also has the potential to greatly alter nutrient availability in these systems. Arctic soils experience extreme seasonal climatic variation and exhibit strong seasonal patterns of biogeochemical cycling. Microbial activity, substrate utilization and nutrient cycling dynamics vary drastically between warm and cold seasons with significant transitions occurring near freezing soil temperatures. Under colder conditions, microbial communities preferentially process labile, N-rich microbial byproducts and net N mineralization dominates during the winter season (Schimel and Mikan, 2005). During the warmer active season, microbial decomposition of recalcitrant SOM and nutrient-poor plant detritus is prevalent (Schimel and Mikan, 2005; Baisi et al. 2005) and net N immobilization occurs. Increased air temperature as well as elevated winter soil temperatures potentially allow for greater N mineralization during the cool seasons (Schimel et al., 2004) when biological N demand is also low. Greater N mineralization during cool seasons could result in greater soil N availability during the active summer season when any available N is readily utilized by both plants and microbes (Jonasson et al., 1999a; Hole, 2004; Schmidt et al., 1997; Schimel & Chapin 1996). Increased N availability

likely would not persist throughout the duration of the active season and may differentially affect early season and peak soil microbial processes.

Available amino acids and inorganic forms of soil N have been shown to vary throughout the active season (Weintraub and Schimel 2005a ;Hobbie & Gough, 2002) with greater N availability early in the growing season followed by a sharp decline mid-season when N demand of vegetation is greatest (Weintraub and Schimel 2005a). This active season decline in available soil N may be attributable to greater plant uptake of soil nutrients during the peak growing season and/or changes in microbial driven N mineralization and immobilization (Weintraub and Schimel 2005b). Seasonal dynamics and temperature effects on Arctic soil microbial activity suggest that soil temperatures may be a primary driver of active season N cycling in these soils. While N has been demonstrated to be limiting to primary productivity and microbial activity in Arctic ecosystems, the microbial drivers and implications of intra-seasonal N variability during the course of the active season are largely unknown.

Before we can predict how Arctic tundra systems may be altered by climate change and assess the fate of the large C stocks contained in Arctic soils, it is first necessary to understand the intricacies of nutrient limitation of decomposition throughout the warm active season. I conducted multiple laboratory soil fertilization incubations to examine how microbial N limitation varies over the course of the active season and how soil temperature affects microbial N limitation. By conducting multiple soil incubations utilizing soils collected over two consecutive years, early after thaw and at the peak of the active season, I aimed to address whether microbial N limitation varies temporally. Specifically, I assessed the variability in microbial N limitation across years and within a single season. With biogeochemical cycling being tightly regulated for much of the year by physical environmental conditions, I

hypothesized that there would not be large annual variability in active season microbial N limitation. However, based on intra-seasonal variability in soil nutrient availability, I expected that there would be greater microbial N limitation seen in the peak season soils and less in the early season soils collected shortly after thaw. Additionally, owing to temperature dependent microbial processes, I predicted that soils incubated at 15^{o}C would exhibit greater N limitation and have a greater response to N amendments than those incubated at 5^{o}C.

Methods

Sample collection, preparation, and storage

Soil samples were collected from a moist acidic tundra (MAT) site east of Toolik Lake at Imnavait Creek (68° 37' 37"N, 149° 19' 11"W) on the north slope of the Brooks Range in Alaska. The site consisted of typical MAT tussock vegetation, which is dominated by the tussock forming sedge *Eriophorum vaginatum L*. with mosses, deciduous shrubs, graminoids, and evergreen shrubs found between tussocks. Globally, MAT covers approximately 900,000 km^2 (Oechel et al., 2003), with areas of tussock-dominated vegetation similar to our research site found throughout the Northern Slope of Alaska, northern Canada, and eastern Siberia (Bliss & Matveyeva, 1992).

MAT tussock soil samples were collected at the Imanvait site over two consecutive years with sample dates targeting the beginning of the active growing season and the peak time of plant productivity and nutrient demand. Five field samples were collected early in the morning on the dates of 06/01/2010, 07/21/2010, 6/8/2011, and 8/8/2011. Tussock soils were collected using 5 cm diameter cores inserted directly beneath tussock vegetation to a depth of approximately 20 cm where possible, or until solid frozen or mineral soil was reached. Each field

sample consisted of 2-3 combined soil cores taken from a single tussock mound. The five field replicate samples were collected from randomly selected tussocks at the Imnavait Creek site and treated as independent laboratory replicates for incubation. Upon collection, all material above the lowest rhizome was removed from each core including any aboveground material and thatch (standing dead tussock material). After aboveground portions were removed, the remaining soil core was measured to 15cm with any excess material from the bottom of the core removed, and the samples were then homogenized by hand. All live *Eriophorum vaginatum* roots and remaining thatch were removed during the homogenization process and the multiple cores of a single sample were combined and thoroughly mixed. Soil samples were immediately weighed into subsamples for initial soil analyses and the subsequent soil incubation was immediately started.

Incubation Set-up and Soil Treatments

Soil incubations were started immediately after soil sampling and homogenization and conducted in a field lab at the Toolik Lake Research Station. A total of four soil incubations were conducted over the course of two years. The soil incubations were set-up in a factorial design with treatments of amended nitrogen or DI water as a control and incubation temperatures of 5 ^{o} and 15^{o} C. Each of the five field replicates was split into four subsamples with each receiving a different treatment (temperature X N). For each incubation sample, 20 g of homogenized field moist soil was weighed into a one pint glass mason jar. Half of the subsamples were treated as controls and received 1 ml of DI water while the other half received a low-level N amendment consisting of 1 ml of ammonium nitrate solution containing 450 ug NH_4NO_3 (equivalent to 112.5 ug N/g dry soil in 1 ml of water). The 1 ml soil amendments were made dropwise, after which the jars were immediately covered loosely with lids to prevent moisture loss. The incubation jars

were then placed in incubators set at 5^{o} or 15^{o} C with a control and amended nitrogen treatment for each field replicate being incubated at each temperature. The incubation set-up resulted in 20 total samples (5 field reps X 2 N treatments X 2 incubation temperatures) with 5 field replicates for each combination of treatments. The soils were incubated at the designated temperature for 5 days with the instantaneous soil respiration rate measured approximately once every 24 hours for the duration of the incubation. After the 5 day incubation period and final respiration measurement, the incubation soils were analyzed for total organic C (TOC), total extractable N (TN), ammonium (NH_4), nitrate (NO_3), total free amino acids (TFAA), microbial biomass C and N, and potential microbial extra-cellular enzyme activities using methods described below.

Soil Respiration

Instantaneous respiration rates were measured daily (but day 3 readings were skipped for some incubations) throughout the 5 day long incubation. Incubation jars were left unsealed for the duration of the soil incubation with jar lids loosely covering the jar tops to prevent loss of soil moisture through evaporation. Prior to measuring respiration rates, the jars were completely uncovered and ambient room air was well intermixed with the air within the jars. After the air was thoroughly mixed the jars were quickly capped and tightened to seal the jars airtight. Each sealed jar was left to continue incubating at the designated temperature for 1 hour before a gas sample was withdrawn via needle through rubber septa in the jar lids. 3 clean, empty jars were treated the same as the incubation jars; the measured CO_2 concentrations of the ambient air in the empty jars were used for "initial" CO_2 measurements in respiration rate calculations. The CO_2 concentrations of gas samples were analyzed immediately after being withdrawn via an Infrared Gas Analyzer (LI-820).

Soil Nutrients and Microbial Biomass

Soil subsamples of 5 g were extracted in 25 ml 0.5 M K_2SO_4, shaken for 1 h, and vacuum filtered to determine TOC, TN, NH_4, NO_3, and TFAA. Soil extracts were analyzed for TOC and TN by the Ecosystem and Soil Ecology Laboratory at the University of Toledo on a Shimadzu TOC/N instrumental analyzer. Soil NH_4 content was determined using a colorimetric microplate assay (Rhine et al. 1998), as was NO_3 (Dopane and Haorwath 2003), and TFAA were measured using a fluorometric assay (Darrouzet-Nardi et al. 2013). Microbial biomass was determined utilizing a method of direct chloroform addition to the study soils described by Scott-Denton et al. (2006) and Weintraub et al. (2007). 5 g soil subsamples with 2ml of ethanol free chloroform added were kept in capped flasks for 24 hours, followed by K_2SO_4 extraction as described for TOC methods. Microbial biomass C and N were determined by calculating the difference in TOC and TN content between the chloroform exposed and corresponding unexposed K_2SO_4 soil extracts. Additional C and N in the fumigated soil extracts is assumed to have come from lysed microbial cells and attributed to microbial biomass content. No extraction efficiency correction factor (e.g. K_{ec}) was used in biomass calculations. Gravimetric soil moisture was determined by drying 5 g field moist soil from each experimental sample at 60^{o} C for 48 hours, and nutrient data is reported on a dry soil weight basis.

Potential Enzyme Activities

Soil enzyme assays were conducted to determine the potential activity of the hydrolytic enzymes b-1,4-glucosidase (BG), b-1,4-N-acetylglucosaminidase (NAG) and phosphatase (PHOS), leucine aminopeptidase (LAP), and oxidative enzyme phenol oxidase (PO) (Table 2.1).

Table 2.1 Assayed extracellular enzymes with their abbreviations and general

Enzyme	Abbreviation	Function
β-Glucosidase	BG	Sugar degradation
Lucine Aminopeptidase	LAP	Protein degradation
N-acetyl-Glucosaminidase	NAG	Chitin degradation
Phosphatase	PHOS	Phosphorus mineralization
Phenol Oxidase	PO	oxidation of phenolics

Each sample was homogenized by adding 2.75 g field moist soil to 91ml of 50mM sodium acetate buffer adjusted to a pH of 4.5 and blending for a total of 2 minutes. Hydrolytic enzymes were assayed following the protocol outlined in Steinweg et al. (2012) with some minor modifications to standard curve concentrations and calculations, incubation times, and sample/plate handling. Substrates used were labeled with the fluorescent indicators of 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC) and the substrates (with their target enzymes) utilized were: 4-MUB-β-D-glucopyranoside (BG), 4-MUB- β -D-cellobioside (CB), 4-MUB-N-acetyl- β -D-glucosaminide (NAG), 4-MUB phosphate (PHOS), L-leucine-7-amido-4-methylcoumarin hydrochloride (LAP). All substrates were added well in excess and were of a 200 μM concentration. A three point standard curve for the fluorescent markers was produced using concentrations of 0uM (DI water), 100 μM, and 200 μM for MUB and 0 μM, 2.5 μM, and 5 μM for MC which is used to determine the very minute LAP concentrations in these soils. Soil slurry, substrates, and standards were added to the deep well plates and were covered with parafilm and gently swirled by hand to mix. The plates were incubated at 10^{o} C for approximately 4 hours after which 250 μM of sample from each of the deepwells was transferred into the corresponding wells of black 96 well microplates. The microplates were then read for

fluorescence on a Bio-Tek Synergy HT Microplate Reader with settings of: excitation= 360/40, emissions=460/40, and sensitivity=46.

The oxidative enzyme phenol oxidase (PO) was assayed utilizing the substrate of 2,2′-azinobis-(-3-ethylbenzothiazoline-6- sulfononic acid) diammonium salt (ABTS) to detect enzyme activity. Assays were conducted according to the protocol outlined by Floch et al. (2007) which has been shown to be as effective as other oxidative enzyme methods (German et al. 2011). The same soil slurry was used for both the fluorometric and oxidative enzyme assays. The PO assay required soil slurry from each sample to be pipetted into two adjacent columns of a deepwell plate with each well receiving 800 μl of slurry. The first two columns of each plate were filled with buffer only and 5 soil slurry samples were dispensed into the remaining 10 columns. For each sample, one column received an addition of 200 μl of 2mM ABTS and the other 200 μl of buffer as a control. Once the soil slurry and ABTS substrate were added to the deep well plates, they were covered with parafilm and gently swirled by hand to mix. The plates were incubated at 10^{o} C for approximately 4 hours after which 250 μM of sample from each deepwells was transferred into the corresponding wells of black 96 well microplates. The microplates were then read for absorbance on a Bio-Tek Synergy HT Microplate Reader at a wavelength of 420nm.

Statistical Analysis

Results were analyzed using a mixed-effects ANOVA in SAS software utilizing the GLIMMIX procedure with fixed effects of season, incubation temperature, and N amendment and random effects of block/sample plot. Some data sets were log transformed prior to statistical analysis to normalize the data distributions. Statistical significance was determined at $p \leq 0.05$.

Results

Soil Respiration/C Mineralization

Instantaneous soil respiration rates measured daily showed distinctive seasonal differences in rates, patterns, and N response over the course of the 5 days of the soil incubation (Figure 2.1). The early season soils had significantly greater respiration rates than the peak

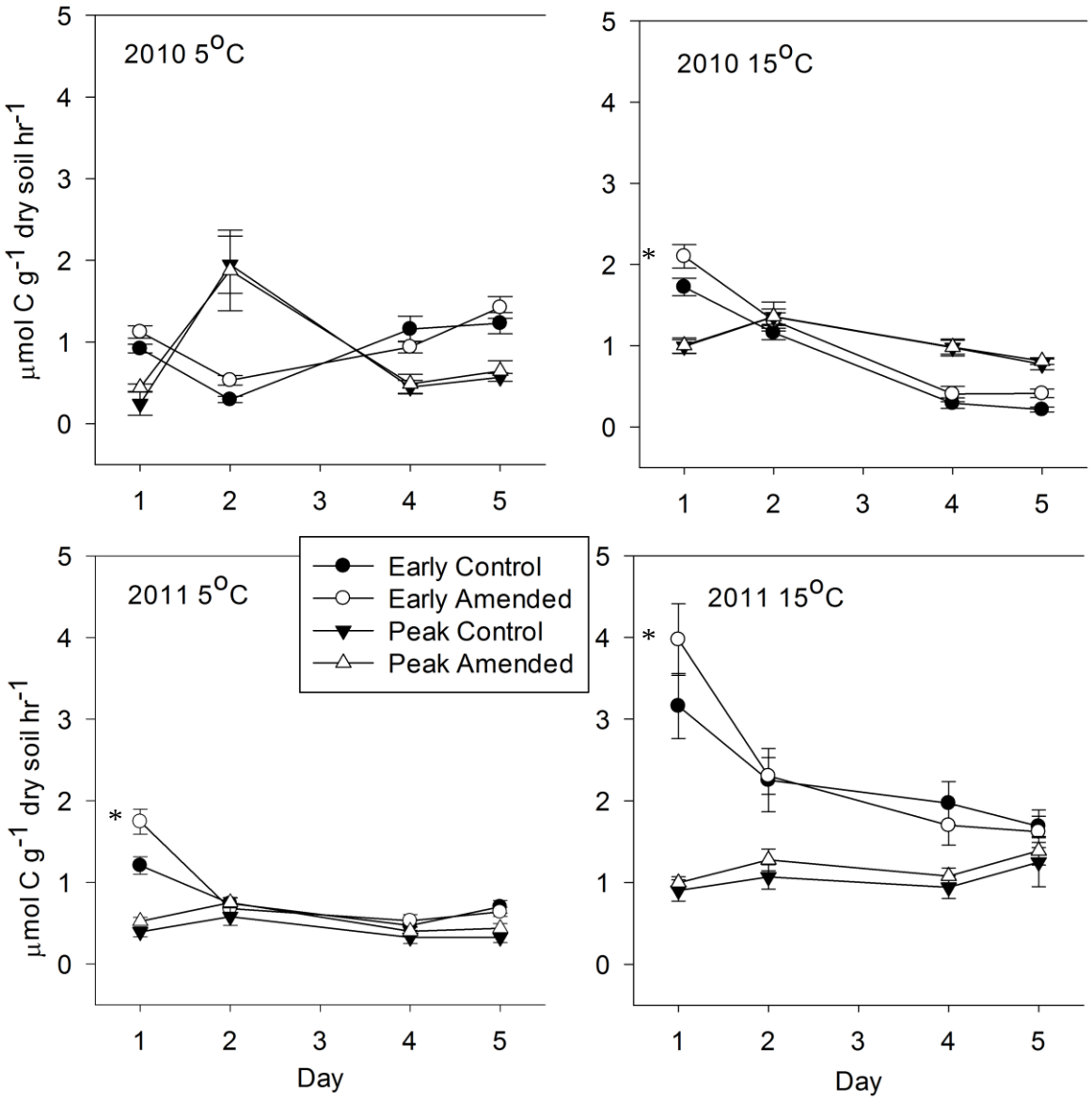

Figure 2.1 Mean (+1 s.e.) of instantaneous soil respiration rates during incubation for 2010 (top) and 2011 (bottom) early and peak season soils incubated at temperatures of 5°C(left) and 15°C (right). Asterisks (*) next to symbols denote significance from the corresponding controls; $P \leq 0.05$.

season soils of comparable treatments after one day of incubation. The early season soil respiration rates declined after day one and converged towards, or even dropped below (2010 soils at 15^{o}C), the lower peak season respiration rates by day 5 of the incubation.

Both the greatest N amendment effect on respiration rates and seasonal differences occurred after 1 day of incubation (Figure 2.1). The day one respiration rates of early season soils were significantly greater (for all cross-seasonal comparisons of similar treatments: $p<0.015$) than the peak season soils of corresponding year and temperature. All of the early season N amended soils had elevated respiration rates over the controls after one day of incubation, with all the day one early season N amended soils respiring at a significantly higher rates except for the 2010 5^{o}C soils (2010 5^{o}C $p=0.248$; 2010 15^{o}C $p=0.023$; 2011 5^{o}C $p=0.052$; 2011 15^{o}C $p=0.004$). These significant N effects were only seen in early season soils on day 1; there were no significant effects of N amendment on instantaneous respiration seen on any other day for any soils.

The average respiration rates over the 5 days of the incubation did exhibit variability across treatments, seasons, and years (Figure 2.2). There are trends of slightly increased respiration rates with N amendments in the early season soils for all temperatures and sample years. N amendment of early season soils in 2010 stimulated a 10% ($p=0.651$) and 25% ($p=0.132$) increase of average respiration rates at 5^{o}C and 15^{o}C respectively. Average respiration rates of peak season soils in 2010 did not respond to N amendments. N amendment of early season soils in 2011 stimulated a 15% ($p=0.441$) increase of average respiration rates at 5^{o}C and had no effect on soils incubated at 15^{o}C. Average respiration rates of peak season soils in 2011 were increased 30% ($p=0.423$) and 14% ($p=0.346$) with N amendment at 5^{o}C and 15^{o}C respectively.

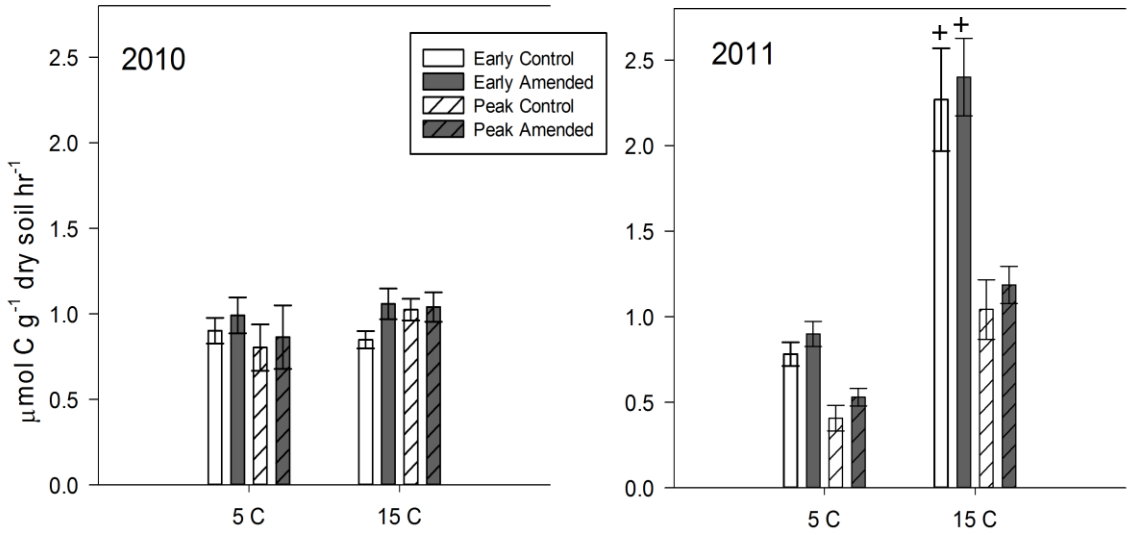

Figure 2.2 Mean (+1 s.e.) of average soil respiration rates for 2010 (top) and 2011 (bottom) early and peak season soils incubated at temperatures of 5oC and 15C. Averages were calculated from instantaneous respiration rates measured on days 1, 2, 4, and 5 of the incubation. Crosses (+) above bars denote significance from peak season soils of similar treatment and temperature; $P \leq 0.05$.

Soils sampled in 2010 maintained fairly consistent average respiration rates regardless of season, N treatment, and temperature. There were no statistically significant differences (at $p \leq 0.05$) in average respiration rates for any of 2010 soils despite N treatment, season, or incubation temperature. Average respiration rates of soils sampled in 2011 were more variable than those of 2010 and exhibited strong seasonal and temperature differences. The respiration rates of early season soils were all greater than the peak season soils at corresponding temperatures for all 2011 samples. The 2011 seasonal differences were greater at higher incubation soil temperatures. When incubated at 15°C, the early season soils had statistically significant (p-value <0.001) greater respiration than the peak season soils regardless of N status. The 2011 early season soils incubated at 5°C had greater average respiration rates than the corresponding peak season soils (control p=0.096: amended p=0.100) although these differences were not as large or statistically significant as those seen in the 2011 soils incubated at 15°C.

Microbial Biomass

Microbial biomass C did not exhibit any consistent trends or treatment effects with regard to N treatment, season, or temperature (Figure 2.3). The 2010 peak season soils incubated at

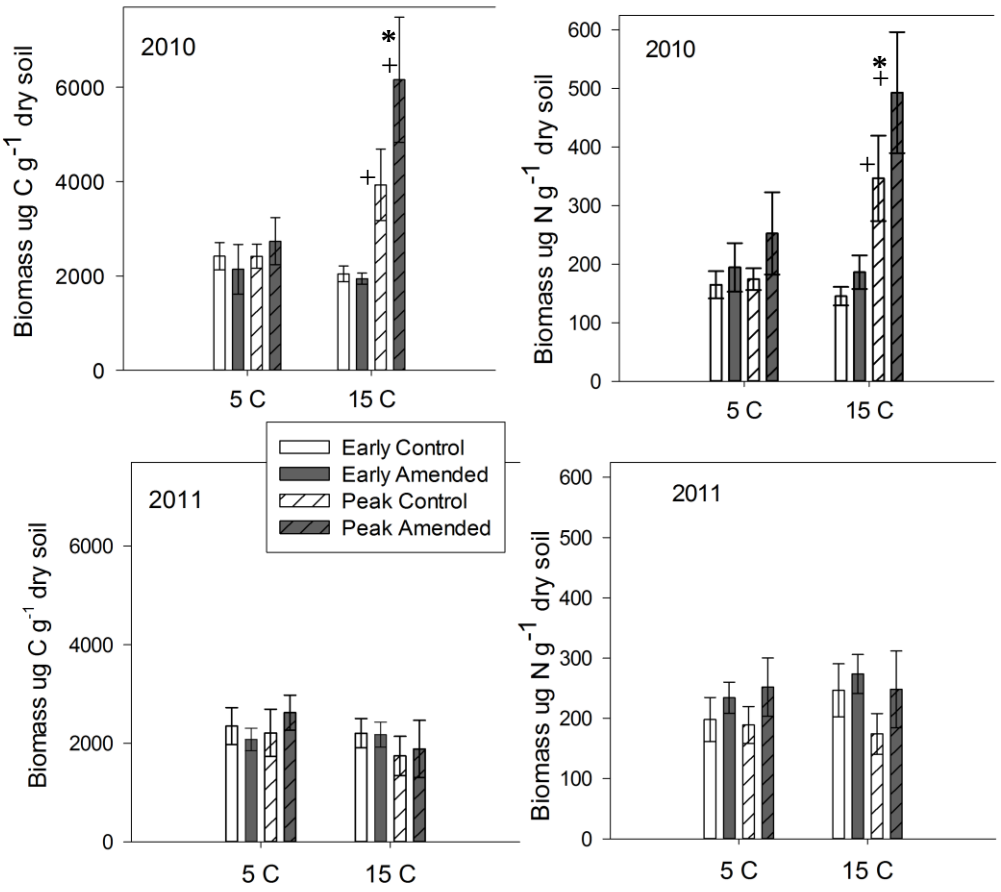

Figure 2.3 Mean (±1 s.e.) of post incubation microbial biomass C (left) and microbial biomass N (right) for 2010 (top) and 2011 (bottom) early and peak season soils incubated at temperatures of 5°C and 15°C. Asterisks (*) above bars denote significance from the corresponding controls; crosses (+) above bars denote significance between early and peak seasons of corresponding treatment and temperature; $P \leq 0.05$.

15°C did have significantly greater microbial biomass C than the early season soils of corresponding treatments (control $p=0.022$; amended $p<0.0001$) and N additions did significantly increase biomass C over the corresponding control ($p=0.002$). For all soils and temperatures microbial biomass N increased with N amendments over the comparable control, however only the 2010 peak soils incubated at 15°C had a significant increase ($p=0.006$). These

same peak season soils also had significantly greater biomass N than the early season soils of similar treatment and temperature (control p=0.005; amended p=0.0002). Season and incubation temperature did not consistently influence amount of biomass N or response to N fertilization (Figure 2.3).

All of the 2010 early season soils responded to N amendment with decreased C:N of microbial biomass (Figure 2.4). The early season 2010 5°C (p=0.003), 2010 15°C (p=0.011), and

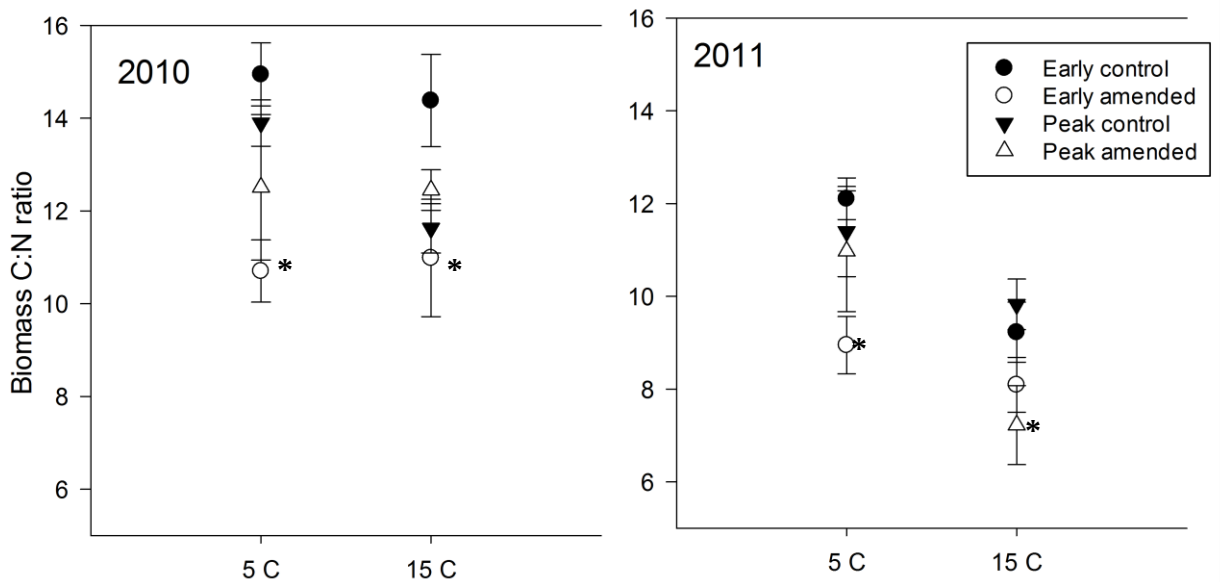

Figure 2.4 Mean (±1 s.e.) of the post incubation ratios of C:N of microbial biomass for the 2010 (left) and 2011 (right) early and peak season soils incubated at temperatures of 5°C and 15°C. Asterisks (*) above bars denote significance from the corresponding controls; P≤0.05.

2011 5°C (p=0.015) all had significantly lower microbial biomass C:N with N amendment. The peak season soils did not have as consistent of a response to N amendment as the early season soils. The only the peak season samples with significantly lowered C:N ratios of biomass was the 2011 15°C (p=0.010) soils. The 2010 peak season soils C:N ratio was reduced by N amendment at the incubation temperature of 5°C, although not significantly, and the remaining peak season soils did not have any stoichiometric response to N amendments. Additionally, incubation temperature did not consistently influence microbial biomass C:N or response to N treatment.

Potential Enzyme Activities

There were no consistent trends in the potential activities of enzymes with respect to incubation temperature, N amendment, season, or year. There was large variability between the two years as well as the early and peak season soils. Potential enzyme activities of incubated soils in 2010 had little variability across N treatment, season, and temperature in 2010 (Figure 2.5). N amendments did result in significant increases in early BG at 5^{o} C (p=0.019) and peak

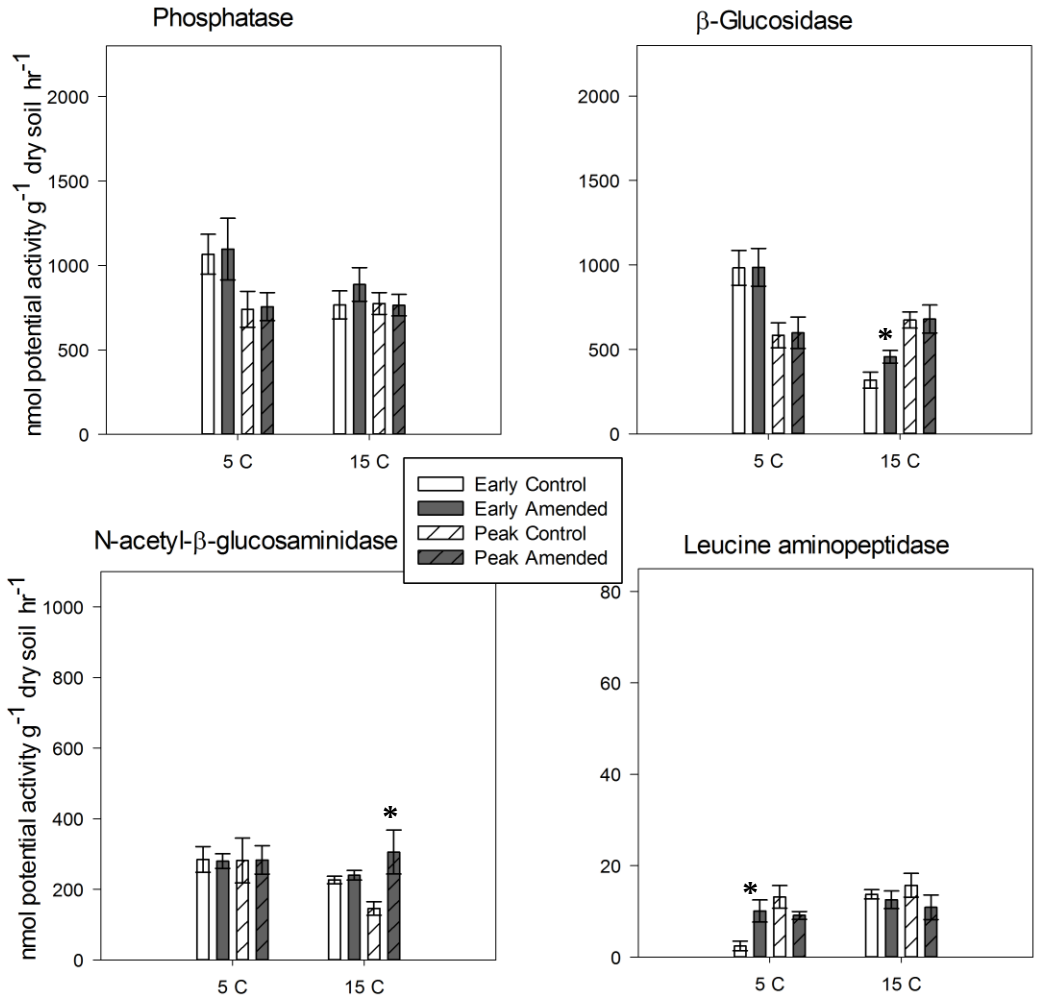

Figure 2.5 2010 Mean (±1 s.e.) of potential enzyme activities assayed post incubation for early and peak season soils incubated at 5oC and 15C. Astericks (*) above bars denote significance from corresponding controls; P≤0.05.

season NAG at 15° C ($p<0.0001$). 2011 early season soils were more variable (Figure 2.6); there

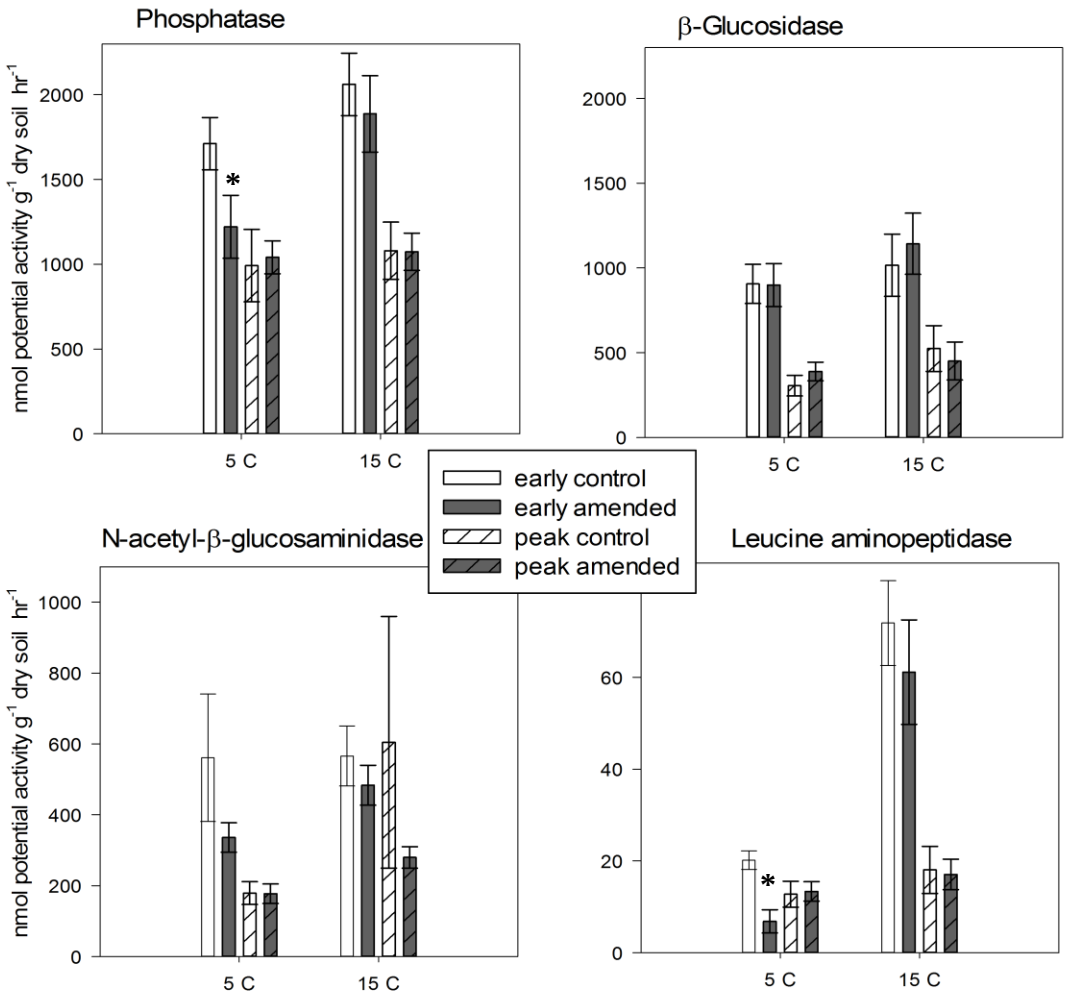

Figure 2.6 2011 Mean (±1 s.e.) of potential enzyme activities assayed post incubation for early and peak season soils incubated at 5oC and 15C. Astericks (*) above bars denote significance from corresponding controls; P≤0.05.

were significant early season decreases of PHOS ($p=0.018$), and LAP ($p=0.0004$) potential activity with N additions at 5° C, while the peak season soils exhibited no N fertilization effects.

Ratios of extracellular enzymes targeting C- and N –rich compounds and phosphorus mineralization provides an index of microbial nutrient demand. In order to consider stoichiometric aspects of microbial resource demands, the ratios of enzymes targeting C-rich substrates (BG) to enzymes targeting N-rich compounds (NAG and LAP) as well as C to

phosphorus mineralizing enzymes (PHOS) was used (Figure 2.7). In 2010, there were significant

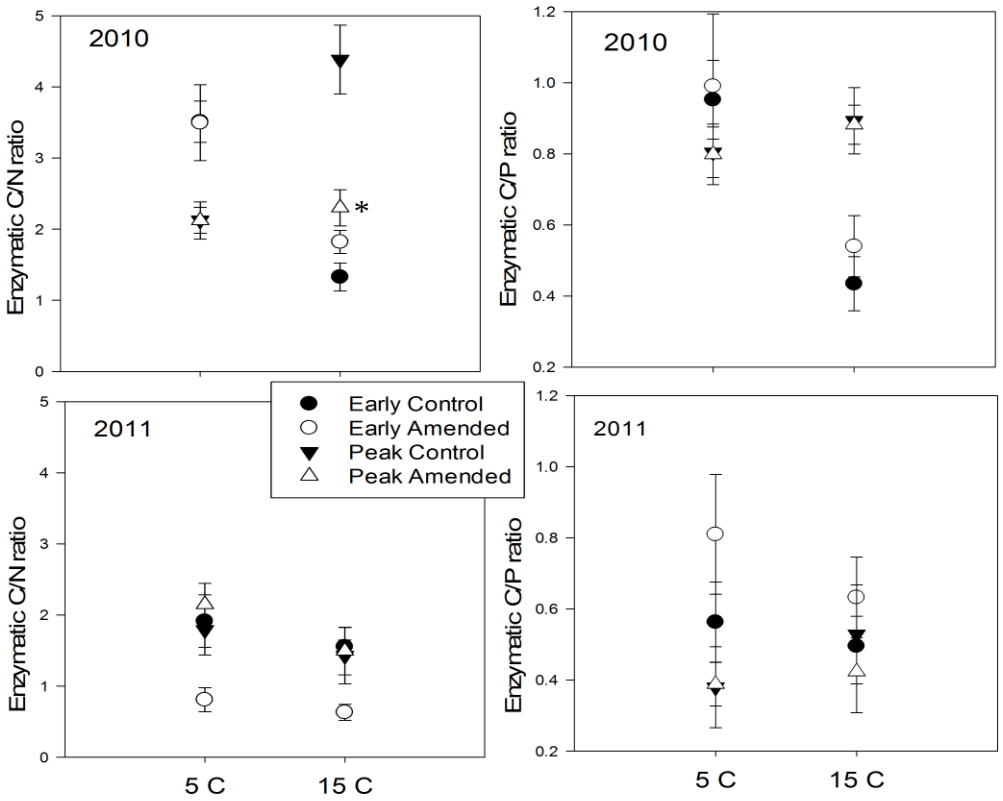

Figure 2.7 Left: Mean (±1 s.e.) of the ratio of C:N acquiring enzyme activities post incubation for 2010 (top) and 2011 (bottom) for early and peak season soils incubated at 5°C and 15°C. Potential activities of BG : LAP and NAG were used to calculate the enzymatic C:N ratios.
Right: Mean (±1 s.e.) of the ratio of C:P acquiring enzyme activities post incubation for 2010 (top) and 2011 (bottom) for early and peak season soils incubated at 5°C and 15°C. Potential activities of BG:PHOS were used to calculate enzymatic C:P ratios. Asterisks next to symbols denote significance from the corresponding control; P-value ≤0.05.

seasonal differences for both ratios C:N and C:P degrading potential enzyme activity, but no consistent effect of N amendment regardless of temperature or season. The 2010 early season soils had significantly reduced enzymatic C:N and C:P activity with increased temperatures, with both ratios being significantly lower at 15°C than at 5°C. The 2010 peak season soils had less enzymatic variability across temperatures.

The 2011 peak season soils, similar to the 2010 peak season soils, exhibited no consistent effects of N amendment or incubation temperature with similar enzymatic C:N and C:P ratios

regardless of N status or temperature (Figure 2.7). The enzyme ratios of 2011 early season samples, unlike the 2010 early season soils, was most responsive to N amendment and not influenced by incubation temperature. The ratio of carbon to nitrogen acquiring enzymes was significantly reduced with N addition in the 2011 early season soils and while also exhibiting trends of increased enzymatic C:P ratios.

Discussion

The current paradigm of Arctic ecosystem function suggests that when environmental conditions are favorable during the summer active season, N is the dominant limiting resource for both primary productivity and microbial decomposition. However, this paradigm is based on only a few previous studies finding that N fertilization stimulated carbon mineralization (via soil CO_2 respiration), increased microbial biomass and altered extracellular enzyme pools (Sistla et al. 2012; Wallenstein et al., 2009). The results of this study cast doubt on the universality of those previous findings, and suggest that N may not exclusively limit microbial activity throughout the active summer season and that N limitation may also vary across years.

This study was designed to address the extent of microbial N limitation over the course of the Arctic active summer season and to assess the significance of temporal variability that exists in these systems. Based on seasonal biogeochemical dynamics and intra-seasonal variability of soil nutrient availability (Weintraub and Schimel, 2005a; Gough 2002), there is potential for microbial N limitation to vary greatly over the course of a single active season. I hypothesized that MAT tussock soils collected early in the active season would respond differently to N amendments than soils collected at the peak of the active growing season. I expected N to be less limiting to microbial activity early in the active season when there is the potential for greater N

availability, and to be a more significant control on microbial decomposition later in the summer. In contrast, I found that low-level N amendments to MAT tussock soils did not stimulate any growth in microbial biomass (except for the 2010 peak season soils), consistent alterations in extracellular enzyme pools or significant stimulation of the average soil respiration rates, regardless of intra-seasonal timing. However, N amendments increased N immobilization rates and stimulated soil respiration for a brief period, with these effects seen more strongly in the early season soils. These results suggest that microbial responses to nutrient amendments do vary over the course of the active season and that early season soils potentially experience greater N limitation. Moreover, microbial responses to N amendments differed among the two consecutive study years, suggesting inter-annual variability in controls on nutrient cycling.

The general lack of microbial response to N amendments in this study contradicts the results of a previous similar study of MAT tussock soil collected near the peak of the active season in July which showed that N additions stimulated microbial biomass growth, increased C mineralization, and increased potential extracellular enzyme activity (Sistla et al. 2012). While Sistla et al (2012) found strong evidence of microbial N limitation for several microbial processes, the effects of N additions in my study were mostly limited to increased N immobilization and short term stimulation of C mineralization in the early season soils. The contradictory results of these two similar soil incubations indicate that microbial nutrient limitation may be dynamic and can exhibit large (annual) and/or small (daily) scale temporal variation. The significant differences seen in early season soils across two consecutive years could result from annually differential early season nutrient and/or substrate availability; this could also arise from small differences in sample timing relative to snowmelt between years in conjunction with rapidly shifting soil conditions and microbial communities.

The limited response to N amendments and seemingly random variability in this soil incubation may be a result of variability of other available soil nutrients. Labile C availability has been found to influence microbial responses to N additions, with greater nutrient immobilization in microbial biomass occurring with greater C availability in the Arctic tundra (Lavoie et al. 2011) and immobilization being strongly dependent on labile C availability in Arctic Heath systems (Jonasson et al. 1999b). Limited labile C availability has also been found to restrict microbial biomass growth in Arctic soils despite nutrient fertilization status (Lavoie et al. 2011; Jonasson et al. 1996, Jonasson et al. 1999a). With no consistent changes in microbial biomass C in my laboratory incubation and the greatest N immobilization seen early in the season, I speculate that limited availability of labile C can inhibit microbial utilization of added available N throughout much of the year.

Further supporting the conclusion of limited substrate and/or labile C availability, the early season soils also exhibited greater soil respiration rates across treatments and a greater respiration response to N amendments than the peak season soils. However, the early season soil respiration rates and N stimulation quickly declined during the incubation with all soil respiration rates converging by the end of the incubation. While the average soil respiration rates were slightly higher with N amendment, the N effects were not as significant as expected and were inconsistent with previous findings of strong N stimulation of C mineralization in laboratory incubations (Sista et al. 2012; Jonasson et al. 1996) and field studies (Mack et al. 2004). Greater average soil respiration rates and brief stimulation of respiration seen in the early season soils may be attributable to increased substrate availability induced by homogenization of the thawing early season soils. The soil homogenization process at the onset of our soil incubation may have increased substrate accessibility in the still thawing early season soils,

while the peak season soils were already depleted of available substrates. The seasonal pattern of respiration rates can also be explained by differential labile C availability. Other studies have found that the degree to which N stimulates respiration is controlled by the availability of labile C, and that N additions can actually inhibit C mineralization when labile C is limiting (Lavioe et al. 2011). The increased N immobilization and short term soil respiration seen in the early season soils are indicative or labile C and/or other soil substrates co-limiting microbial activities.

The lack of effects of N additions on extracellular enzyme production also suggests that other factors limit microbial activities. While previous studies of N effects on potential extracellular enzyme activity have concluded that N availability does influence microbial enzyme activity (Sistla et al., 2012; Wallenstein et al., 2009), there were no consistent effects of N fertilization on extracellular enzyme activity in this study. N amendments did sporadically alter potential enzyme activities, but with no consistent trends in direction, season, temperature, or year. The lack of consistent response in extracellular enzyme activity to N addition further supports the hypothesis of a co-limiting factor to microbial activity during the active season. Production of extracellular enzymes is not only N intensive, but also has energetic requirements which may not be able to be met without access to adequate labile C.

While many of the results of this study are contradictory to previous studies and indicative of microbial co-limitation, effect on N immobilization and microbial C:N stoichiometry were consistent with prior findings. The early season soil samples consistently responded to N amendments with lowered C:N ratios, but this response was not as consistent in the peak season soils. N additions have previously been shown to increase N immobilization rates and decrease C:N ratios of microbial biomass (Lavoie et al. 2011; Jonasson et al. 1996; Sistla 2012; Jonasson et al. 1999; Hole 2004) in laboratory and field studies, as I observed in this

study. While a common effect of Arctic soil fertilization studies, the mechanism driving lowered C:N ratios of biomass is not well understood. Altered stoichiometry of biomass could indicate stoichiometric flexibility of the existing microbial community (Sistla & Schimel, 2012) or a change in the relative abundance of bacteria and fungi (Strickland & Rousk, 2010). Existing microbes may possess enough biological flexibility to be able to store excess N within biomass for later utilization when soil conditions are more favorable. Conversely, increased N availability could alter microbial communities by allowing for greater growth of bacteria, which have a high N demand, over more C-rich fungal biomass. The consistently greater initial C:N ratios of microbial biomass which decline with incubation regardless of nutrient treatment indicates a shift in fungal:bacterial ratios. Altered microbial community composition within soils carries large implications for biogeochemical cycles due to functional and stoichiometric differences between fungi and bacteria (Strickland & Rousk, 2012). A better understanding of the mechanism driving greater N immobilization and reduced C:N ratios of microbial biomass under N amended conditions could help explain variability seen in microbial activities under nutrient fertilization and should be further studied.

I originally hypothesized that seasonal variation in soil N availability and corresponding variable microbial N limitation may be driven by soil temperature. Previous studies of biogeochemical cycling in Arctic tundra soils have found N cycling and microbial activity to be strongly affected by soil temperature, with increased soil temperatures potentially inducing greater N limitation. However, I did not observe any effect of temperature on microbial N utilization, growth, or immobilization in this study, except for a positive effect on microbial biomass in the 2010 peak season soils. The lack of temperature effects on microbial growth and N immobilization has also been previously observed in long term field fertilization studies

(Jonasson et al. 1999) and could be attributed to limited availability of labile C (Jonasson et al. 1996; sources). Potential extracellular enzyme activities also showed no consistent trends of temperature dependence. There were some significant differences seen across incubation temperatures, occurring more often in early season soils, although temperature effects were not consistent in direction or across years and specific enzyme type. Other studies have shown that temperature can affect in situ enzyme activities and that enzyme activities vary seasonally (Weintraub and Schimel, 2005b), however my results suggest that temperature is not a direct driver of enzyme production for my soils. The lack of consistent temperature effects in this soil incubation indicate that factors other than temperature and N limit microbial activity during much of the active season in the Arctic. Temperature appears to act similarly to any other resource, in that it does not stimulate microbial activity when other resources are limiting.

Lack of microbial response to N amendments in my incubations may be due to the short incubation time utilized of one week. It is possible that microbial exploitation of added N for growth and enzyme production may occur over a longer time frame than our incubation period. However, effects of N addition on microbial activities in similar soil incubations have been seen over the short time period of 2 weeks (Sistla et al. 2012) and the greatest N stimulation of soil respiration in my incubations occurred immediately after the soil amendments and dissipated within a few days.

In addition to conflicting results possibly arising from temporal variability and co-limitation, site-based variability could also potentially explain incompatible findings of microbial nutrient limitation. Much of the existing research suggesting microbial N limitation in MAT soils has originated from areas near the Toolik Lake Research Station in Alaska; with research conducted out of the Arctic Long Term Ecological Research sites at Toolik Lake contributing to

a large portion of the current understanding of Arctic ecosystem ecology. Despite unique soil properties supporting the assumption of uniformity within MAT tussock soils, the lack of apparent N limitation in soils collected from Imnavait Creek presents the possibility of site driven variability in microbial function and nutrient limitation. The soils utilized in my incubations were collected from a site of close physical proximity which shares similar topography, climate, and vegetation as the more extensively studied MAT tussock soils near Toolik Lake, AK. Despite similar soil properties and climate, contrasting observations of microbial N limitation indicate that site-based variability within these soils may be more prevalent than previously thought. The potential for site-based variability with a singular Arctic soil type has never been directly addressed and is poorly understood. Limited accessibility of Arctic regions has led to information arising from a few locations and time points being extrapolated to much larger areas and time scales. However, this extrapolation of data may be inaccurate and misleading in light of the possibility of significant, unexplainable temporal and/or site based variability.

Large temporal variability in microbial responses to added N, which cannot be attributed to soil temperature effects, demonstrates the need for a better understanding of the factors controlling Arctic soil microbial activities. These results support co-limitation of microbial activities by labile C for portions of the active season and may indicate the existence of site-based variability as well. The current paradigm of N limitation in Arctic ecosystems needs to be refined to reflect variability and include potential co-limitation by other resources. A greater comprehensive knowledge of active season microbial dynamics and nutrient limitation is needed to effectively predict the fate of large Arctic SOC stores.

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CHAPTER 3

CO-LIMITATION OF MICROBIAL ACTIVITY IN ARCTIC TUSSOCK SOILS BY CARBON AND NITROGEN DIFFERS AMONG NEARBY SITES

Introduction

The large amount of soil organic matter (SOM) contained in high latitude areas (Tarnocai et al., 2009) coupled with the disproportionate effects of climate change and potential for conflicting feedbacks (McBean et al., 2005; Anisimov et al., 2007) complicates predictions of the vulnerability of Arctic SOM to increased decomposition. Much of the SOM contained in Arctic systems is thought to be readily mineralizable (Weintraub & Schimel, 2003; Shaver et al., 2006), but has historically been protected from decomposition by inhibitive physical environmental conditions. Slow decomposition has in turn resulted in extremely nutrient poor soils, creating a negative feedback to SOM decomposition. Limited decomposition, low soil N availability (Hole, 2004; Hobbie and Gough, 2002; Weintraub and Schimel, 2005), and low N mineralization rates (Marion and Miller, 1982) have made these systems extremely N limited. N amendments have been shown to increase primary productivity and alter plant community composition (Chapin et al. 1995; Shaver et al., 2001; Jonasson et al., 1999b). N fertilization has also been shown to increase microbial immobilization and biomass (Sistla et al., 2012; Jonasson et al., 1996; Jonasson et al., 1999a; Jonasson et al. 1999b; Lavoie et al., 2011), alter extracellular enzyme activity (Wallenstein et al. 2009; Sistla et al., 2012, Marklein et al. 2012), and increase decomposition rates (Mack et al., 2004; Sistla et al., 2012).

Altered climatic conditions are not only reducing physical inhibition of decomposition, but also threaten to ease nutrient limitation of microbial activities. Climate warming may result

in increased soil N availability resulting from greater annual mineralization (Elberling, 2007; Schimel et al. 2004) while also potentially increasing and/or altering distribution within the soil profile of inputs of labile compounds into the rhizosphere (Drake et al., 2011; Phillips et al., 2011). The C and N biogeochemical cycles of Arctic ecosystems are tightly coupled, and labile C availability has been shown to be an important factor in the response of microbial activities to nutrient amendments. Climate change has the potential to simultaneously alter soil C and increase N availability in Arctic tundra systems, however little is known about how these nutrients may interrelate to affect microbial activities and decomposition.

Despite the large amounts of soil organic carbon (SOC) contained in Arctic tundra soils, microbial activities may still be limited by the availability of labile, C-rich compounds. Labile, low molecular weight compounds are passively released into the soil by plant roots undergoing active growth, and constitute the most important C source for soil microbes during the active summer season (Loya et al. 2004). Exudates are assimilable by soil microbes without utilization of extracellular enzymes (Bremer and van Kessel, 1990; Bremer and Kuikman, 1994) and preferential use of carbon provided through root exudation may inhibit decomposition and alter nutrient partitioning (Loya et al. 2004; Schmidt et al. 2012). C additions may also induce localized extreme microbial N limitation, prompting increased decomposition of N-rich proteins (Weintraub and Schimel, 2005) and greater net N mineralization (Phillips et al., 2011; Drake et al., 2011). Additionally, synthesis of microbial biomass has been shown to be dependent on labile C availability (Jonasson et al., 1996). These findings suggest that inputs of labile C may be able to influence microbial driven biogeochemical cycles and drive microbial nutrient limitation status.

The potential for climate change to simultaneously alter N and labile C availability in Arctic soils necessitates a better understanding of how these interactively influence soil microbial activities. Knowing how labile carbon may influence microbial activity and microbial N limitation is crucial to fully understanding how increased early season N may alter seasonal ecosystem nutrient dynamics of Arctic tundra soils. Coupling of N and C biogeochemical cycles in Arctic tundra soils can potentially result in co-limitation of decomposition, however the currently understanding of how N and labile C interact to influence soil microbial processes is lacking.

My previous study found temporal and the potential for intersite variability of microbial nutrient limitation (Chapter 2). Physical climatic conditions are being uniformly altered by climate change across large Arctic regions, however the potential for microbial driven site-based variability of biological responses to these changes remains largely unknown. Due to the remote nature and inaccessibility of much of the Arctic, most research and knowledge synthesized regarding Arctic tundra ecosystems has originated from relatively few research locations. However, conflicting findings of microbial responses to N amendments and poorly understood drivers of Arctic soil microbial nutrient dynamics (Chapter 2) indicate that nutrient limitation of microbial activities may be more variable than the current paradigm would suggest.

The vast global expanse of moist acidic tundra has similar climate and vegetative community composition, however areas in close physical proximity may differ in other soil formation factors. Soil chronology is a prominent factor in formation and function of soils (Jenny, 1941) and varied glacial geologic histories have resulted in a large range of effective soil ages across the Alaskan Arctic Tundra (Hamilton, 2002). Soil chronology may be an important factor influencing Arctic soil microbial ecology; significant differences in decomposition and

microbial activities have been observed between MAT and non-acidic moist tundra (NAMT) sites physically close together but with very different glacial geologic histories (Hobbie and Gough, 2004; Hobbie et al., 2002; Wittinghill and Hobbie, 2011). Differences in decomposition across sites could not be fully explained by variability in soil properties or vegetation (Hobbie and Gough 2002) suggesting that differential microbial processes may be driving site-based differences in decomposition rates.

While glacial geology/soil chronology has been considered across differing soil types, the unique features of MAT tussock soils would suggest greater homogeneity among sites regardless of glacial geology and chronological differences. MAT systems are dominated by the tussock forming sedge *Eriophorum vaginatum L*. Soils sampled directly below *Eriophorum vaginatum* (tussock) vegetation are highly organic and composed of biomass in various stages of decay (Figure 3.1). The self-forming properties of MAT tussock soils and consistent nutrient content of tussock vegetation across sites (Hobbie and Gough, 2002) raise the question of whether the microbial communities and function in these soils are dictated primarily by vegetation and uniform across sites. This question of uniformity within MAT tussock soils across sites has never been directly addressed. Arctic tundra soils occupying small geographic areas can possess widely varied glacial geologic histories which allow for experimental control of topography, vegetation, and climatic conditions, while maintaining different effective soil age. This allows for

Figure 3.1. Homogenized MAT tussock soil. Soil structure and formation is driven by the tussock forming *Eriophorum vaginatum L.* vegetation. Photograph of approximately 25 x 25 cm area of soil.

examination of soil microbial nutrient limitation across sites of varied glacial histories with limited variability of other site factors. Site based variability of microbial activities and nutrient limitation may provide insight into how glacial history may influence microbial driven biogeochemical cycles.

My primary objective was to determine how labile C alters microbial N limitation and utilization in early season MAT tussock soils. I incubated early season soils for two weeks in a factorial laboratory experiment with N and glucose (a labile C source) amendments. By utilizing soils from two similar sites in this incubation, I was able to assess whether these effects were consistent among two sites in close geographic proximity which experience similar climate, topography, and vegetation. I hypothesized that labile C availability would alter microbial responses to increased N by allowing for greater growth of biomass and stimulating C mineralization. Due to the homogenous properties of MAT tussock soils I anticipated that the microbial response to N and labile C treatments would be similar across sample sites.

Methods

Site descriptions

Soil samples were collected from two MAT sites near the Toolik Lake Research Station on the north slope of the Brooks Range in Alaska. Samples were collected from a designated destructive harvest area within the Arctic Long Term Ecological Research (LTER) site adjacent to Toolik Lake (68° 38'N / 149° 34'W) and from a nearby site east of Toolik Lake at Imnavait Creek (IMN) (68° 37' 37"N, 149° 19' 11"W). The soil dynamics of the LTER site have been well studied and previously described (Mack et al., 2004; Sistla et al., 2012; Weintraub & Schimel, 2005a; Hobbie et al., 2002; Hobbie & Gough, 2004), while there is little research originating out

of the IMN site (Chapter 2). The two sample sites have different glacial geologic histories with the LTER site occupied by the Itkillik I glacial drift approximately 50,000-120,000 years ago while the IMN site was occupied by the Sagavanirktok glacial drift occurring 120,000-600,000 years ago (Hamilton, 2002). Glacial histories of Arctic soils directly influence soil development and Arctic glacial geology can be used as a proxy for soil chronology (Hobbie & Gough, 2002; Hobbie et al., 2002; Hobbie & Gough, 2004; Wittinghill & Hobbie, 2011), as it is in this study. Despite differences in glacial geology, the two sites share similar climate, vegetation, topography, and parent material. Both sites consist of typical MAT tussock vegetation which is dominated by the tussock forming sedge *Eriophorum vaginatum L*. with mosses, deciduous shrubs, graminoids, and evergreen shrubs found between tussocks. Globally, MAT covers approximately 900,000 km^2 (Oechel et al., 2003), with areas of tussock dominated vegetation similar to our research sites found throughout the Northern Slope of Alaska, northern Canada, and eastern Siberia (Bliss and Matveyeva, 1992).

Sample collection, preparation, and storage

Early season MAT tussock soil samples were collected at both the IMN and LTER sites shortly after snowmelt in the spring of 2012. Both IMN and LTER soils were sampled 5/29-5/30/12 when the soils first began to thaw at the start of the active season. Samples were collected by removing entire tussocks and the soil below from randomly chosen locations within destructive harvest plots at both field sites. Whole tussocks were cut from the ground by hand with large serrated knives to a depth of about 25 cm or until solid frozen ground was reached. Approximately 5 tussocks were sampled from each site. Upon sampling, all aboveground material, thatch, live roots, and shrub roots were removed and soils from each site were then homogenized by hand resulting in one homogenous soil sample from each sample site.

Immediately after homogenization, soils were frozen and shipped to the Natural Resources Ecology Laboratory at Colorado State University. Upon arrival they were stored frozen at -20^{o} C for approximately 1-2 months prior to the soil incubation beginning.

Incubation Set-up and Soil Treatments

Prior to the start of the soil incubation soils were thawed refrigerated at about 5 ^{o} C for approximately 24 hours. Once thawed, each soil type from the two sample sites (IMN and LTER) were subsampled by weighing out 50 g subsamples into 1 pint glass mason jars secured within ½ gallon glass mason jars. Prior to the start of the incubation, soils were weighed into the incubation jars and loosely covered, then left for a pre-incubation period of 3 days at the temperature of 10 ^{o} C to allow for any spike in soil respiration due to disturbance to subside. The soil incubation was run in two rounds because of laboratory limitations. Each soil incubation round was maintained for a total of 12 days, running from 7/4/12-7/16/12 and 7/18/12-7/30/12. Each round of the incubation included 3-4 laboratory replicates of each treatment and combination. The soil incubation was set-up in a factorial design with treatments of amended N, amended glucose (labile C source) and DI water as controls. 50 g of field moist soil were weighed into the jars, half received a dropwise addition of 1 ml of NH_4NO_3 solution containing 450 μg NH_4NO_3 (equivalent to 112.5 μg N g^{-1} dry soil in 1ml of water) or 1 ml of DI water at the onset of the incubation. After the N treatment was applied, the large ½ jars were sealed for the duration of the soil incubation. Microlysimeters were placed to fit through the center of the jar lids allowing for them to be inserted into the center of the incubating soils (Figure 3.2). The Microlysimeters were injected daily for the first 10 days with 1 ml of glucose solution providing 144 μg C g^{-1} dry soil or 1 ml or DI water for a control. Jars were incubated over 12 days at 10 ^{o} C with daily C amendments and respiration readings and flushing of the gas in the jar's headspace

Figure 3.2 Soil incubation jars with microlysimeters implanted in incubating soils allowing for daily C additions simulating the delivery method of biologic root inputs of labile C. Septa in the lid allowed for respiration readings and flushing of the head space without opening the jars and disturbing the soils or mycrolysimeters.

approximately every 3-4 days. Soil analyses were conducted with the initial samples obtained after the completion of the pre-incubation period and the post-incubation samples were harvested immediately upon the completion of the incubation.

Soil Respiration

Cumulative soil C respired was measured approximately every 3-4 days for the duration of the soil incubation. Gas samples from each sealed jar were obtained by syringe inserted through the rubber septa of the jar lids and injected into an IRGA. After CO_2 concentrations were read, the gas within the sealed jars was flushed by continuously injecting CO_2 free gas scrubbed by soda lime; jars were flushed to prevent excessive buildup of CO_2 which could eventually inhibit microbial activity. Jars were flushed for about 30 minutes and then sampled again to determine the CO_2 concentrations of each incubation jar post gas flush. The jars remained sealed and air tight for the entire duration of the soil incubation.

Soil Nutrients and Microbial Biomass

Soils (5 g subsamples) were extracted in 25 ml 0.5 M K_2SO_4, shaken for 1 h, and vacuum filtered to determine total organic carbon (TOC), total extractable nitrogen (TN), ammonium (NH_4), nitrate (NO_3), and total free amino acids (TFAA). Soil extracts were analyzed for TOC and TN by the Ecosystem and Soil Ecology Laboratory at the University of Toledo on a

Shimadzu TOC/N instrumental analyzer. Soil NH_4 content was determined using a colorimetric microplate assay (Rhine et al., 1998), as was NO_3 (Dopane and Haorwath, 2003), and TFAA were measured using a fluorometric assay (Darrouzet-Nardi et al. 2013). Microbial biomass was determined utilizing a method of direct chloroform addition to the study soils described by Scott-Denton et al. (2006) and Weintraub et al. (2007). 5 g soil subsamples with 2ml of ethanol free chloroform added were kept in capped flasks for 24 hours, followed by K_2SO_4 extraction as described for TOC methods. Microbial biomass C and N were determined by calculating the difference in TOC and TN content between the chloroform exposed and corresponding unexposed K_2SO_4 soil extracts. Additional C and N in the fumigated soil extracts is assumed to have come from lysed microbial cells and attributed to microbial biomass content. No extraction efficiency correction factor (e.g. K_{ec}) was used in biomass calculations. Gravimetric soil moisture was determined by drying 5 g field moist soil from each experimental sample at 60^{o} C for 48 hours, and nutrient data is reported on a dry soil weight basis.

Potential Enzyme Activities

Soil enzyme assays were conducted to determine the potential activity of the hydrolytic enzymes b-1,4-glucosidase (BG), cellobiohydrolase (CB), b-1,4-N-acetylglucosaminidase (NAG) and phosphatase (PHOS), leucine aminopeptidase (LAP), and the oxidative enzyme phenol oxidase (PO) (Table 3.1).

Table 3.1 Assayed extracellular enzymes with their abbreviations and general functions.

Enzyme	Abbreviation	Function
β-Glucosidase	BG	Sugar degradation
β-D-Cellubiosidase	CB	Cellulose degradation
Lucine Aminopeptidase	LAP	Protein degradation
N-acetyl-Glucosaminidase	NAG	Chitin degradation
Phosphatase	PHOS	Phosphorus mineralization
Phenol Oxidase	PO	oxidation of phenolics

Each sample was homogenized by adding 2.75 g field moist soil to 91ml of 50mM sodium acetate buffer at a pH of 4.5 and blending for a total of 2 minutes. Hydrolytic enzymes were assayed following the protocol outlined in Steinweg et al. (2012) with some minor modifications. Substrates used were labeled with the fluorescent indicators of 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC) and the substrates (with their target enzymes) utilized were: 4-MUB-β-D-glucopyranoside (BG), 4-MUB- β -D-cellobioside (CB), 4-MUB-N-acetyl- β -D-glucosaminide (NAG), 4-MUB phosphate (PHOS), L-leucine-7-amido-4-methylcoumarin hydrochloride (LAP). All substrates were added well in excess and were of a 200 μM concentration. A three point standard curve for the fluorescent markers was produced using concentrations of 0μM (DI water), 100 μM, and 200 μM for MUB and 0 μM, 2.5 μM, and 5 μM for MC which is used to determine the very minute LAP concentrations in these soils. The plates were incubated at 10^{o} C for approximately 4 hours after which 250 μM of sample from each of the deepwells was transferred into the corresponding wells of black 96 well microplates. The microplates were then read for fluorescence on a Bio-Tek Synergy HT Microplate Reader with settings of: excitation= 360/40, emissions=460/40, and sensitivity=46.

The oxidative enzyme phenol oxidase (PO) was assayed utilizing the substrate of 2,2′-azinobis-(-3-ethylbenzothiazoline-6- sulfononic acid) diammonium salt (ABTS) to detect PO activity. Assays were conducted according to the protocol outlined by Floch et al. (2007) which has been shown to be as effective as other oxidative enzyme methods (German et al. 2011). The same soil slurry was used for both the fluorometric and oxidative enzyme assays. The soil slurry for one sample was pipetted into two adjacent columns of a deepwell plate with each well receiving 800 μl of slurry. The first two columns of each plate were filled with buffer only with 5 soil samples dispensed into the remaining 10 columns. For each sample, one column received the

addition of 200 μl of 2mM ABTS and the other 200 ul of buffer as a control. Once the soil slurry and ABTS substrate were added to the deep well plates they were covered with parafilm and gently swirled by hand to mix. The plates were incubated at 10^{o} C for approximately 4 hours after which 250 μM of sample from each deepwells was transferred into the corresponding wells of black 96 well microplates. The microplates were then read for absorbance on a Bio-Tek Synergy HT Microplate Reader at a wavelength of 420nm.

Statistical Analysis

Results were analyzed using a mixed-effects ANOVA in SAS software utilizing the PROC MIXED procedure with fixed effects of soil site, glucose amendment, and N amendment and random effects of block/sample plot. Some data sets were log transformed prior to statistical analysis to normalize the data distributions. Statistical significance was determined at $p \leq 0.05$.

Results

Cumulative Soil Respiration/C Mineralization

The IMN and LTER soils had similar cumulative respiration over the two week long incubation (Figure 3.3). The only treatment in which cumulative respiration differed between the two soil types of same treatment occurred was the glucose and N treatment, with the IMN soils having greater respiration than the LTER soils ($p=0.015$).

N--only amendments increased soil respiration 20% ($p=0.020$) for IMN soils and 11% for LTER soils ($p=0.170$). Glucose amendments stimulated IMN respiration 54 % ($p <0.0001$) over the control and LTER respiration 44% ($p <0.0001$) over the control. IMN soils had the greatest respiration with the combined N and glucose treatment which was 69% greater than the control,

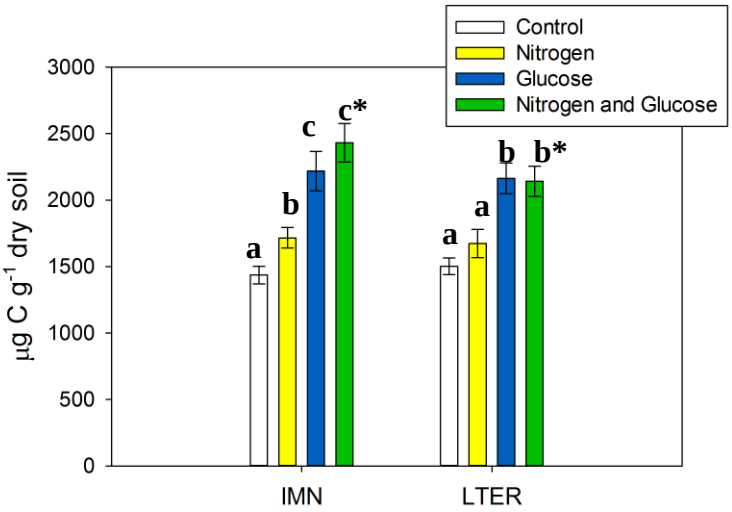

Figure 3.3 Mean (±1 s.e.) of cumulative C respired during soil incubation for IMN and LTER soils. Letters at the top of bars denote significance with individual soil type only. Asterisks denote significance across soil types of the same treatment; P≤0.05

although not significantly greater than the glucose only treatment. The cumulative respiration of the LTER soils treated with glucose and N was the same as soils amended with glucose only.

Microbial Biomass

Control soils from both sites had similar microbial biomass C and N content (Figure 3.4).

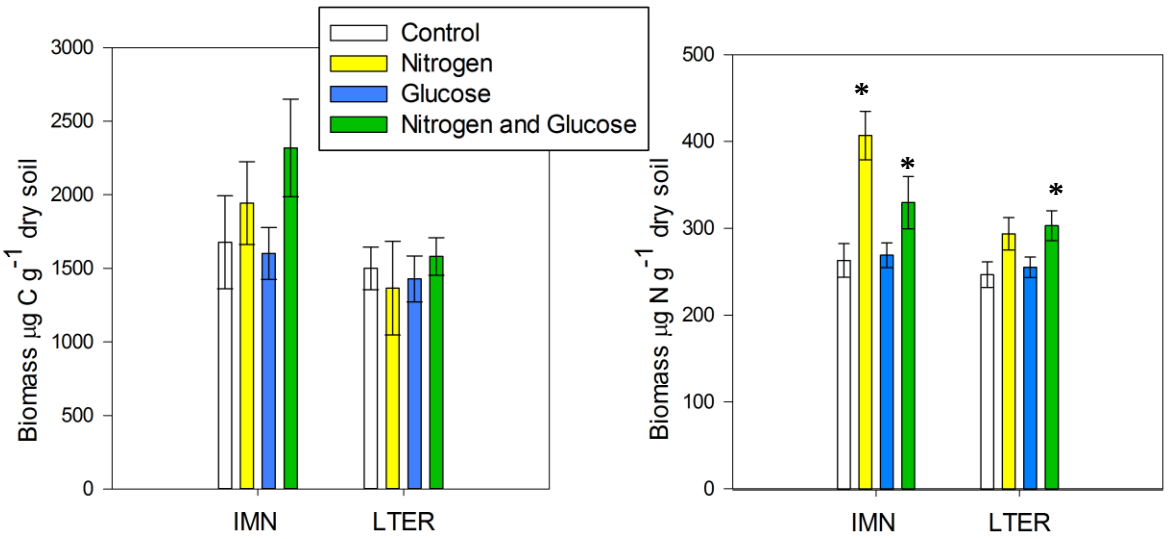

Figure 3.4 Mean (±1 s.e.) of microbial biomass C (left) and microbial biomass N (right) for IMN and LTER soils post incubation. Asterisks (*) above bars denote significance from the corresponding control; P ≤0.05.

IMN soils had a 16% increased microbial biomass C with N amendments only (p=0.379) and the

greatest growth of biomass C with N/glucose treatments which increased 38% (p=0.079). Glucose treatments did not affect microbial biomass C in IMN soils. Microbial biomass C of the LTER soils was not affected by any of the applied treatments.

All N additions, regardless of soil site and glucose amendment, increased microbial biomass N (Figure 3.4). IMN soils had the greatest change in biomass N when amended with N only, with biomass N increased by 55% (p-value: <0.0001), while N/glucose treatments resulted a smaller increase in biomass N of 38% over the control (p=0.014). The N only and N/glucose amendments to LTER samples resulted in similar increases in biomass N, with 19% (p=0.065) and 23% (p=0.027) increase over the control respectively. Glucose amendments alone did not alter microbial biomass N in either soil. The N content of microbial biomass in the two soil types had the greatest differential response to N only treatments. Immobilization of N in microbial biomass was much greater in the IMN soils than the LTER soils when amended with N only (p=0.001).

In order to address stoichiometric aspects of soil microbial biomass, the ratio of C:N of biomass was considered (Figure 3.5). Despite growth of biomass C with N amendment in the IMN soils, N immobilization resulted in a decrease in the ratio of C:N ratio of microbial biomass. None of the other treatments altered the C:N ratio of biomass in the IMN soils. LTER soils also showed a decrease in the C:N ratio of microbial biomass with N

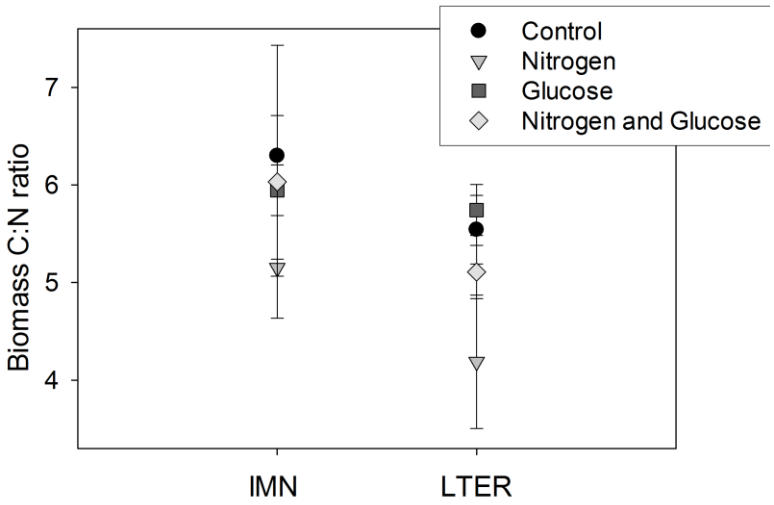

Figure 3.5 Mean (±1 s.e.) of the C:N ratio of microbial biomass post incubation in IMN and LTER soils.

amendment, there was a trend of decreased C:N of biomass with the N/glucose treatment as well. None of the reductions in biomass C:N were significant from the corresponding control at the 0.05 level.

Potential Enzyme Activities

Six individual extracellular enzymes were assayed (Table 3.1). LTER soils had greater activity of C degrading enzymes than IMN soils (Figure 3.6). LTER soils had increased BG

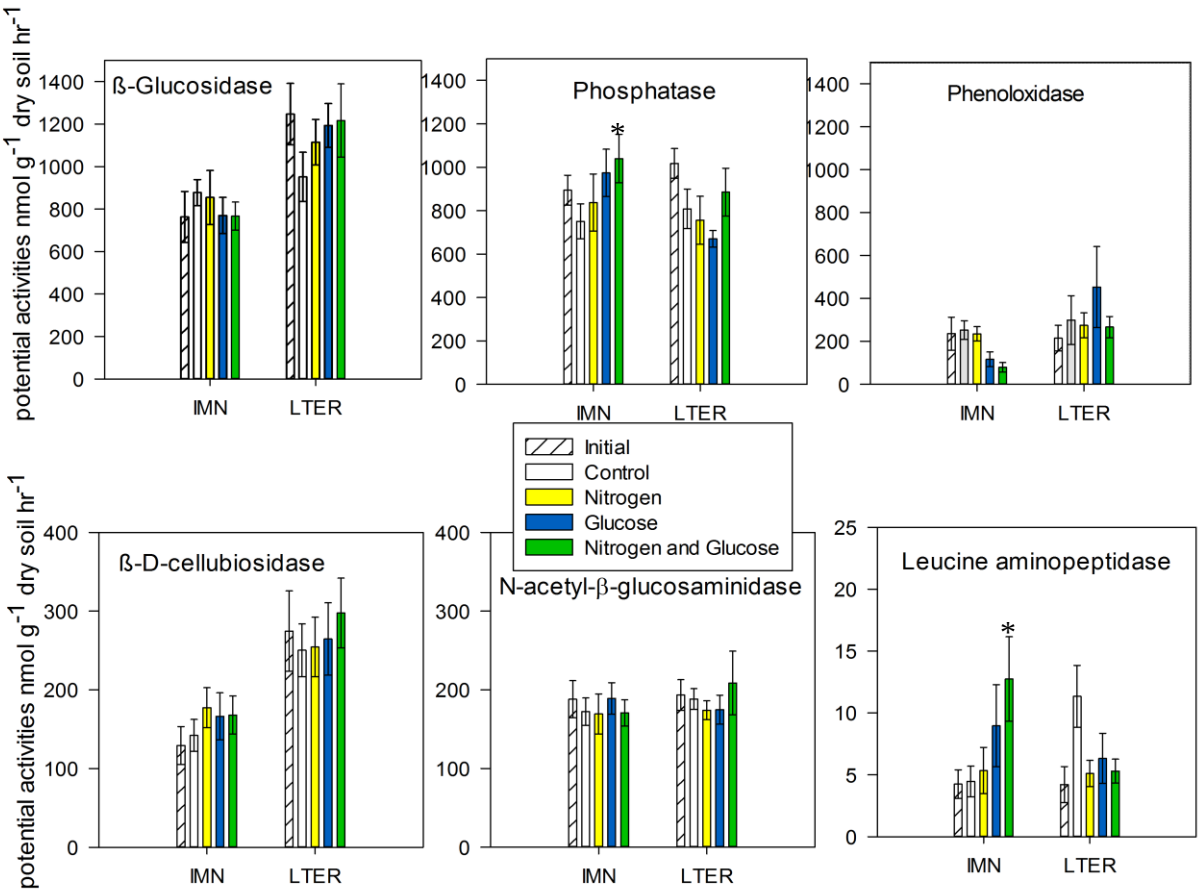

Figure 3.6 Mean (±1 s.e.) of potential enzyme activities pre (initial) and post incubation for IMN and LTER soils. Asterisks (*) above bars denote significance from the corresponding control; P-value ≤0.05.

activities with all treatment amendments and increased CB activities under the combined N and glucose treatment. Potential BG activities of IMN soils were unresponsive to soil amendments while CB activities were slightly increased with amendments. All amended LTER soils had

greater C acquiring enzyme activities (BG and CB) than the control soils, although not statistically significant. Potential NAG activities of IMN and LTER soils were fairly consistent across soils and treatments and no significant effects of nutrient amendments were seen. IMN soils amended with glucose had insignificantly increased LAP activity and the combine N and glucose treatment doubled LAP activities ($p=0.014$). LAP activity of LTER soils was slightly decreased from the control with all fertilization treatments, although the LTER control had much greater LAP activity than the IMN control. IMN soils had trends of increasingly greater potential PHOS activity with N, glucose, and combined N and glucose treatments respectively with the combined N and glucose treatment being significantly greater than the control ($p=0.051$). LTER soils had decreased PHOS measured in the glucose treated soils and slightly increased potential PHOS was seen with both N and C treatment.

Ratios of extracellular enzymes targeting C- and N –rich compounds provides an index of microbial nutrient demand. The ratio of BG and CB to NAG and LAP was used to indicate the ratio of C acquiring enzymes to N acquiring enzymes and represents relative C:N demand of soil microbes (Figure 3.7). IMN soils responded to glucose and N/glucose amendments with a decrease in C demand relative to N (decreased C:N enzyme ratio). Glucose-only additions resulted in the greatest decrease in the IMN enzyme C:N ratios, although not significant There was no shift in the enzymatic C:N acquisition ratio with N-only amendments in the IMN soils. The LTER soils responded differently than the IMN soils, with trends of increased C:N enzyme ratios with all nutrient amendment treatments, and significantly increased enzymatic C:N over the control with glucose only ($p=0.002$).

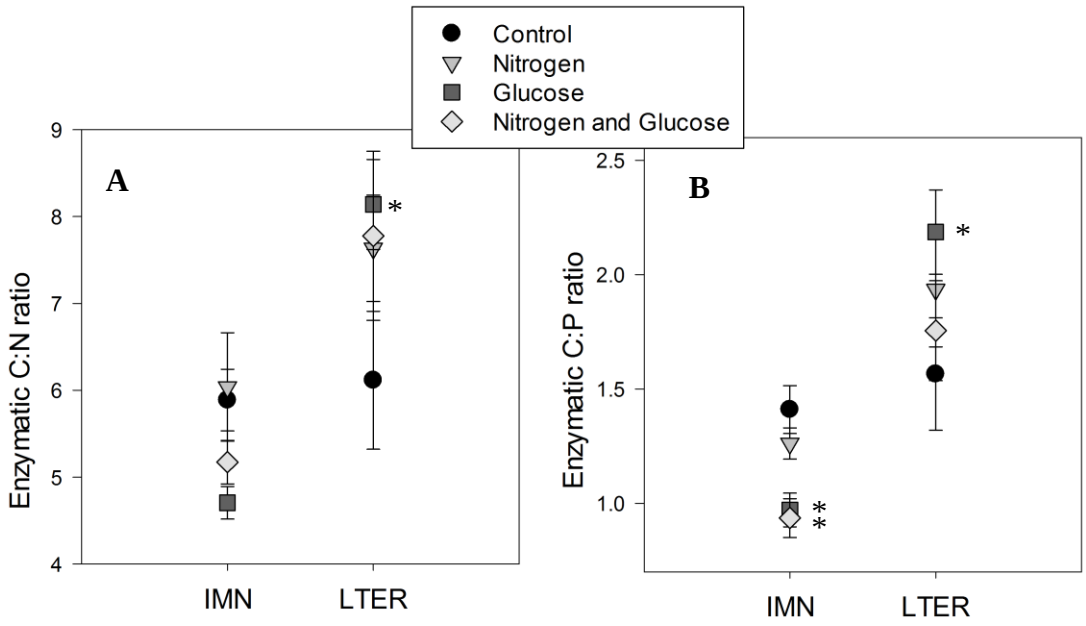

Figure 3.7 A: Mean (±1 s.e.) of the ratio of C:N acquiring enzyme activities post incubation for IMN and LTER soils. Assayed potential activities of C degrading BG and CB and N degrading NAG and LAP were used to calculate enzymatic C:N rations.
B: Mean (±1 s.e.) of the ratio of C:P acquiring enzyme activities post incubation for IMN and LTER soils. Assayed potential activities of C degrading BG and CB and P mineralizing PHOS were used to calculate enzymatic C:P rations. Asterisks next to symbols denote significance from the corresponding control; P ≤0.05.

Ratios of extracellular enzymes targeting C-rich compounds (CB and BG) and mineralizing phosphorus (PHOS) provides another index of microbial nutrient demand (Figure 3.7). IMN soils had a slightly decreased C:P enzymatic ratio with N amendment only and larger, significantly decreased ratios with the glucose and N/glucose treatments (p= 0.041 and p=0.021 respectively). LTER had trends of increased enzymatic C:P ratios with all nutrient amendments, with the glucose treatment yielding the greatest increase for the LTER soils (p=0.021).

Discussion

The current paradigm of Arctic ecosystem ecology posits that both aboveground productivity and belowground activity are primarily N limited for the duration of the active summer season. However, observations linking Arctic soil microbial activity and N utilization to

other soil factors (Jonasson et al., 1999a) and intra-seasonal and annual variability of soil N availability and microbial response to N amendments (Chapter 2) indicate that the current understanding of active season belowground biogeochemical cycling needs to be refined. Contradictory effects of N amendments on microbial activity in these nutrient poor systems and tightly coupled biogeochemical cycles suggest that labile C availability may be an integral determinate of how soil microbes utilize soil N sources. I hypothesized that microbial activity in MAT tussock soils is strongly co-dependent on labile C availability and microbial utilization of N is influenced by labile C inputs. I found that labile C additions influenced microbial activities and altered utilization of added N. C mineralization and microbial biomass C of IMN soils exhibited signs of N limitation as well as strong co-limitation of labile C. For example, N amendments slightly increased soil respiration and microbial biomass, while combined N and labile C inputs strongly stimulated both soil respiration and growth of microbial biomass C. Additionally, labile C inputs reduced microbial N immobilization in these N fertilized soils. Microbial responses to N and C additions differed between the "older" IMN soils compared to the "younger" LTER soils suggesting that nutrient limitation of microbial activities does vary across sites. Respiration of LTER soils was only slightly increased by N amendment and showed no combined effect of N and labile C, while microbial biomass was not significantly increased under any treatment. N immobilization was also found to be independent of labile C availability in these soils. The responses to nutrient treatments suggest that labile C availability can alter microbial response to increased N and that belowground nutrient limitation may be more variable across sites than expected.

The effects of N and C additions on extracellular enzyme activities were also different between the sites, further suggesting intersite differences in microbial resource limitation.

Despite similar enzymatic C:N and C:P ratios of the control soils, when provided additional N and/or labile C, LTER soils exhibited greater potential activity of C acquiring enzymes relative to N and P acquiring enzymes. IMN soils exhibited an opposite response to labile C additions with greater activity of N and P degrading enzymes relative to C acquiring enzymes and enzymatic ratios did not shift with N-only additions. Microbial communities from the LTER soils appeared to consistently devote greater resources to promote C acquisition whereas the IMN soils responded to added labile C with reduced C acquisition activities relative to N and P and no response to N. The lack of N effects on enzymatic ratios of IMN soils suggests that available substrates may be limiting. Additionally, the differential effects of N amendments seen with and without glucose amendment of IMN soils further support co-limitation. The opposite effects of amendments on enzymatic C:P ratios indicates that P may be more limiting at the older IMN site and additional resources may stimulate greater P mineralization, while P mineralization of LTER soils may decline with additional resource availability. The consistently greater C acquiring activities of LTER soils with amendments are indicative of greater microbial C demand and/or greater C limitation relative to other nutrients at the LTER site (Allison, et al., 2011).

Despite strong site differences in microbial nutrient limitation, C amendments induced similar responses of microbial biomass and C mineralization for both soils. Microbial biomass was unaltered by additions of labile C suggesting that microbial biomass synthesis is not limited by labile C availability alone. Additionally, greatest increases in soil respiration were attributable to labile C inputs in both soils. The increase in C mineralization may be due to an increase in microbial waste respiration occurring with regular soil inputs of labile C (Schimel and Weintraub, 2003). This contradicts findings of a similar soil incubation of soils near my LTER

sample site in which labile C additions did not stimulate C mineralization (Sistla et al. 2012). This discrepancy could be ascribed to the method of soil amendment. In an attempt to simulate the delivery method of the most significant source of soil labile C inputs, I designed C additions to simulate the delivery method of root exudates and provided daily C amendments. The repeated delivery of small C inputs into the soil "rhizosphere" could have allowed for greater C mineralization than a single large C addition would. The prolonged fertilization method may have may have resulted shifts of microbial community composition and the proliferation of microbial communities with higher C maintenance demands (Bird et al., 2011).

The large stimulation of C mineralization with labile C amendments carries significant implications when considering larger ecosystem processes. The greatest source of labile soil C during the Arctic active season is from vegetative root exudation of low weight molecular compounds (Loya et al. 2004). Inputs of labile C provided via root exudation are dependent on the vegetative species as well as physical conditions such as atmospheric CO_2 and soil N availability (Phillips et al., 2011; Drake et al., 2011); all of which are also vulnerable to being influenced by altered climatic conditions. The potential of climate change to alter soil N processes and labile C inputs simultaneously could have significant repercussions on belowground processes. These types of interactions should be better understood and incorporated into analyses assessing the vulnerability of Arctic C stores to climate change.

The two sample sites of this study were selected for their uniformity of soil properties and differing glacial histories. While potential differences within a single soil type have not been previously addressed, biogeochemical cycles along glacial geologic gradients in Alaska have been previously examined across differing soil types. There is greater annual N mineralization, soil respiration, and rates of decomposition in older MAT soils when compared to younger non-

acidic moist tundra (NAMT) sites (Hobbie et al. 2002; Hobbie & Gough 2004). These functional soil differences cannot be fully attributed to site differences in vegetation, soil moisture, or soil temperature (Hobbie & Gough 2002), but may be explainable by variability of microbial nutrient limitation between sites. By collecting soils directly below tussock vegetation, which maintain consistent foliar nutrients regardless of soil conditions (Hobbie & Gough 2002), I was able to exclude the effects of intersite differences in plant community structure. After controlling other factors, one primary remaining difference between sites is glacial geology, although intersite differences may be driven by other soil factors not considered in this study. Based on glacial geology, my results may suggest that older Arctic MAT soils may have greater N limitation and that labile C inputs may be a greater determinant of microbial N utilization at older soil sites. Additionally, the responses of potential extracellular enzyme activities to C and N additions may indicate that older MAT microbial communities devote greater resources to active degradation of N and P mineralization while younger MAT microbial communities invest more heavily C acquisition. Long-term field studies of younger MAT at the LTER site have shown significant loss of SOC under fertilized conditions (Mack, 2004) which could be explained by my results of greater activities of C acquiring enzymes at the LTER site. As MAT develops through time, remaining SOC may become more recalcitrant and N limitation and labile C availability become more significant controls of microbial activity. Additionally, previous studies concluding that Arctic SOM is largely mineralizable (Weintraub & Schimel, 2003; Shaver et al., 2006) were also conducted near the younger LTER site and may not hold true for older MAT sites.

While Arctic tundra covers large geographic areas globally and stores disproportionately large amounts or SOC, most of the knowledge of Arctic ecosystem ecology has been synthesized from relatively few study sites. Intersite differences in N limitation and labile C co-limitation

have important implications for larger ecosystem processes. Attempts to determine how altered climatic conditions may influence Arctic belowground C sequestration using data extrapolated from studies of a limited spatial scope is questionable given the observed intersite variability of my study. The ability of climate change to differentially influence microbial decomposition through alteration of multiple factors which may have site-specific responses necessitates a better understanding of belowground controls and variability in nutrient limitation of microbial activities.

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CHAPTER 4

CONCLUSIONS

The current paradigm of Arctic ecosystem ecology suggests that active season productivity and microbial activities are primarily N limited. My thesis research reveals that temporal and intersite variability is more significant in Arctic tundra systems than previously thought and that N limitation may not be as universal in these systems as previous studies indicate. I began my thesis trying to assess intricacies of microbial N limitation and drivers of microbial N cycling in MAT tussock soils and found that Arctic microbial activities and response to nutrient amendments seem to be intrinsically variable.

Chapter 2 of my thesis attempted to assess how N limitation of microbial activities may vary from the onset of the active season to the peak season when ecosystem N demand is greatest. I also wanted to assess whether seasonal variability of microbial N limitation was driven by soil temperature altering microbial processes. I found that there were indeed significant differences in early and peak season microbial activities and response to N amendment, but contradictory to other studies, did not find extracellular enzyme activities, microbial biomass, or long term C mineralization to be N stimulated. Instead, surprisingly large, seemingly sporadic variability was seen annually as well as intra-seasonally. Additionally, temperature was not a driver of microbial N limitation in this incubation. The extensive temporal variability which could not be attributed to soil temperature emphasizes the need for a better understanding of the drivers of microbial activities and nutrient limitation.

The unexpected results of Chapter 2 led to speculation that microbial activities of MAT soils may be co-limited by N and availability of labile C and also brought into question the

possibility of site-based variability within soils of uniform properties. Subsequent attempts to assess how N and labile C may interact to influence soil microbial activities in Chapter 3 revealed that microbial nutrient limitation appears to be site-dependent and the significance of N limitation and labile C co-limitation of microbial decomposition can vary over small geographic areas.

The large, unexpected variation in microbial N limitation of these soil incubations contradicts the current understanding of controls of microbial decomposition. Going forward, a better understanding of what drives microbial mediated biogeochemical cycles as well as temporal and site-based variability is needed. Attempts at predicting the future fate of large Arctic SOM stores in light of climate change based on the assumption of uniform N limitation may be woefully inadequate.