BIOGEOCHEMICAL CONSEQUENCES OF SHRUB EXPANSION IN ARCTIC TUNDRA

by

CARLY ANNE PHILLIPS

(Under the Direction of Nina Wurzburger)

ABSTRACT

The response of arctic ecosystems to global change will have critical effects on future climate. These ecosystems are experiencing the greatest rates of warming globally and store over half the world's soil carbon (C). Climate warming has already triggered the expansion of shrubs across tundra, raising questions about how shrubs will affect ecosystem C balance. Shrub functional traits like litter quality and mycorrhizal symbionts may accelerate the activity of soil microorganisms by increasing the rate of C inputs and changing the chemical composition of soil organic matter (SOM). In this dissertation, I investigated four mechanisms by which shrubs may affect the activity of soil microorganisms by comparing shrub and non-shrub soils using a combination of manipulative lab incubations and field experiments Specifically, I tested whether shrubs stimulate heterotrophic activity and C loss by 1) their litter inputs; 2) increasing the availability of soil nitrogen (N) and phosphorus (P); 3) promoting more labile SOM relative to non-shrub species; and 4) producing root systems with stronger stimulatory effects on microbial activity. I found evidence that shrubs stimulate soil heterotrophic respiration, but microbes responded uniformly to litter addition, regardless of soil origin. I found no evidence that shrubs alter microbial N and/or P limitation on soil microbes, but are limited by P. I also found that long-term warming reduced C and N stocks in sub-arctic tundra, but did not uniformly stimulate

increases to microbial activity or C loss. My final dissertation chapter demonstrated that roots are the primary drivers of microbial activity in arctic soils, regardless of species identity. I also found evidence that shrub-induced changes to soil organic matter quality may increase microbial activity in organic soils. Further, I found that the relationship between soil C content and root growth was strongly horizon dependent, with increasing root growth leading to greater C content in organic soils and lower C content in mineral soils. Interestingly, shrub roots appear to ameliorate this negative relationship in the mineral horizon. Collectively, my results suggest that shrubs are fundamentally modifying C cycling, and are changing the chemical composition and spatial distribution of C stored in arctic ecosystems.

INDEX WORDS: shrub expansion, carbon cycle, ecosystem ecology, global change, tundra, soil carbon

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DEDICATION

To the women who gave, and continue to give, me life - my grandmothers, Elaine Phillips and Mary Flom, and my mother, Brenda Phillips

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Arctic ecosystems store a substantial fraction of terrestrial biosphere carbon (C) (Hugelius et al. 2014; Tarnocai et al. 2009), and are experiencing the greatest rates of warming across Earth's surface (IPCC 2014). Rising temperatures may alleviate thermal constraints on microbial activity and stimulate soil C loss. However, rising temperatures have also been linked to an increase in the range and cover of woody, deciduous species across tundra (Beck et al. 2011; Blok et al. 2011; Loranty & Goetz 2012; Myers-Smith et al. 2011; Tape et al. 2006), which are more productive than the vegetation they replace. Thus, climate change may have direct and indirect effects on arctic ecosystems, and the belowground consequences of these effects remain a looming unknown for the arctic and global C balance.

Belowground interactions between shrubs, soil microbial communities, and soil organic matter (SOM) will ultimately determine how arctic ecosystems store and release C. The aboveground consequences of this vegetation shift have been widely addressed from the ecological impacts of increased snow trapping (Liston et al. 2002; Myers-Smith & Hik 2013; Sturm et al. 2001; Zamin & Grogan 2012) and changing albedo (Loranty et al. 2011; Sturm et al. 2005) to the impact on arthropod communities (Rich et al. 2013) and herbivores (Christie et al. 2014; Plante et al. 2014; Speed et al. 2013). However, shrubs appear to modify critical aspects of plant-soil relationships that have historically led to accumulations of C in high-latitude ecosystems (Loranty & Goetz 2012; Myers-Smith et al. 2011). Specifically, there are four pathways by which shrubs may alter C cycling in arctic ecosystems relative to other tundra

vegetation. Shrubs may stimulate heterotrophic activity and C loss by 1) their litter inputs to soils (Myers-Smith et al. 2011); 2) increasing the cycling rates and availability of nitrogen (N) and phosphorus (P) (Buckeridge et al. 2010, Weintraub et al. 2005), which can both limit microbial processes (Mack et al. 2004, Sistla et al. 2012); 3) producing higher quality SOM relative to non-shrub species (DeMarco et al. 2011), as a result of the long-term interactions of shrub litter and microbial activity; and 4) producing root systems with stronger stimulatory effects on microbial activity. Each of these pathways threaten the stability of vast stores of arctic C, making it critical to understand the mechanisms driving C dynamics.

First, shrubs are highly productive compared to other tundra vegetation types (Weintraub & Schimel 2005), which increases the rate of C inputs to the soil system (McLaren et al. 2017). The low productivity of arctic plants, in conjunction with cold temperatures, short growing seasons and saturated soils, have contributed to historical accumulations of C in these soils (Shaver et al. 1992). In addition to greater biomass production, shrubs also increase the quality and quantity of C inputs coming into the soil system (McLaren et al. 2017). While shrubs produce highly recalcitrant woody stems (De Deyn et al. 2008), their leaf and root litter has lower C:N ratios than other tundra species (Weintraub & Schimel 2003; Weintraub & Schimel 2005). Although arctic soils are highly organic, arctic microbial communities seem to rely on fresh plant inputs for their activity (Melle et al. 2015, Weintraub et al. 2003). As a result, increases in the rate of litter inputs may promote heterotrophic respiration and soil C loss. Further, although greater production and biomass may enhance or stabilize soil C stocks (Lynch et al. 2018; Ravn et al. 2017; Sistla et al. 2013), they may also stimulate decomposition of SOM via positive priming, whereby heterotrophic microbial communities use fresh plant C to fuel the breakdown of SOM (Blagodatsky et al. 2010; Fontaine et al. 2004; Kuzyakov 2010). Thus, C

appears to constrain the activity of microbes suggesting that higher productivity in arctic ecosystems could unexpectedly lead to net C loss.

Independent of the rate of shrub litter addition, microbes may adapt to repeated exposure to the same resource (Strickland et al. 2009, Wallenstein et al. 2013). This change may lead to greater decomposition in response to these familiar litter inputs (Ayres et al. 2009; Veen et al. 2015). In the arctic, the adaptation of such a specialized soil microbial community may foster a positive relationship between plant productivity and heterotrophic activity. Indeed, molecular evidence suggests that shrubs modify the structure of microbial communities (Wallenstein et al. 2007), and that this compositional shift may also modify microbial function (Buckeridge et al. 2010, DeMarco et al. 2014, Myers-Smith et al. 2013, Weintraub et al. 2005). If microbes beneath shrubs are adapted to shrub litter, then we would expect the addition of shrub litter to produce higher rates of heterotrophic respiration in shrub-conditioned soils compared to those conditioned by other vegetation.

Arctic shrubs may also stimulate soil heterotrophs by enhancing the cycling rates and availability of N and P (Buckeridge et al. 2010, Weintraub et al. 2005), which can inhibit microbial activity (Mack et al. 2004, Sistla et al. 2012). While all arctic plants store most of their biomass belowground (Iversen et al. 2015), shrubs have a larger proportion of biomass in roots (~70-80% (Chapin et al. 1980)) and produce new roots earlier during spring thaw (Wang et al. 2016), enhancing their ability to access soil nutrients. Since tundra has traditionally been dominated by non-mycorrizhal and ericoid mycorrhizal (ERM) species (Myers-Smith et al. 2011), greater prevalence of ectomycorrhizal (ECM) fungi, which use plant C to create powerful enzymes to liberate N and P from organic matter, may stimulate decomposition (Lindahl & Tunlid 2015). Beyond ECM activity, rapid turnover of ECM biomass itself appears to promote

heterotrophic activity and thus lower C sequestration, relative to ERM dominated systems (Clemmensen et al. 2015). Such an effective means for capturing nutrients, along with increased litter input rates (Shaver et al. 1991) and litter lability (DeMarco et al. 2014, McLaren et al. 2017) may locally enrich pools of available soil nutrients and stimulate microbial decomposition of organic matter. If shrubs increase nutrient availability, we would expect shrub microbial communities to be less nutrient-limited, and thus display a smaller heterotrophic response to the addition of those nutrients, as compared to non-shrub soils.

Shrubs may also fundamentally alter the composition and quality of SOM (DeMarco et al. 2011) through the combination of their high-quality inputs and abiotic modifications of their local environment. Such differences in SOM may be due to the composition of plant C inputs (Buckeridge et al. 2010) and the microbial assemblages that comprise shrub soils and drive decomposition processes (Wallenstein et al. 2007). Indeed, the composition of the vegetative community is a primary determinant of SOM decomposability in tundra (Hobbie 1996; Shaver et al. 2006). Shrubs also produce labile litter (Weintraub & Schimel 2005), which may enhance decomposability of SOM by reducing the soil C:N ratio and increasing nitrogen (N) mineralization (Weintraub & Schimel 2003), and thus promote long term losses of C (Mack et al, 2004). Due to their large stature relative to other tundra species, shrubs trap snow, which insulates soil during winter months (Sturm et al. 2001), creating favorable environments for nutrient mineralization. The cumulative effects of these changes may modify SOM to promote greater heterotrophic C losses.

Shrubs also modify plant-soil interactions through their distinct rooting characteristics, compared to their graminoid and sedge counterparts (Shaver et al. 1992; Shaver & Chapin 1991). The production of shrub roots occurs earlier in the growing season (Wang et al. 2016), increasing

the time during which decomposition can occur. Shrubs produce long-lived roots relative to the annually produced roots of graminoids (Sullivan et al. 2007). While lower rates of root turnover may reduce rates of C inputs, these long-lived roots may exude labile C to the rhizosphere (Iversen et al. 2015; Pries et al. 2013). Arctic microbial communities appear to be limited by available C (Melle et al. 2015), and thus, may increase their activity in response to litter and exudates (Phillips, Chapter 2). Deciduous shrubs also directly provide plant C to ECM fungi (Clemmensen & Michelsen 2006; Myers-Smith et al. 2011), which decompose SOM using a suite of powerful enzymes (Finlay 2008; Lindahl & Tunlid 2015) that may aid in depleting soil C stocks (Parker et al. 2015). Additionally, shrubs in the genus *Alnus*, associate with N-fixing bacteria (Tape et al. 2006; Tape et al. 2012), which can locally enrich soil N pools (Mitchell & Ruess 2009), and suppress decomposition (Frey et al. 2014). Such changes to the quantity, timing and associations of shrub roots may result in changes to the rate of C cycling, feeding back to climate warming and thus greater shrub expansion.

The goal of this dissertation is to investigate these four pathways by which shrubs may modify C cycling and storage across arctic ecosystems, specifically focusing on the role of soil microbial communities in modulating this response. In Chapter 2, I address pathways 1 and 2 by examining differences in the rate of C loss, and mechanisms governing these processes in shrub and non-shrub areas. I incubated soils and measured heterotrophic respiration alone, and in response to litter and nutrient additions. In Chapter 3, I investigate pathway 1 and the direct effects of warming by working with samples from a 16-year warming and litter addition experiment. Further, in Chapter 4, I investigate pathways 3 and 4 by addressing the role of SOM and shrub root growth in mediating microbial processes and C cycling in arctic soils. The results of this research will expand our understanding of how arctic ecosystems respond to global

change, and provide insight into the drivers of C loss across these soils. Broadly, this work will inform earth system models that incorporate feedbacks between the atmosphere and terrestrial ecosystems to better predict future climate.

CHAPTER 2

ELEVATED RATES OF HETEROTROPHIC RESPIRATION IN SHRUB-CONDITIONED ARCTIC SOILS[1]

[1] Phillips, C.A. and Wurzburger N. Elevated rates of heterotrophic respiration in shrub-conditioned arctic soils. Submitted to *Pedobiologia*, July 2018.

Abstract

The response of arctic ecosystems to global change will be critical for future climate, due to their vast stores of carbon. Climate warming appears to be linked to the expansion of shrubs across tundra, but it is unclear how shrubs affect the activity of soil microbes. We investigated three potential mechanisms by which shrubs may stimulate soil microbial activity, by 1) increasing the rate of litter inputs, 2) promoting soil microbial adaptation to litter and 3) reducing nutrient limitation. We created microcosms of root-free soils collected from shrub (*Alnus fruticosa, Betula nana,* and *Salix pulchra*) and non-shrub areas in arctic Alaska and conducted two experiments. We quantified heterotrophic soil respiration rates in response to litter inputs (experiment 1), nitrogen, phosphorus, or both nutrients together (experiment 2). We found that shrub-conditioned soils maintained higher rates of soil respiration in both experiments. Shrub litter increased respiration in both soil types, but the relative response was greater in non-shrub soils. We found no evidence that shrubs reduce nutrient limitation to heterotrophic respiration, although we observed a short-term increase in respiration after phosphorus addition in both soil types. Collectively, our results suggest that higher rates of respiration in shrub-conditioned soils may be the result of higher litter input rates, but other factors such as organic matter quality or microbial community structure may also contribute to our observed differences in respiration.

Keywords: biogeochemistry, shrub expansion, nutrient limitation, litter addition, arctic ecology, heterotrophic respiration

Introduction

Arctic soils store a substantial amount of terrestrial biosphere carbon (C) (Hugelius et al. 2014), and are experiencing the greatest rates of warming globally (IPCC 2014). In tundra ecosystems, warmer temperatures have been linked to an increase in the range and cover of woody, deciduous shrub species (Beck et al. 2011, Blok et al. 2011, Loranty et al. 2012, Myers-Smith et al. 2011, Tape et al. 2006). Warming has also stimulated greater shrub productivity (Bret-Harte et al. 2001, DeMarco et al. 2014), which is fostering structural and biogeochemical changes to arctic ecosystems. While shrub expansion increases ecosystem C fixation (Shaver et al. 1991) and C inputs below ground (Sistla et al. 2013), it is unclear how these changes may affect the availability of C and nutrients for soil microbes, thereby increasing or decreasing their activity.

Deciduous shrubs (hereafter shrubs) appear to modify the interactions between plants and soil microbial communities that historically led to large accumulations of soil C in arctic tundra (Loranty et al. 2012, Myers-Smith et al. 2011). Shrubs are highly productive compared to other tundra vegetation (Weintraub et al. 2005), which increases the rate of C inputs to the soil system (McLaren et al. 2017). Despite the highly organic nature of tundra soils, arctic soil microbial communities seem to rely on fresh plant C for their activity (Melle et al. 2015, Weintraub et al. 2003). If C limitation is a general phenomenon in arctic soils, we would expect litter inputs to stimulate soil microbes and increase heterotrophic respiration.

Independent of the rate of shrub litter inputs, microbes may respond to repeated exposure to the same resource (Strickland et al. 2009, Wallenstein et al. 2013), leading to greater decomposition and heterotrophic respiration in response to familiar litter (Ayres et al. 2009; Veen et al. 2015). In the arctic, the creation of such a specialized soil microbial community may

foster a positive relationship between plant productivity and heterotrophic decomposition. Indeed, molecular evidence suggests that distinct microbial communities develop beneath shrubs (Wallenstein et al. 2007), and that this compositional shift also represents modified function within the community (Buckeridge et al. 2010, DeMarco et al. 2014, Myers-Smith et al. 2013, Weintraub et al. 2005). Alternatively, a specialized microbial community could lead to lower respiration rates due to increasing microbial use efficiency of C compounds (Sinsabaugh et al. 2013). In either case, if microbes become specialized to shrub litter, we would expect distinct relative responses to the addition of shrub litter in shrub-conditioned soils compared to soils conditioned by other vegetation.

Arctic shrubs may also stimulate soil microbes by increasing the cycling rates and availability of nitrogen (N) and phosphorus (P) (Buckeridge et al. 2010, Weintraub et al. 2005), which can both limit microbial processes (Mack et al. 2004, Sistla et al. 2012). Shrubs have a larger proportion of biomass in roots (~70-80% (Chapin et al. 1980)) relative to graminoid and ericaceous plants, and produce new roots earlier in the spring thaw (Wang et al. 2016), suggesting that their capacity to forage for soil nutrients in organic soils may exceed that of other arctic vegetation. Further, shrubs associate with ectomycorrhizal fungi (unlike non-mycorrhizal graminoids and ericoid mycorrhizal, prostrate, evergreen shrubs), which connect them to mycelial networks that help mobilize nutrients from organic matter (Finlay 2008, Hobbie et al. 2006). Such an effective means for capturing nutrients, along with increased litter input rates (Shaver et al. 1991) and litter lability (DeMarco et al. 2014, McLaren et al. 2017) may locally enrich pools of available soil nutrients and stimulate microbial decomposition of organic matter. If shrubs increase nutrient availability for soil microbes, we would expect shrub microbial

communities to be less nutrient-limited, and thus display a smaller response in heterotrophic respiration to the addition of those nutrients, as compared to non-shrub conditioned soils.

Here we investigate three potential mechanisms for how shrubs might stimulate the activity of soil microbes and increase heterotrophic respiration. To this end, we conducted two experiments where we isolated soils from shrub and non-shrub areas of arctic tundra and performed a series of laboratory manipulations. First, to test the idea that soil microbes are C limited, we added litter to soils to determine if it increased heterotrophic respiration. In the same experiment we tested the second idea, that microbes beneath shrubs respire relatively more when exposed to shrub litter, by comparing how shrub vs. non-shrub conditioned soils responded to shrub litter addition. In a second experiment we tested the third idea, that shrubs reduce nutrient limitation on heterotrophic microbes. In this experiment we compared the response of shrub vs. non-shrub conditioned soils to the addition of N, P, or both. Overall, we expected to observe higher rates of heterotrophic respiration in shrub-conditioned vs. non-shrub-conditioned soils. To explain this pattern, we hypothesized that 1) litter, both leaf and root, stimulates heterotrophic respiration, but that 2) the response to shrub litter would be relatively greater in shrub soils, and 3) the response of heterotrophic respiration to soil N, P and both would be lower in shrub vs. non-shrub-conditioned soils indicating that shrub microbes are less nutrient limited.

Materials and Methods

Experiment 1 – Litter addition

Sampling

To test our first two hypotheses relating to the response of soils to shrub litter addition, we sampled soils from moist acidic tundra in mid-July across 9 sites located within 50 km of Toolik Lake Field Station (68° 38' N, 149° 36' W) on the North Slope of the Brooks Range in arctic

Alaska. At each site, we established a pair of plots (each 10 x 10 m) that were within 20 m of each other, where one (i.e. "shrub" plot) was dominated by either *Alnus fruticosa*, *Betula nana*, *Salix pulchra*, or (hereafter alder, birch, and willow respectively), while the other plot consisted of other tundra vegetation including prostrate ericaceous *spp* and sedges (i.e., "non-shrub" plot). Species in the non-shrub plots included *Carex bigelowii, Eriophorum vaginatum, Empetrum nigrum, Vaccinium uliginosum, Vaccinium vitis-idaea* and *Rhododendron lapponicum* among others. For each shrub species, we established 3 paired plots, for a total of 9 paired plots or 18 total plots. In each plot, we sampled 3 soil cores (7 cm diameter and 25 cm depth), and separated organic and mineral horizons due to inherent differences in organic matter, moisture and rooting density. For each shrub species, we collected foliage and fine roots (< 2 mm diameter) from each plot. All plant and soil material was kept cool but not frozen and shipped to the University of Georgia, where we dried root and leaf tissues until stable at 60°C, and then fragmented it into ~1 cm^2 pieces. For soil samples, we removed roots and maintained them at 4°C until we initiated laboratory incubations within 4 weeks of sampling.

Experimental design

We homogenized cores by shrub species (i.e., combining soils across the 3 sites for each species) but separated soils based on origin (shrub vs. non-shrub plot) and horizon (organic vs. mineral) for a total of 12 homogenized samples. We used this approach to isolate the shrub (i.e., soil origin) effect, and shrub species served as replicates. This approach, however, prevented us from isolating differences between the species and across the 3 sites from which we sampled. As a result, our experiment was a modified 3-way factorial (soil origin by soil horizon by litter) design where soil factors of origin (shrub vs. non-shrub) and horizon (organic vs. mineral) were replicated by three shrub species (alder, birch, willow) for a total of 12 homogenized soil

treatment combinations. From each of these 12 soil treatment combinations, we created 24 experimental microcosms for a total 288 microcosms. We applied our final factor of litter addition (+/-) to these microcosms leading to 12 technical replicates of each soil treatment by litter combination. In our statistical analysis (see below), we used the mean of these technical replicates to preserve the replication level our original sampling design. Although traits of individual shrub species may also play a role in mediating soil heterotrophic activity, using species as replicates in our experiment allowed us to test for general shrub effects, but not species-specific effects.

We created experimental microcosms in 237 cm^3 containers to isolate the effect of soil origin (i.e., shrub vs. non-shrub plots), soil horizon and leaf litter inputs on heterotrophic activity. Moist soils were added to microcosms at the dry equivalent of 5 g of organic and 15 g of mineral soil to each respective treatment. To microcosms receiving litter (dried material from above), we added 0.12 g of dry, fragmented litter to using species-specific application. For example, soils from birch paired plots received birch litter. While roots may persist in both organic and mineral soil (Wang et al. 2016), we added leaf litter to organic soil and root litter to mineral soil, and litter was gently mixed into the surface. This allowed us to understand how microbes respond to two types of plant tissue. We dried a subset each litter type at 60°C until stable and ground to a fine powder in a ball-mill grinder. Samples were then analyzed for total C and N content by combustion (CHN Carlo-Erba Elemental Analyzer, NA 1500, Carlo-Erba Instruments, Milan Italy).

Microcosms were covered with perforated parafilm to allow gas exchange but to minimize drying, and placed in a laboratory incubator at 15°C. We added DI water weekly to maintain soils at a constant gravimetric moisture content of 63% and 40%, for organic and

mineral soils, which represented the average field soil moisture content upon collection. Microcosms were sampled for respiration rates after 1 month of incubation.

Respiration measurements

To determine the response of the microbial community, we quantified rates of heterotrophic respiration. Prior to gas sampling, we flushed each microcosm with N_2, and then capped them with a gas-tight lid equipped with a septum. Flushing briefly with N_2 reduced the concentration of CO_2, but did not create anoxic conditions in our microcosms. To sample, we thoroughly mixed headspace, sampled 2 mL gas, and analyzed sample CO_2 concentrations on an infrared gas analyzer (LiCor 6252, LiCor, Lincoln, NE). Over 8 hours, we sampled three times and generated slopes for each microcosm to determine respiration rate ($\mu mol\ CO_2 \cdot g\ soil\ day^{-1}$). We excluded samples that generated non-linear accumulation rates of CO_2 because these microcosms were not gas-tight over the course of the incubation period. To determine the total C content in each microcosm we dried a subset of each soil type at 60°C until stable and ground to a fine powder in a ball-mill grinder. Samples were then analyzed for total C and N content as above. To normalize for the different amounts of soil C in each sample (including the additions of litter treatments), we expressed respiration rates per g C in each microcosm, including C from both soil organic matter and added litter where appropriate (i.e., $\mu mol\ CO_2 \cdot g\ soil\ C\ day^{-1}$).

Data analysis

We first averaged the technical replicates from each combination of our experimental treatments (soil origin, litter treatment, horizon), and used the resulting 24 means in our statistical models. This ensured that the replication in our models (12 no litter, 12 litter added) matched the replication of our sampling design. We analyzed respiration data separately for each horizon with a two-way ANOVA using soil origin (shrub, non-shrub), litter (+/-), and their interactions, with

the 12 soil treatment combinations (6 organic, 6 mineral) as blocks to control for the relatedness of soils with and without litter. By analyzing horizons separately, we also accounted for the two different types of litter added to each horizon. We conducted F tests ($\alpha = 0.05$) and post-hoc tests using Tukey's Honest significant difference test (R for Mac, version 3.1.2). We square-root and log transformed data where required to meet assumptions of normality and heteroscedasticity. We tested our first hypothesis that litter addition simulated respiration by isolating the single factor fixed effect of litter to compare microcosms receiving litter to those that did not. We also determined if the addition of litter to shrub soils resulted in a higher rate of respiration compared to that of non-shrub soils (i.e., an interaction of soil origin and litter).

To further test for differences in the response to litter from each soil origin, we also calculated the relative response to litter as a percent increase from basal respiration. We analyzed these data using a two-way ANOVA with soil origin and horizon as the predictor variables. We did not include the block from the respiration measurements because our calculations of relative response already accounted for the relatedness of soils with and without litter. We also used a paired t-test to compare bulk density, %C, %N, and C:N of shrub and non-shrub soils, and of root and leaf litter.

Experiment 2 – Nutrient addition

Sampling

To understand if shrubs reduce nutrient limitation to microbes, we sampled soils from paired shrub and non-shrub plots (each 10 x 10 m within 20 m of each other) distributed across 7 sites within 50 km of Toolik field station in mid-July. For each shrub species (alder, birch, willow), we collected soils from paired plots at 4 different sites, but these species were not present at all sites. 5 sites contained plot pairs for two species and 2 sites contained plot pairs for one species.

At each plot, we sampled three cores (7 cm diameter and 25 cm depth) which were homogenized by species and horizon. Thus, we collected soil samples that represented three species, by two soil origins (shrub and non-shrub areas), and two horizons (organic and mineral) from four sites. We transported soils back to the University of Georgia on icepacks to maintain cold, but not freezing conditions. We removed roots from soil and maintained soils at 4°C until the initiation of the experiment, 4 weeks after collection.

Experimental design

Our experimental design was a 3-way factorial including soil origin (shrub vs. non-shrub plots), N addition (+/-) and P addition (+/-), replicated 6 times for each of 3 species. This design was repeated in both organic and mineral soils for a total of 288 experimental units. We established experimental microcosms in 50 cm^3 containers with field moist soil that contained either 4 g of organic or 2 g of mineral dry-equivalent soil. These soils were maintained at field moisture and 15°C in a laboratory incubator for one month to stabilize microbial respiration before the application of the treatments. We applied four nutrient treatments: control (deionized water), N alone (150 μg N · g dry $soil^{-1}$ as NH_4NO_3), P alone (300 μg P · g dry $soil^{-1}$ as NaH_2PO_4), and N+P (both 150 μg N · g dry $soil^{-1}$ as NH_4NO_3 and 300 μg P · g dry $soil^{-1}$ as NaH_2PO_4) in 0.5 mL aqueous solution. These supply rates of N and P corresponded to approximately three-times field-extractable concentrations (Weintraub et al. 2005), extrapolate to field addition rates of ~ 9 g · m^{-2} and 6 g · m^{-2} respectively, which are within the range of other field and laboratory studies (Hartley et al. 2010, Mack et al. 2004, Melle et al. 2015, Sistla et al. 2012).

Respiration measurements

We applied nutrient treatments to the soil surface and quantified heterotrophic respiration rates five times (30 min, and 2, 3, 5 and 8 days after adding nutrients) over the course of 8 days.

Techniques for quantifying and expressing respiration (i.e., on a per gram soil C basis) were the same in experiment 1. Additionally, we calculated total CO_2 loss over the 8-day incubation period by extrapolating our measured rates between sampling points.

Data analysis

We analyzed respiration data with a linear mixed effects model, using soil origin (shrub, non-shrub), shrub species (alder, birch, willow), N addition (+/-) and P addition (+/-) as fixed effects, and site as random effects, for the organic and mineral horizons separately. To test our third hypothesis, we analyzed cumulative C loss to determine if nutrients stimulate greater respiration in non-shrub soils. We also used a repeated measures analysis with data from our 5 sampling points to determine if nutrient limitation modified respiration rates over time, and analyzed single time points if we observed a significant interaction with time. To understand differences in %C, %N, C:N, and bulk density, we used species, soil origin, and horizon as fixed effects with site and microcosm as random effects . Data were square root transformed to achieve normality when necessary. We calculated p values ($\alpha = 0.05$) using an F test (package nlme, R for Mac, version 3.1.2) (Pinheiro et al. 2014), and initially included interactions among fixed effects, but removed them if they were not significant.

Results

Experiment 1 –

In our first experiment, we observed that shrub-conditioned soils had consistently higher rates of heterotrophic respiration in both organic ($F_{1,5} = 19.16$, $p = 0.008$) and mineral soils ($F_{1,5} = 55.17$, $p < 0.001$) (Fig. 1). We found that litter addition had an positive effect on respiration in both organic ($F_{1,5} = 18.11$, $p = 0.007$) and mineral soils ($F_{1,5} = 18.89$, $p = 0.007$).

We next determined if the addition of shrub litter had a stronger positive effect on soils conditioned by shrub vs. non-shrub vegetation by testing for an interaction between soil origin and litter addition. We observed the same stimulatory effect of litter in both shrub and non-shrub soil (organic $p > 0.05$, mineral $p > 0.05$, Fig. 1, S1). In the organic horizon, this same trend appeared in our analysis of relative response data ($p > 0.05$). However, in the mineral horizon, we saw a greater relative response to litter in non-shrub vs. shrub soils ($F_{1,4}$ =14.13, $p = 0.019$) (Fig. 2).

We next determined differences in elemental composition between leaf litter treatments and quantified the rate at which we added C and N to microcosms. Leaf litter had higher %N ($t(2) = 9.456$, $p=0.011$) and modestly lower C:N ($t(2) = 3.932$, $p = 0.059$) relative to root litter (Table 1). Leaf litter inputs supplied an average addition of 63.3 mg of C (range 48.7 – 74.7 mg) and 3.23 mg N (range 2.57 – 4.02 mg), and root litter treatments applied an average of 64.0 mg C (range 55.1 - 74.1 mg) and 1.99 mg N (range 1.20 - 2.61 mg) to each microcosm (Table 1). These inputs of litter represent an average percent (± SE) increase of 5.7 ± 0.5 and 3.5 ± 0.03 for C and N, respectively, relative to the soil C and N in the microcosms. Interestingly, our comparison of shrub and non-shrub soils from the field revealed no differences in soil bulk density, total %C, total %N, and C:N (all $p > 0.05$) (Table 2).

Experiment 2 - We first analyzed cumulative respiration over the course of the experiment, and observed higher respiration in shrub vs. non-shrub soils in the organic horizon ($F_{1,123} = 13.21$, $p < 0.001$) (Fig. 3a), consistent with our first experiment. However, we also observed a significant interaction of soil origin and species, where respiration rates in birch and willow areas were higher in shrub vs. non-shrub soils, while alder respiration rates were unaffected by soil origin ($F_{2,123} = 3.56$, $p = 0.032$) (Fig. 3a). In the mineral horizon, we only observed a mild species

effect, with respiration rates modestly higher in willow vs. alder soils ($F_{2,83}$ = 2.71, p = 0.073) (Fig.3c).

We observed no differences in nutrient response between shrub and non-shrub soils for either nutrient (nutrient by soil origin interactions: p > 0.05). Instead, we found that P, but not N, increased the cumulative loss of C by 12 % in organic soils (Fig. 3b). This response however, was not statistically significant due to high variation between sites ($F_{1,123}$ = 3.56, p = 0.062). In mineral soils, nutrient addition did not affect cumulative flux (Fig. 2d, p > 0.05).

We then analyzed respiration over the 8-day experiment using a repeated measures analysis. In organic soils, respiration rates were generally higher in shrub vs, non-shrub soils ($F_{1,130}$ = 21.50, p < 0.001), even as respiration rates declined over the course of the incubation. However, we also observed an interaction of soil origin and species ($F_{2,130}$ = 3.15, p = 0.046), where shrub soils had consistently higher respiration than non-shrub soils in birch and willow, but not alder areas (Fig. 4a,b,c). In contrast, soil origin did not affect respiration in the mineral horizon overall, but we observed an interaction among soil origin and species ($F_{2,129}$ = 6.99, p = 0.001). This interaction was driven by differences among species within a given soil origin, rather than within species effects (Fig. 4e,f,g). However, we did find consistent species differences in the mineral horizon ($F_{2,129}$ = 3.29, p = 0.040), where alder areas had lower respiration rates than those of birch and willow (both p < 0.05).

We also observed that the response to P addition changed over time in the organic horizon (P by time interaction; $F_{1,564}$ = 26.05, p < 0.001) (Fig. 4d). P addition stimulated respiration on the first day ($F_{1,125}$ = 4.78, p = 0.031), but not on subsequent days of the incubation (p > 0.05). For mineral soils, our repeated measures analysis also demonstrated a significant interaction between P addition and time ($F_{1,490}$ = 7.64, p = 0.006) (Fig. 4h). However, this

interaction was driven by the dramatic decline in respiration rate in P-addition soils between days 1 and 2 of the experiment, rather than a stimulatory effect of P addition.

In our analysis of soil properties in shrub and non-shrub plots, we found that soil %C and %N depended on species, soil origin, and horizon (%C: $F_{2,125} = 4.74$, $p = 0.0104$; %N: $F_{2,125} = 4.75$, $p = 0.0104$) (Table 3), where patterns in the mineral horizon appear to be driving the interactions (Table 3). For both of these interactions, differences among species were observed in non-shrub soils, suggesting greater heterogeneity in non-shrub soil properties across our plots. For C:N, we observed a species by soil origin interaction in both organic ($F_{2,59} = 11.50$, $p < 0.001$) and mineral soils ($F_{2,60} = 7.23$, $p = 0.002$). In the organic horizon, willow soils had higher C:N in non-shrub vs. shrub soils, while in the mineral horizon, birch soils had higher C:N in shrub vs. non-shrub.

Discussion

We found that soils beneath deciduous shrubs support higher rates of heterotrophic respiration relative to non-shrub soils, supporting field observations that woody vegetation increases *in situ* soil respiration rates (Grogan et al. 1999, Grogan et al. 2005). Here, we tested three potential mechanisms that may explain this pattern - that shrubs increase the activity of soil microbial communities by 1) producing more litter, 2) stimulating greater respiration in response to shrub litter or 3) alleviating nutrient limitation to heterotrophs. We only found support for one of these hypotheses, that shrubs increase heterotrophic respiration via their litter, suggesting that labile C limits the activity of soil microbes in arctic soils.

In both experiments, we observed higher heterotrophic respiration in shrub-conditioned organic soils, but we only observed this in mineral soils in the first experiment (Fig. 1). The consistency of response in organic soils suggests that shrubs have a stronger effect on microbial

activity in the upper, organic horizon. Such effects may be explained by the lateral structure of shrub roots (Mack et al. 2004), and thus a greater concentration of shrub roots in upper layers of soil relative to other plant species (Wang et al. 2016). However, since we only added leaf litter to organic soils in our experiment, it remains unclear if shrub root or leaf litter plays a more important role in regulating the activity of heterotrophic microbial communities. While the organic horizon also contains more C than the mineral horizon, we normalized respiration rates per gram soil C, which indicates that shrub conditioning, and not soil C content, drives microbial activity in our study. The inconsistent shrub effect in the mineral horizon in our study may be explained by the recalcitrance of root litter relative to leaf litter (Table 2; McLaren et al. 2017) or that variable shrub size across our sampling plots determined the magnitude of shrub-effects on soils with depth (Bonfils et al. 2012). Another possibility is that shrubs support a larger pool of microbial biomass, leading to greater activity.

In our first experiment we found that leaf and root litter addition had a positive effect on heterotrophic respiration (Fig. 1). Upon analyzing interactions between soil origin and horizon, we found no evidence that soil microbial communities beneath shrubs were better adapted to shrub litter (Fig. 1). In contrast, the relative response of non-shrub, mineral soils was greater than that of shrub soils (Fig. 2), suggesting that these microbial communities are more C limited or support a larger pool of biomass. Given the divergent rooting strategies of shrubs and historically-dominant graminoids (i.e., shrubs root laterally, grasses root deeply) (Wang et al. 2016), this heightened response suggests that roots may be driving microbial processes in these soils. Alternatively, this pattern could reflect greater C use efficiency in shrub-conditioned soil microbial communities, where more C is used towards microbial growth than lost to respiration (Sinsabaugh et al. 2013). In the context of litter responses, we acknowledge the possibility that

our experiment induced a higher response of microbial activity than would be observed in nature, because our litter samples were collected fresh and likely contained a higher concentration of N and P than would senesced leaf and root tissues.

Results from our second experiment suggest that species play a role in modulating the observed "shrub" effect, particularly in organic soils (Figs. 3,4). We found higher respiration rates in the presence of birch and willow shrubs but not alder (Figs. 3b, 4). While this suggests a lack of a stimulatory affect, respiration rates in non-shrub alder areas were higher than those of non-shrub areas of willow and birch. Alder may not stimulate heterotrophic activity, as do birch and willow because it associates with N-fixing bacteria (Mitchell et al. 2009), and the enriched soil N pool may suppress decomposer activity (Frey et al. 2014; van Diepen et al. 2017). Regardless of the mechanism, our findings highlight the importance of species-level differences in shrub effects across the landscape of arctic tundra, and emphasize the role that root symbionts may play in mediating this response.

In contrast to our expectation that shrubs increase soil nutrient availability, the results from our second experiment suggest that shrubs do not alleviate nutrient limitation for heterotrophic microbes. Instead, we observed evidence of mild P limitation on microbial activity in the organic horizon, regardless of the presence of shrubs (Figs. 3, 4). Although arctic soils are rich in organic P (Weintraub et al. 2003), these findings suggest that the release of phosphate from organic molecules via phosphatases may constrain microbial activity. Since P is a critical element for growth, reproduction and energy production (Sterner et al. 2002), the rapid response to P additions suggests that heterotrophic communities may be limited by P in respect to population growth. Our finding of P limitation contrasts with the long-standing view that arctic ecosystems, and specifically plants, are N limited (Chapin III et al. 1986). However, our findings

align with recent work that highlights the importance of P for microbes in arctic ecosystems, specifically during thaw (Buckeridge et al. 2016) and in response to fertilization (Koyama et al. 2013). The heterogeneity of arctic ecosystems may explain why N (Chapin III et al. 1986, Sistla et al. 2012, Weintraub et al. 2003), P (Weintraub 2011), and C (Melle et al. 2015) can limit soil microbial communities depending on the plant community and time of sampling. Interestingly, the lack of a differential respiration response to N and P in shrub vs. non-shrub soils, but the positive response to litter addition in our first experiment and other work (Hartley et al. 2010, Melle et al. 2015), suggests that C limitation may be a stronger driver of microbial activity in these soils.

The findings from our short-term incubation experiments suggest that shrubs stimulate the interaction of heterotrophic microbes with organic matter. However, the lack of a difference in bulk density and % C between shrub and non-shrub soils in our sampling plots (Tables 1,3) suggests that patterns of respiration in our study may be more indicative of faster C turnover rates in shrub versus non-shrub soils, rather than a reduction of the soil C stock. Testing this hypothesis would require in situ examination of litter inputs and both heterotrophic and autotrophic loss rates, as turnover would depend on a suite of biotic and abiotic factors (Hartley et al. 2012, Myers-Smith et al. 2013, Parker et al. 2015). For example, in addition to litter inputs (Iversen et al. 2015), shrubs may intensify heterotrophic activity through root exudation of C (van der Putten et al. 2013) and through associations with root symbionts that drive greater plant C allocation belowground (i.e., ectomycorrhizal fungi and N-fixing bacteria, respectively) (Deslippe et al. 2011, Mitchell et al. 2009). Shrubs may also change the abiotic environment by trapping snow and insulating soils from low winter temperatures (Sturm et al. 2005), while

reducing albedo (Loranty et al. 2011) and increasing the depth of permafrost thaw (Bonfils et al. 2012) during the growing season.

In conclusion, our results suggest that shrubs stimulate microbial activity in tundra soils and our findings points to interactions between soil microbes, shrub litter, and soil organic matter as a possible driver of this pattern. Although we found support for the idea that shrubs increase heterotrophic respiration via their litter inputs, our study does not eliminate other potential explanations for high respiration rates in shrub-conditioned soils, such as differences in microbial community composition or soil organic matter quality. Future work is necessary to determine if our findings from our microcosms are consistent with patterns in the field, and how increased rates of heterotrophic respiration affect the soil C balance.

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Table 2.1 Leaf and root litter total C, N and C:N from plant material used in experiment 1. Leaf litter had greater N content ($p < 0.05$) and modestly lower C:N ($p = 0.06$) than did root litter.

Leaf litter					Root litter				
	Alder	**Birch**	**Willow**	**Means**		**Alder**	**Birch**	**Willow**	**Means**
C (%)	48.72	49.96	49.50	49.39 ± 0.36	C (%)	45.89	49.40	46.43	47.24 ± 1.09
N (%)	2.57	2.68	2.31	2.52 ± 0.11	N (%)	1.75	1.15	0.86	1.25 ± 0.26
C:N	18.99	18.64	21.44	19.69 ± 0.88	C:N	26.19	43.00	54.28	41.16 ± 8.16

Table 2.2 Soil properties from shrub and non-shrub soil combinations in experiment 1, including total C, N C:N and bulk density, where species means are from 3 technical replicates. We found no significant differences in soil properties between shrub and non-shrub soil combinations via t-tests. Values are means ± SE.

	Shrub soils				Non-shrub soils			
Organic Horizon	**Alder**	**Birch**	**Willow**	**Means**	**Alder**	**Birch**	**Willow**	**Means**
C (%)	34.36	32.49	21.86	29.57 ± 3.89	26.91	23.07	24.45	24.81 ± 1.12
N (%)	1.94	1.44	1.29	1.56 ± 0.20	1.34	1.26	1.29	1.30 ± 0.02
C:N	17.70	22.50	16.89	19.03 ± 1.75	20.04	18.34	18.96	19.11 ± 0.49
Bulk density ($g \cdot cm^{-3}$)	0.10	0.11	0.16	0.12 ± 0.02	0.12	0.14	0.16	0.14 ± 0.01
Mineral Horizon								
C (%)	4.56	10.01	9.27	7.95 ± 1.71	5.79	6.89	14.73	9.14 ± 2.81
N (%)	0.31	0.53	0.55	0.47 ± 0.08	0.36	0.43	1.03	0.60 ± 0.21
C:N	14.52	18.95	16.73	16.73 ± 1.28	16.16	16.09	14.34	15.53 ± 0.59
Bulk density ($g \cdot cm^{-3}$)	0.85	0.40	0.56	0.60 ± 0.13	0.71	0.52	0.30	0.51 ± 0.12

Table 2.3 Total C, N C:N and bulk density values from shrub and non-shrub plots used in experiment 2. Significant differences among species by soil origin and horizon ($\alpha = 0.05$) are designated by lowercase letters. Values are means ± SE.

	Shrub soils						Non-shrub soils					
Organic Horizon	**Alder**		**Birch**		**Willow**		**Alder**		**Birch**		**Willow**	
C (%)	30.63 ± 5.33	*a*	31.28 ± 3.53	*a*	26.04 ± 5.79	*a*	25.74 ± 5.88	*a*	36.19 ± 3.62	*a*	29.86 ± 3.94	*a*
N (%)	1.86 ± 0.34	*a*	1.98 ± 0.26	*a*	1.67 ± 0.43	*a*	1.39 ± 0.28	*a*	1.63 ± 0.23	*a*	1.73 ± 0.16	*a*
C:N	16.59 ± 1.29	*a*	16.05 ± 1.11	*a*	16.08 ± 0.97	*a*	17.98 ± 1.58	*a*	23.04 ± 2.32	*b*	17.11 ± 1.20	*a*
Bulk density ($g \cdot cm^{-3}$)	0.21 ± 0.06	*a*	0.19 ± 0.01	*a*	0.35 ± 0.16	*a*	0.31 ± 0.12	*a*	0.15 ± 0.02	*a*	0.23 ± 0.03	*a*
Mineral Horizon												
C (%)	8.30 ± 3.37	*bc*	5.81 ± 2.16	*bc*	10.12 ± 5.75	*bc*	11.7 ± 5.01	*b*	9.26 ± 4.65	*bc*	4.20 ± 1.41	*c*
N (%)	0.57 ± 0.24	*bc*	0.36 ± 0.12	*bc*	0.64 ± 0.35	*bc*	0.72 ± 0.29	*b*	0.65 ± 0.31	*bc*	0.27 ± 0.07	*c*
C:N	14.03 ± 1.36	*cd*	16.05 ± 1.02	*d*	14.55 ± 1.64	*cd*	15.65 ± 0.48	*cd*	13.70 ± 2.22	*c*	14.54 ± 1.55	*cd*
Bulk density ($g \cdot cm^{-3}$)	1.01 ± 0.33	*b*	1.08 ± 0.21	*b*	0.92 ± 0.31	*b*	0.76 ± 0.20	*b*	1.08 ± 0.31	*b*	1.29 ± 0.21	*b*

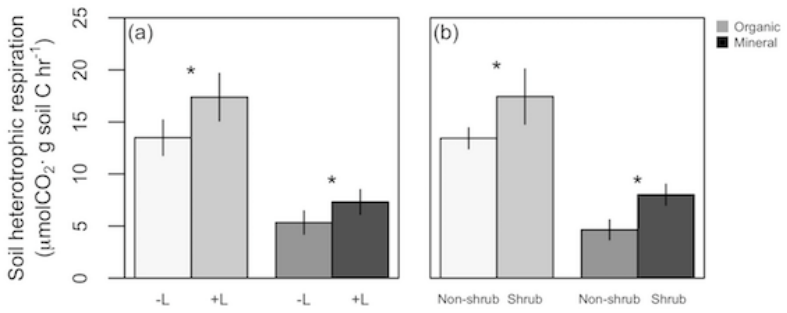

Fig. 2.1 Heterotrophic respiration in response to a) litter addition in experiment 1 in b) shrub and non-shrub soils. Data from each horizon were analyzed in separate ANOVA models. Bars represent means ± standard error. Significant differences ($\alpha = 0.05$, single factor effects) are indicated by asterisks

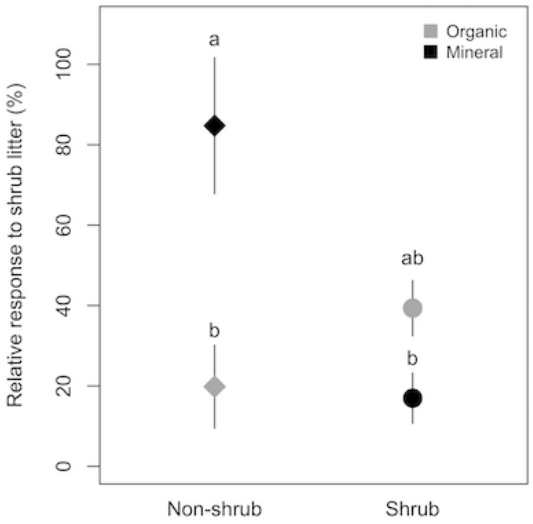

Fig. 2.2 Relative response to shrub litter addition in shrub and non-shrub soils. Points represent means ± standard error. Significant differences ($\alpha = 0.05$, two-way interaction of soil origin and horizon) are indicated by lowercase letters

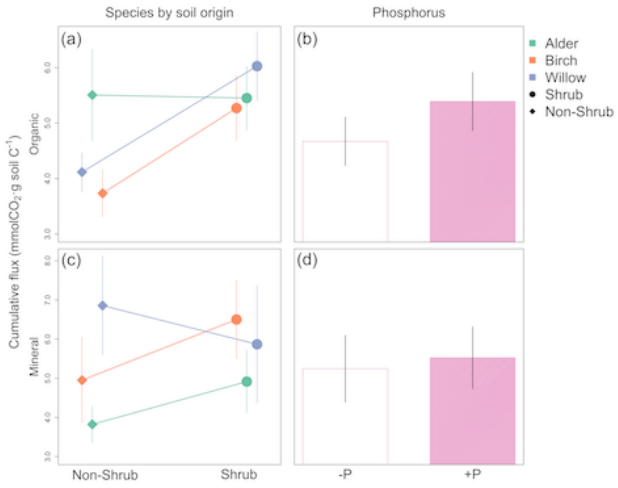

Fig. 2.3 Cumulative heterotrophic respiration over 8-days in experiment 2 showing the interaction of species and soil origin in a) organic horizon and c) mineral horizon and responses to P addition in b) organic and d) mineral horizons. Values represent treatment means ± standard error

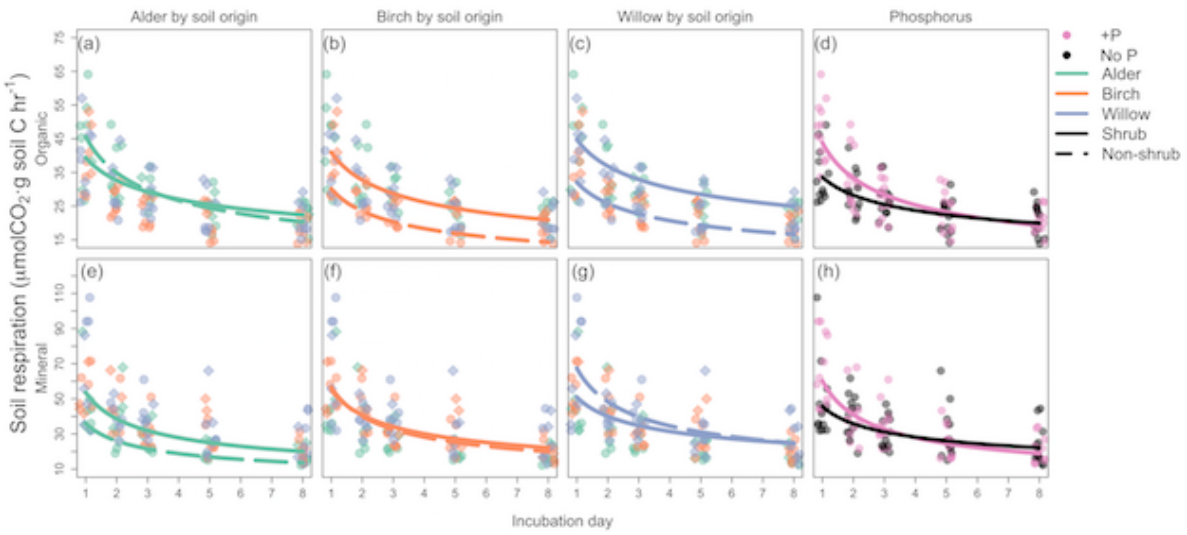

Fig. 2.4 Heterotrophic respiration in soil microcosms over time in experiment 2, where panels a, b and c display effect of alder, birch and willow (respectively) by soil origin in the organic horizon, and panels e, f, and g display the effect of alder, birch and willow (respectively) by soil origin in the mineral horizon, and panels d and h display the effect of phosphorus in organic and mineral horizons, respectively. In the organic horizon, birch, willow but not alder differ between shrub and non-shrub soils ($p < 0.05$). In the mineral horizon, no species by soil origin differences were observed, but alder areas had significantly lower respiration rates than birch and willow areas ($p < 0.05$). A transient effect of P addition (Day 1, $p < 0.05$) in both shrub and non-shrub organic soils (P by time of sampling interaction, $p < 0.05$) was observed in both organic and mineral horizons.

CHAPTER 3

SOIL CARBON AND NITROGEN STOCKS AND TURNOVER FOLLOWING 16 YEARS OF WARMING AND LITTER ADDITION[2]

[2] Phillips, C.A., Elberling, B., and Michelsen, A. Soil carbon and nitrogen stocks and turnover following 16 years of warming and litter addition. *Ecosystems*.
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Abstract

Soils in northern latitudes store more than twice the amount of carbon (C) currently in the atmosphere, and are warming faster than the rest of the globe. Warming has been linked to an expansion of woody vegetation across tundra, raising questions about how these two phenomena interact to modulate C stocks and turnover. We investigated how long-term warming and litter addition have modified microbial processes, soil characteristics, and C and nitrogen (N) stocks. We hypothesized that warming and litter would interact to amplify soil C losses, and would be accompanied by increases in microbial activity. Using soil samples from a 16-year warming and litter addition field manipulation, we measured soil C and N stocks, heterotrophic respiration, extracellular enzyme activity, and microbial stoichiometry. We found that warming decreased C and N stocks across the entire soil profile. Depth-specific analyses illustrated that these changes are driven by increasing microbial activity at 5-10 and 10-15 cm depth, and trends towards higher dissolved organic C and N at 5-10 cm depth. This emphasizes the potential for increased leaching losses with warming and additional litter. While litter addition did not change overall C and N stocks, it appears to modify the ecosystem by adding nutrients and C to the soil. Collectively, these findings highlight the vulnerability of northern soils to continued warming with respect to nutrient and C turnover, and provide insights into the mechanistic responses of tundra soil to prolonged global change.

Keywords: experimental warming, extracellular enzymes, tundra, litter addition, carbon cycling

Introduction

Soils in northern latitudes store a large proportion of terrestrial carbon (C) (Hugelius and others, 2014), and are also subject to higher rates of warming than other ecosystems globally (IPCC, 2014). Higher temperatures stimulate microbial activity and can lead to greater soil nutrient availability and heterotrophic C losses to the atmosphere (Rustad and others, 2001), creating a feedback loop between climate warming and C loss (Crowther and others, 2016). In arctic regions, climate warming is also linked to expansion of woody vegetation and increases in biomass and litter (Stow and others, 2004; Tape and others, 2006; Myers-Smith and others, 2011; Naito and Cairns, 2011; Sorensen and others, 2012), which fundamentally change the physical structure, abiotic environment, and biotic interactions that historically characterize tundra ecosystems. Despite the co-occurrence of these two phenomena and their potential to modify critical ecosystem functions, we lack evidence regarding how they interact to modify soil C loss. The net effect of these interactions remains a key unknown in our predictions of how warming and soil C turnover will feedback to a warmer climate.

Due to the projected increase of warming in arctic regions, the temperature response of these ecosystems is critical for our understanding of global C cycling (Rustad and others, 2001; Lu and others, 2013). Soil respiration, a primary pathway of ecosystem C loss (Bardgett and others, 2008), generally increases with warming, but may return to non-warmed levels after several years of experimental warming (Allison and others, 2010). In northern latitudes, by contrast, soil microbial respiration does not acclimate to higher temperatures (Hartley and others, 2008; Ravn and others, 2017), suggesting that prolonged warming may enhance soil C loss. However, complex relationships and feedbacks between plant communities, soil biota, moisture, and organic matter may counterbalance enhanced heterotrophic C losses (Sistla and others,

2013). For example, warming enhances nitrogen (N) and phosphorus (P) mineralization (Lu and others, 2013), which can heighten competition between plants and microbes for nutrients, and in some cases stimulate plant productivity (Melillo and others, 2011). However, greater mineralization also suggests an increase in microbial decomposition and release of C, underscoring the tight and intricate coupling of these biogeochemical cycles (Eliasson and others, 2005). Further, the dearth of long-term experimental field data, specifically in northern latitudes, continues to obscure our understanding of ecosystem responses to warming (Knorr and others, 2005; Rinnan and others, 2008; Sistla and others, 2013). In addition, the effects of warming and global change often cascade through the soil profile (Mack and others, 2004; Sistla and others, 2013), raising questions about the responses of soil in deeper horizons, that are often overlooked in high latitude ecosystems. Thus, while warming may enhance heterotrophic activity and C losses, interactions with plant communities, soil biota, and organic matter at depth complicate predictions of soil C dynamics.

Similarly, the ecological consequences of range expansions of woody plants in high latitude ecosystems may lead to large scale changes in ecosystem fluxes (Sturm and others, 2005; Wookey and others, 2009; Myers-Smith and others, 2011; Rundqvist and others, 2011; Bonfils and others, 2012; Cahoon and others, 2012; Loranty and Goetz, 2012). In Subarctic Sweden, we see an inverse relationship between plant biomass and soil C storage. In areas of highly productive mountain birch forest, soils store less C than the soils beneath their less productive counterparts; a consequence of a higher growing season input (litter) from birch which facilitates the decomposition of old soil organic matter (SOM) (Hartley and others, 2012). Furthermore, shrubs and trees produce a larger quantity of higher quality litter (Weintraub and Schimel, 2005a), increasing the potential for soil priming, whereby new plant-derived C inputs

(i.e. leaf and root litter, exudates) enhance the decomposition of old SOM (Kuzyakov and others, 2000; Kuzyakov, 2002; Nottingham and others, 2009). Litter inputs also provide soil nutrients, like N and P that may enhance microbial activity (Buckeridge and others, 2010). Thus, by creating a favorable environment for decomposition (Bonfils and others, 2012) and increasing labile C inputs (Hartley and others, 2012), the expansion of high productivity species into heath and tundra ecosystems may reduce soil C stocks.

Due to the specific ways in which warming and woody vegetation encroachment modify microbial activity and biogeochemical cycles, these two factors may interact to further exacerbate soil C losses. Woody biomass expansion has already been linked to warming (Sturm and others, 2001; Tape and others, 2006; Elmendorf and others, 2012a; Elmendorf and others, 2012b; DeMarco and others, 2014) suggesting that the two phenomena should not be viewed in isolation of each other. Warming may increase the activity of soil microbes (Melillo and others, 2002; Allison and others, 2010) and in conjunction with more labile litter from shrubs (Weintraub and Schimel, 2005a), the interaction between woody expansion and warming may enhance soil C losses. Emphasis on individual factor effects may overlook potential synergisms between warmer temperatures, increased C inputs, and changes in C chemistry. These interactions present a critical unknown in predicting C storage and dynamics in high latitude ecosystems with fundamental implications for global climate.

Here we present results from a 16-year warming and litter addition field manipulation of wet heath tundra in the Swedish Subarctic. To investigate how warming and litter addition interact to modify C and N stock and turnover in subarctic soils, we assessed soil nutrient concentrations, quantified soil C and N stocks, and characterized microbial biomass C,N, and P and microbial activity (soil respiration, extracellular enzyme assays) in the entire soil profile. We

hypothesized that warming and litter addition would decrease soil C stocks because warming alleviates thermal constraints, litter alleviates energetic constraints, and together they synergistically interact to enhance microbial activity. We also hypothesized that effects of warming and litter addition would be linked to litter and root input and would thus decline with greater soil depth.

Materials and Methods

Experimental design – We sampled soils from a manipulative, long-term field experiment near Abisko Scientific Research Station in subarctic Sweden (68° 21'N, 18° 49'E), where mean air temperatures during June-August are on average 10.0°C, and the coldest month is February with -10.0°C. The mean annual air temperature is 0.2°C and the mean annual precipitation is 337 mm (1986-2015, Abisko Scientific Research Station 2016). Soils have a near-neutral pH of 6.9, and are highly organic with an average SOM content of 88.5% in the top 10 cm (Rinnan and others, 2008). Dominant plant species include graminoids like *Carex vaginata,* deciduous low shrubs like *Vaccinium uliginosum*, evergreen low shrubs like *Empetrum hermaphroditum* and *Andromeda polifolia*, and mosses (Sorensen and Michelsen, 2011). Low shrub is covering about 23% of the land area in the Low Arctic (Bliss and Matveyeva, 1992) and forms a mosaic of wetter and drier heath types in which various types of low and dwarf shrubs together with grasses, sedges and mosses dominate. The investigated heath is the wetter end of this continuum of heath tundra types.

The experiment, initiated in 1999, consists of a fully factorial completely randomized block design crossing warming and birch (*Betula pubescens* ssp. *tortuosa*) litter addition in 1m^2 plots

of wet heath, replicated across six experimental blocks. These manipulations simulate predicted increases in temperature (IPCC, 2014), and expansion of local deciduous species (Myneni and others, 1997; Rinnan and others, 2007; Myers-Smith and others, 2011; Hartley and others, 2012) in this region. Litter of Mountain birch (*Betula tortuosa*) was chosen for the experiment as this species is forming the tree-line in most of the Scandinavian subarctic, and is expected to expand into tundra areas. The addition of litter is simulating the impacts of increased nutrient input with this litter source. The added litter contains approximately 518 mg C g^{-1} dry weight, 9.8 mg N g^{-1} dry weight and 1.2 mg P g^{-1} dry weight. These values are equivalent to 46.6 g C, 0.88 g N and 0.108 g P m^{-2} added annually with litter, or a total addition of 745 g C, 14.1 g N and 1.73 g P m^{-2} during the 16 years with experimental manipulations. Warming is achieved using open top plastic tents (Ravn and others, 2017). The open top tents have been erected in Spring (late May) and removed by the end of Summer (late August or early September) each year. These tents raise summer air temperatures by 3°C, and increase sub-canopy and soil temperature by 1°C (Sorensen and Michelsen, 2011). In 2015, ambient sub-canopy and soil temperatures were increased by 1.0 ± 0.2 °C and 0.9 ± 0.1 °C, respectively ($p < 0.001$) in warming compared to control plots during the growing season (26 June to 28 August). Addition of birch litter (90 g dry weight/m^2) collected from the surrounding open birch forest-tundra occurs annually during three weeks in the period from late August to mid-September to mimic natural litter input amounts and timing at the end of the growing season.

Soil Collection – In July 2015, we sampled soils at 4 depths (0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm) using a standard soil auger. The organic soil profile is approximately 20 cm deep and underlain by large stones or bedrock. In each treatment plot, we sampled two cores measuring 37

mm in diameter, weighed each individual 5 cm depth section, and immediately removed roots by hand. We froze approximately 2 g fresh weight soil for subsequent enzyme analysis, used 4 g fresh weight to determine soil microbial biomass and extractable nutrients, and shipped the remainder of soils on ice back to the University of Copenhagen for further analysis.

Microbial Biomass C, N and P – We used a standard chloroform fumigation method (Michelsen and others, 1999) to determine microbial biomass C, N and P from 3 soil depth-intervals (0-5 cm, 5-10 cm, 10-15 cm) from each treatment. We used approximately 2 g root free, fresh weight soils and 20 mL of 0.1M K_2SO_4 for both the fumigated and unfumigated extractions. Extracted samples were frozen and shipped to University of Copenhagen on ice for total dissolved C (TDC) analysis on a Shimadzu TOC-5000A (Shimadzu, Kyoto, Japan) and total dissolved N (TDN) and total dissolved phosphorus (TDP) analysis on a flow injection analyzer (FIASTAR 5000, Höganäs, Sweden). Unfumigated extractions were also analyzed for ammonium (NH_4^+), nitrate (NO_3^-), and phosphorus (PO_4^{3+}), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON).

Fine Root Biomass – We removed roots manually from each sample. Fine roots (<1 mm diameter) were separated from coarse roots, cleaned, and dried at 70°C for 48 hours, and weighed for total biomass.

Soil Incubations for CO_2 analysis –We created mesocosms to measure respiration by adding 5 g root-free, fresh weight soil at field moisture capacity to a 50 mL glass vial. We pre-incubated soils for 7 days to reduce the potential impact of initial disturbance effects from transport and

set-up on flux measurements. Incubations were conducted over 5 days to account for the size of the vials and thus significant changes in headspace gas concentrations. We chose 5°C to simulate an environment closer to that of soil temperatures than air temperatures. June-August soil temperatures at the site ranged from 3-13°C at 3 cm depth (Pedersen et al., 2017) and our incubations included soils from deeper layers, with lower temperatures. We capped vials with air-tight, crimp caps fitted with rubber septa. To take a measurement, we vigorously mixed head space, sampled 0.5 mL of gas and analyzed for CO_2 concentrations on an infrared gas analyzer (LI-COR 840A, LI-COR Lincoln, Nebraska, US). Over a 5-day incubation period, we measured CO_2 three times to determine rates of CO_2 flux from soil kept at 5°C. For our analysis, we excluded samples in vials that leaked gas over the course of the incubation period. To normalize for the amount of soil and C in each sample, we expressed flux rates as μmol $CO_2 \bullet g^{-1}$ soil C day^{-1}.

Soil Analyses – Soil moisture was determined by weight difference before and after drying at 70 °C until stable. The latter weight was used to calculate soil bulk density based on volume-specific samples. To determine soil C and N content, we dried root-free samples at 70°C, and ground them to a fine powder. Soils were then weighed into tin capsules and measured for total C and N on an Isoprime isotope ratio mass spectrometer coupled to a Eurovector CN elemental analyzer. To determine bulk density, we used the initial weight of each soil core divided by the total volume of the core, separately for each depth.

Extracellular enzyme assays – To quantify the rate limiting step of SOM decomposition, we measured microbially derived extracellular potential enzyme activity for 6 enzymes using a

modified fluorometric assay (Bell and others, 2013). We measured three C-cycling enzymes (β-glucosidase, BG; β- xylosidase, BX; and cellobiohydrolase, CB) and three N and P-cycling enzymes (N-acetyl-glucosaminidase, NAG; acid phosphatase, AP; leucine-amino-peptidase, LAP) using both methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (AMC) linked substrates (Table 1). Six samples from 0-5 cm depth were not assayed for enzyme activity due to the low bulk density and thus limited mass of soil at this depth. Because we flushed soils with substrate and eliminate moisture and diffusion constraints on decomposition, we can only assess potential activity. Data are expressed per gram dry soil C (nmol • g soil C^{-1} h^{-1}).

Data analysis – All data were analyzed using a linear model. For our initial analysis, we analyzed total C and N across the entire soil profile. We crossed litter and warming, and included block as a term in the model. Following the results of this analysis, we analyzed data from each depth separately to elucidate the mechanisms driving the overall patterns in C and N stocks. For each depth, we crossed litter and warming (Table 3.2), and included block as a term in the model (R for Mac, version 3.1.2). Nonsignificant interactions and block remained in the model if $p < 0.2$. After testing for the normality of each model's residuals and heteroscedasticity, we used an F test to calculate p values using a significance level of $\alpha = 0.05$ and a near-significance level of $p < 0.10$. Data were square root or log transformed where needed. Further, we performed a 3-way ANOVA crossing litter, warming, and depth including plot as an error term to account for the non-independence of each depth within a given core (Table S3.2).

Results

Soil characteristics

Warming significantly reduced total soil C by 872.2 ± 263.3 g/m^2 ($F_{1,16}$ = 4.928, p = 0.041) and N by 43.9 ± 15.7 g/m^2 ($F_{1,16}$ = 7.080, p = 0.017) stocks across the entire soil profile (Fig. 3.1). These losses represent 18. 6 ± 6.4% and 18.8 ± 6.9% reductions in C and N stocks, respectively. No changes in soil bulk density were noted with any experimental treatments (Table S3.1). For C, the stock change was primarily driven by C losses at 5-10 cm depth ($F_{1,16}$ = 6.418, p = 0.022), however warming did not significantly increase N losses at any specific depth interval (Table 1; Table S2).

Soil moisture content ranged from 45 to 90% of wet weight, but did not significantly change with either warming or litter addition, in contrast to previous investigations of the same plots (Rinnan and others, 2008). Additionally, we observed low concentrations of NO_3^- at all depths (Fig. 2), with litter addition significantly decreasing NO_3^- concentrations at 5-10 cm depth ($F_{1,16}$ = 4.577, p = 0.048) while litter addition and warming interacted to modify NO_3^- concentrations at 10-15 cm depth ($F_{1,20}$ = 6.307, p = 0.021). We saw no significant effects of warming or litter addition on NH_4^+ concentrations (Fig. 3.2). At 0-5 cm depth, litter additions increased PO_4^{3-} concentrations ($F_{1,15}$ = 5.197, p = 0.038) and interactions with warming intensified this trend ($F_{1,15}$ = 10.857, p = 0.005).

Warming increased DOC concentrations at 5-10 cm depth ($F_{1,16}$ = 5.636, p = 0.030), while at 10-15 cm depth, interactions between litter and warming reversed this trend ($F_{1,20}$ = 4.878, p = 0.039) (Fig. 3.3). Warming and litter also interacted to enhance DON concentrations in the top 5 cm of soil ($F_{1,15}$ = 4.915, p = 0.004).

Microbial biomass and fine root biomass

We saw no significant main factor effects for fine root biomass (Fig. S3.1) and microbial biomass C and N (Fig. 3.4), but warming reduced microbial C:N in 0-5 cm soil, suggesting slightly increased bacterial dominance of the microbial community (Fig. S3.2). Warming and litter interacted to increase microbial P biomass in the upper horizon of soil ($F_{1,15}$ = 7.110, p = 0.018). At 5-10 cm depth, warming alone increased microbial P ($F_{1,20}$ = 11.530, p = 0.003). However, there were no microbial responses at greater depth, leading to significant interactions between warming, litter, and depth on microbial N and P (Table S3.2).

Heterotrophic respiration and enzyme activity

Warming increased heterotrophic respiration rates in soils at both 5-10 cm ($F_{1,4}$ = 17.397, p = 0.014) and 10-15 cm ($F_{1,5}$ = 7.092, p = 0.045) depths (Fig. 3.5).

We observed greater responses to warming and litter addition in N and P cycling enzymes than in those involved in C cycling (Figs. 3.6, 3.7). We found no significant differences with warming or litter addition in BG and CB potential enzyme activity at any depth (Fig. 3.6). At 0-5 cm depth, warming stimulated NAG ($F_{1,15}$ = 6.313, p = 0.036), AP ($F_{1,14}$ = 6.284, p = 0.025), and LAP ($F_{1,10}$ = 8.113, p=0.017) potential enzyme activity, while litter addition stimulated only NAG potential activity ($F_{1,15}$ = 5.318, p = 0.036) (Fig. 3.7). Litter addition stimulated AP potential enzyme activity at 5-10 cm ($F_{1,19}$ = 9.001, p = 0.007) and 10-15 cm ($F_{1,16}$ = 5.720, p = 0.029). At 0-5 cm depth, litter addition stimulated BX activity ($F_{1,19}$ = 4.521, p = 0.047), and an interaction of litter and warming decreased NAG potential activity at 5-10 cm depth ($F_{1,18}$ = 5.596, p = 0.029).

Discussion

Our results demonstrate that long-term experimental warming may reduce soil C and N stocks in subarctic ecosystems, but raise questions about the mechanisms driving this response (Fig. 1). Soils lost C and N in similar proportions (Table 3.1), suggesting that warming enhances the rate of microbial processes like decomposition in these soils. Broadly, these losses suggest that warming stimulates overall mineralization more than greater inputs of leaf and root litter.

While the stimulatory effects of warming are well documented (Rustad and others, 2001), the results of this study suggest that the primary effects of warming manifest only at specific depths within the soil profile. The overall reduction in total profile C stocks from warming appears mainly at 5-10 cm depth (Fig. 3.1). While we did not observe a significant experimental effect on C stocks at 15-20 cm depth, litter and warming interacted to reduce C stocks by over 50% (Table 3.1, Fig. 3.1). In the 5-10 cm horizon, warming enhanced both heterotrophic C losses (Fig. 3.5) and DOC concentrations (Fig. 3.3), suggesting that warming decreases C stocks through both lateral and atmospheric pathways. Indeed, soil water in the same experimental plots in 2015 showed higher DOC concentrations in warmed plots (Pedersen and others, 2017) while four years prior, warming stimulated both ecosystem and soil respiration (Ravn and others, 2017). Taken together, these studies suggest that while our results capture important effects of warming, these responses may vary across seasons and years.

As expected, warming stimulated microbial respiration rates (Fig. 3.5), but results from this study indicate that experimental treatments for 16 years have not modified microbial biomass (Fig. 3.4). This suggests that increased respiratory and lateral C losses with warming may be linked to heightened microbial activity, not population growth. However, microbial biomass and respiration may respond to seasonal and annual variation, suggesting that these

single point measurements may not describe the full effects of warming. Additionally, increased respiration (per g soil C) suggests greater lability of C substrates in warmed soil, providing evidence for soil priming via greater plant biomass and soil inputs in warmed plots. Thus, despite increasing biomass and C inputs with warming (Sorensen and others, 2012), we observed greater turnover that resulted in a net loss of C.

Observed N losses complicate the interpretation of biological changes on soil C turnover induced by warming. While changes in C stocks seem largely regulated by microbial processes at 5-10 cm depth, results here do not suggest a single mechanism by which warming modifies N losses in any horizon. Indeed, reductions in N stocks appear to be an emergent effect of ecosystem warming, where incremental losses across the soil profile accumulate to reduce total N. In contrast to the overall patterns of N loss, warming decreased NAG activity at 0-5 cm depth (Fig. 3.7). Because microbes produce enzymes in response to nutrient constraints (Sinsabaugh and others, 2008), this suggests that warming may decrease microbial N demands. However, N-cycling enzyme activity is often highest during the late winter (Wallenstein and others, 2009; Sistla and Schimel, 2013), prior to our measurements presented here, suggesting that our study may have overlooked warming-mediated pulses of enzyme activity. We also see no changes with respect to N immobilized in microbial biomass (Fig. 3.4). Conversely, in parallel with increased DOC we observed a trend of increased DON with warming at 5-10 cm depth (Fig. 3.3), suggesting that lateral N loss may be responsible for reducing N stocks. In addition, our single time point measurements may not have captured the full effect of warming on N losses. Warming could increase losses during spring melt events, when lateral losses of N to streams and rivers is highest (McNamara and others, 2008; McClelland and others, 2014). In highly N-limited systems (Sistla and others, 2012), these results suggest that complex, competitive

interactions between plant roots and soil microbial communities may contribute to observed patterns of C loss, despite increased plant growth, and thus aboveground N demand, with climate change (Lett and Michelsen, 2014). Furthermore, although N may be lost from the soil due to warming, the presence of roots throughout the soil profile may prevent ecosystem leaching losses of N, although lateral transfers and increased plant uptake cannot be excluded.

Warming did, however, modify key elements of the P cycle. We observed an increase of microbial immobilization (Fig. 3.4), and a simultaneous decrease in AP potential activity (Fig. 3.7) in the upper soil layer, likely due to microbial down-regulation of AP production. These results suggest that warming may periodically have increased soil phosphate availability, which is also supported by higher moss P concentration with warming (Sorensen and Michelsen, 2011) and higher available P in plots with warming and litter addition combined (Fig. 3.2). Indeed, in tundra soils, most plant P comes from the breakdown of SOM (Giblin and others, 1991) by microbial (Weintraub and Schimel, 2005b) or, in some cases, plant derived phosphatases (Moorhead and others, 1993; Moorhead and Linkins, 1997), suggesting that greater microbial activity or greater vegetative biomass may ultimately be driving P dynamics in warmed soils. Further, additions and greater availability of P have been linked to both reductions in C stocks (Mack and others, 2004; Hartley and others, 2010) and changes in SOM chemistry (Bradford and others, 2008), emphasizing the tight coupling of these cycles.

In further support of this, litter addition led to lower extracellular potential enzyme activity for AP (Fig. 3.7), suggesting that addition of birch litter, which is particularly P-rich (Sorensen and Michelsen, 2011), increases P availability and may reduce competition for labile C and P by microbes. Over the course of the 16 year experiment, we added 745 g C, 14.1 g N and 1.73 g P m^{-2} as litter. Our findings suggest that these C and N inputs did not increase C or N

stocks or SOM (Fig. 3.1, Fig. S3.3). However, we did not observe priming effects, as the C from added litter does not appear to stimulate decomposition of SOM. Future measurements immediately preceding and following litter addition may further illuminate the role of this litter and develop our temporal understanding of soil P dynamics. Additionally, previous studies found that litter alone and its interaction with warming lowered soil moisture content (Rinnan and others, 2007; Rinnan and others, 2008; Ravn and others, 2017). Added litter may increase interception of rainfall and subsequent evaporation, which could lower soil moisture. While we did not observe this trend in soil moisture, a legacy of this reduction in soil moisture may partly explain the elevated concentrations of DOC, DON, and rates of heterotrophic respiration reported here (Figs. 3.3, 3.5). We also observed lower nitrate concentrations (Fig. 3.2) reinforcing how litter stoichiometry may influence nutrient demands and competition between plant roots and soil microbial communities. However, although litter addition may modify these interactions, it does not appear to promote broad scale changes in soil C and N storage.

Similarly, interactions between litter additions and warming produced a biogeochemical signature distinct from litter and warming alone, specifically in regards to N and P cycling. At 10-15 cm depth, their interactions increased nitrate levels (Fig. 3.2), while in the top 5 cm of soil they interacted to increase phosphate, microbial P, and DON concentrations (Figs. 3.2,3.3,3.4). In addition, they interacted to reduce NAG activity at 5-10 cm depth (Fig. 3.7). Taken together, these suggest that warming and litter interact to modify competitive interactions between plant roots (i.e. their release of exudates) and microbial communities to produce depth-specific patterns in N availability (Figs. 3.2, 3.3). Further, while warming appears to increase activity and litter appears to supplement soil nutrients, the expected synergism is noted only in the top 5 cm of soil for DON and at 10-15 cm depth for nitrate. This suggests that the combination of

warming and birch litter addition modifies C and N cycling, but introduces new constraints to their loss.

Our findings add novel insights to the growing body of literature exploring the way global change will disrupt arctic ecosystems, specifically in regards to soil C storage. A recent synthesis study (Crowther and others, 2016) concluded that across sites globally, near-surface C losses may be significant and in the order of 0-0.3 kg m^{-2} yr^{-1} per degree warming depending on the initial C stock. While this study only includes few arctic sites, our loss rates (approximately 0.05 kg m^{-2} yr^{-1} fall within the lower range of this estimate, which may reflect a smaller initial C stock at this site. It should be noted that some recent synthesis studies (Crowther and others, 2016; van Gestel and others, 2018) which discuss potential soil C losses due to warming both focus on the upper 10 cm of soil. Although we have measured the entire soil profile in our study, it was only 20 cm before reaching the bedrock, and we should therefore only with caution extrapolate our results to areas with deeper soil profiles. For true arctic studies, some have reported a significant C loss (Welker and others, 2000; Welker and others, 2004), while most studies have not detected changes in C stocks (Strebel and others, 2010; Lamb and others, 2011; Sistla and others, 2013). Our results highlight the heterogeneity of arctic ecosystems, while underscoring their potential vulnerability to global change. Investigation of our same plots 4 and 10 years prior showed no differences in C or N stocks, but provided evidence of stimulated microbial process rates that foreshadowed the observed losses in the present study (Rinnan and others, 2007; Rinnan and others, 2008; Ravn and others, 2017). While accelerated loss of C due to continued warming cannot be excluded, the results presented here call for caution in the evaluation of changes in soil C stocks, particularly in areas with uneven soil depth and bedrock, and patchy vegetation composition. In addition, this study underlines the potential importance of

lateral transfers as a mechanism by which C and N can be lost from these soils and highlights the continued threat of warming in northern ecosystems.

Our results also emphasize the need to study the entire soil profile repeatedly, as the biology of the tundra heath changed dramatically with depth and time. The mechanisms driving patterns across the soil profile derived from deeper soil horizons, suggesting that warming may have cascading effects through an ecosystem. Our results demonstrate that long-term warming may lead to C loss in high latitude soils, adding nuance to our biogeochemical understanding of these vulnerable ecosystems, and providing further support of long-term experimental manipulations and ecological monitoring programs.

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Table 3.1: Two-way ANOVA results of responses of soil properties, microbial biomass, heterotrophic respiration, and enzyme activity to experimental warming and litter addition. F values are shown for main factor effects (warming and litter addition) and their interaction in a two-way ANOVA for each depth. We crossed warming, and litter addition, but removed interactions if $p > 0.2$. Block remained in the model if $p < 0.2$. Significant effects are indicated in bold and by $+p < 0.1$, $*p<0.05$, $**p<0.01$.

F values and significance levels

	0-5 cm			5-10 cm			10-15 cm			15-20 cm		
Variable	**Warming**	**Litter**	**LxW**	**Warming**	**Litter**	**LxW**	**Warming**	**Litter**	**LxW**	**Warming**	**Litter**	**LxW**
Soil C ($g \cdot m^{-2}$)	1.107	0.43	---------	**6.418***	0.004	---------	0.361	0.845	---------	2.656	1.597	---------
Soil N ($g \cdot m^{-2}$)	0.117	0.015	---------	2.87	0.036	---------	0.41	0.55	---------	1.615	**3.848+**	---------
% Carbon	0.21	1.449	---------	0.934	2.727	---------	0.95	0.013	---------	0.314	1.142	---------
% Nitrogen	0.448	0.578	---------	0.589	0.021	---------	1.034	1.975	---------	0.697	2.693	---------
Soil C:N	0.749	0.041	---------	0.749	0.304	---------	0.385	1.506	---------	1.693	**5.46***	---------
Bulk density ($g \cdot cm^{-3}$)	0.523	0.001	---------	1.61	0.73	---------	0.393	0.479	---------	0.01	3.407	---------
Soil moisture (%)	0.414	2.899	---------	1.435	0.922	---------	1.372	0.067	---------	0.241	2.198	---------
Soil respiration ($\mu mol\ CO_2 \cdot g\ soil\ C^{-1}\ day^{-1}$)	0.52	0.222	---------	**17.379****	2.344	---------	**7.092***	0.551	---------	0.694	0.339	---------
NH_4^+ ($\mu g \cdot g\ soil\ C^{-1}$)	1.118	1.424	---------	0.080	**3.326+**	---------	0.495	0.220	---------	---------	---------	---------
NO_3^- ($ug \cdot g\ soil\ C^{-1}$)	2.791	2.062	---------	1.403	**4.577***	---------	2.075	**3.292+**	**6.307***	---------	---------	---------
PO_4^{3-} ($ug \cdot g\ soil\ C^{-1}$)	0.685	**5.197***	**10.857****	0.010	0.540	---------	0.398	0.031	---------	---------	---------	---------
DOC ($\mu g \cdot g\ soil\ C^{-1}$)	0.788	**2.992+**	---------	**5.636***	0.005	---------	0.000	0.929	**4.878***	---------	---------	---------
DON ($\mu g \cdot g\ soil\ C^{-1}$)	2.924	**3.912+**	**4.915***	**2.991+**	0.537	---------	1.174	0.983	**3.17+**	---------	---------	---------
Microbial C ($\mu g \cdot g\ soil\ C^{-1}$)	0.605	0.038	---------	0.236	0.647	**3.399+**	0.143	0.069	---------	---------	---------	---------
Microbial N ($\mu g \cdot g\ soil\ C^{-1}$)	1.519	0.002	**3.927+**	1.430	0.000	---------	0.135	0.000	---------	---------	---------	---------
Microbial P ($\mu g \cdot g\ soil\ C^{-1}$)	**3.993+**	**7.849***	**7.11***	**11.53****	**3.843+**	---------	2.764	0.003	---------	---------	---------	---------
Microbial C:N	**5.411***	1.037	---------	0.1	0.005	---------	0.115	0.184	---------	---------	---------	---------
Microbial C:P	**4.106+**	1.137	---------	**8.998****	**3.319+**	---------	**5.527***	0.271	---------	---------	---------	---------
Microbial N:P	0.591	**6.359***	---------	**8.167***	**8.216***	---------	2.795	0.756	---------	---------	---------	---------
Microbial C:N:P	**7.751***	0.074	---------	2.762	**8.634****	---------	0.052	1.113	---------	---------	---------	---------
BX potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	0.015	2.052	---------	0.172	**4.521***	---------	0.654	0.017	---------	0.293	1.705	---------
BG potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	0.305	0.516	---------	2.597	0.346	---------	0.324	0.327	---------	0.002	0.001	---------
CB potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	0.095	0.174	---------	0.262	2.332	---------	0.096	0.027	---------	0.032	0.256	---------
NAG potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	**6.313***	**5.318***	---------	0.001	0.075	**5.596***	1.186	0.147	---------	0.238	0.234	---------
AP potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	**6.284***	0.084	---------	0.102	**9.001****	---------	0.031	**5.720***	---------	0.121	1.273	---------
LAP potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	**8.113***	2.740	---------	0.611	0.489	---------	0.281	0.052	---------	0.000	0.830	---------

Table 3.2: Name and function for each measured enzyme (Adapted from Wallenstein and others, 2009)

Enzyme	Abbreviation	Function
β-glucosidase	BG	releases glucose from cellulose
β- xylosidase	BX	degrades hemi-cellulose
Cellobiohydrolase	CB	releases disaccharides from cellulose
N-acetyl-glucosaminidase	NAG	degrades chitin
Acid phosphatase	AP	phosphorus mineralization
Leucine-amino-peptidase	LAP	degrades protein into amino acids

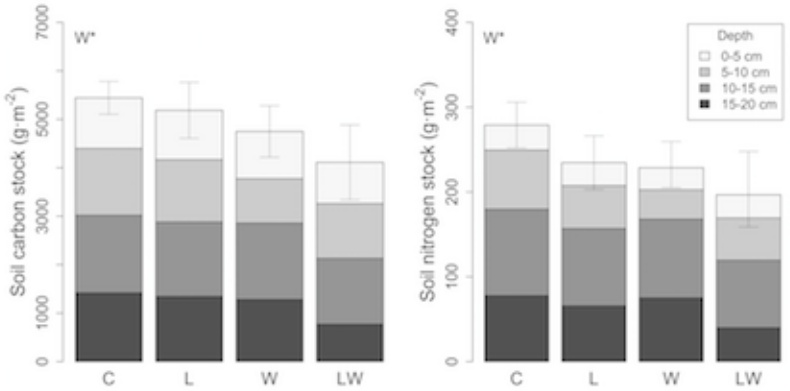

Figure 3.1: Soil carbon and nitrogen stocks by treatment - control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW), showing stocks by depth. Vertical bars represent means ± SE. Levels of significance determined by a two-way ANOVA. Significant effects are indicated by $+p < 0.1$, $*p < 0.05$.

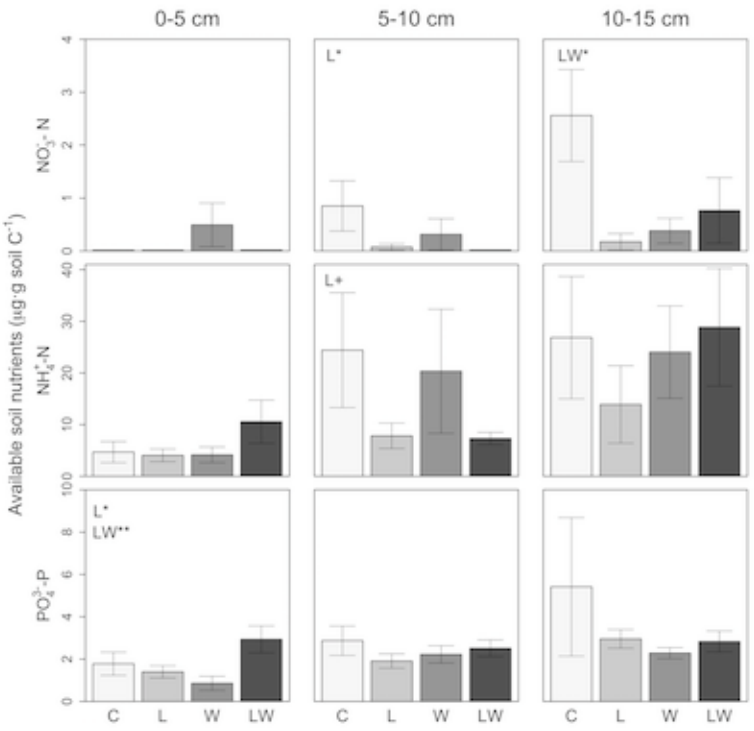

Figure 3.2: Extractable nutrient concentrations in three depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). Bars represent means ± SE. Significant effects are indicated by +p < 0.1, *p<0.05, **p<0.01.

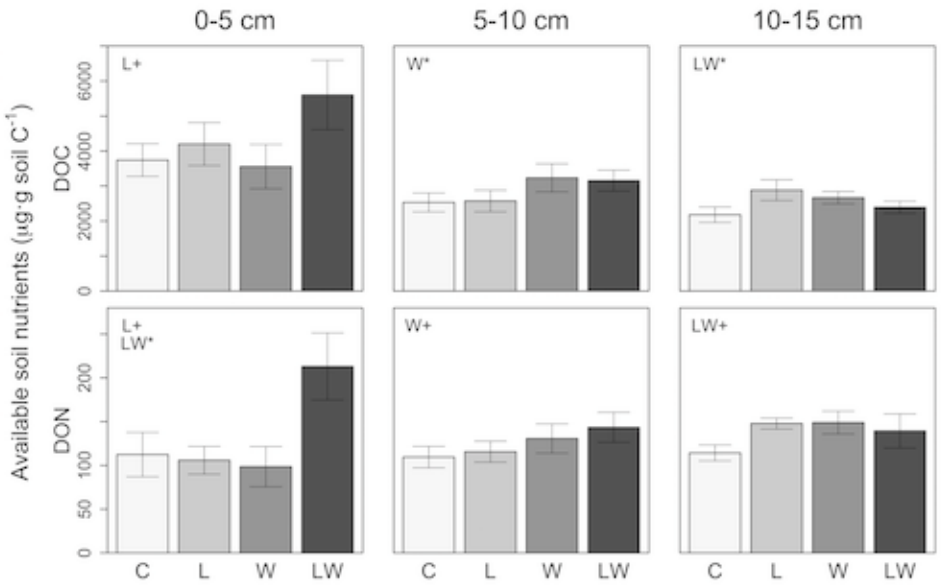

Figure 3.3: Dissolved organic carbon and nitrogen at three depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). Bars represent means ± SE. Significant effects are indicated by +$p < 0.1$, *$p<0.05$.

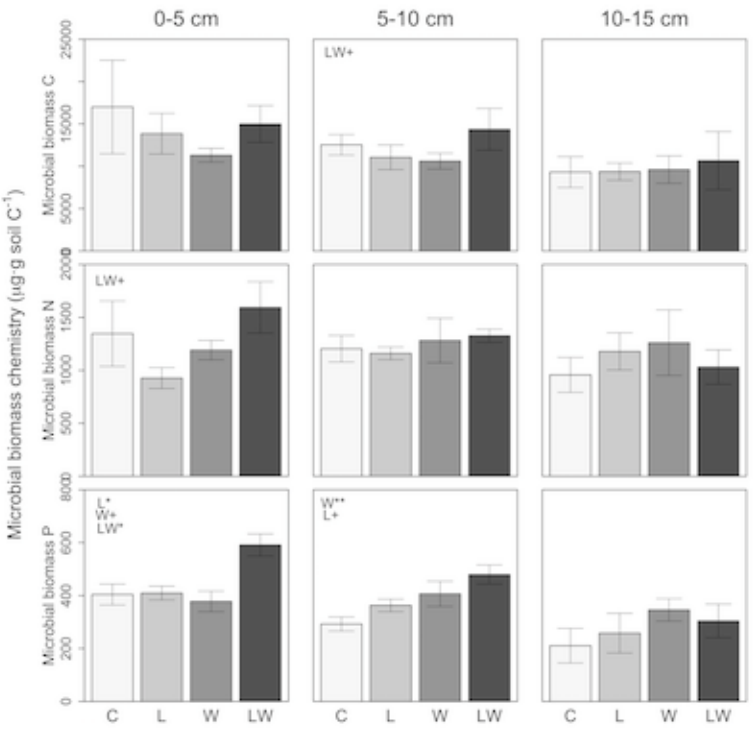

Figure 3.4: Microbial biomass carbon, nitrogen, and phosphorus by three depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming plots (LW). Bars represent means ± SE. Significant effects are indicated by +$p < 0.1$, *$p<0.05$, **$p<0.01$.

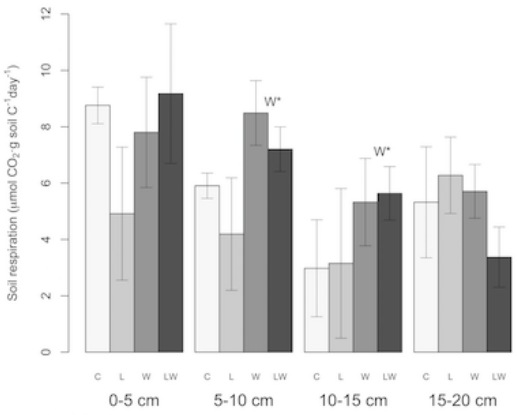

Figure 3.5: Rate of heterotrophic respiration at 4 depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). Bars represent means ± SE. Significant effects are indicated by *$p<0.05$.

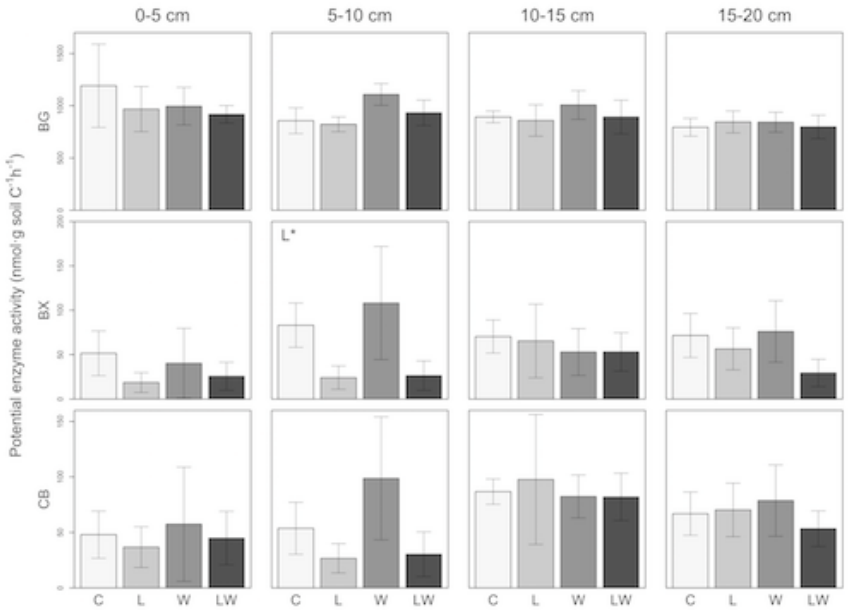

Figure 3.6: Potential enzyme activity at 4 depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). BG, BX, and CB are a carbon cycling enzymes. Bars represent means ± SE. Significant effects are indicated by *p<0.05.

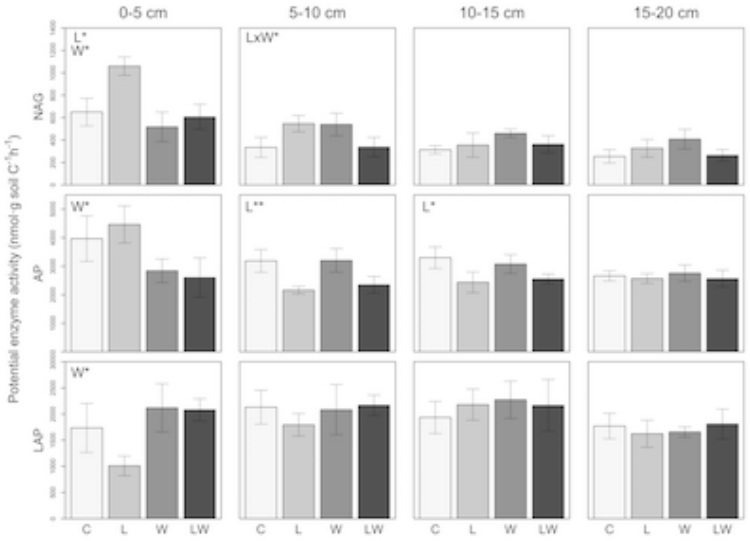

Figure 3.7: Potential enzyme activity at 4 depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). NAG, AP, and LAP are involved in nitrogen and phosphorus acquisition. Bars represent means ± SE. Significant effects are indicated by *p<0.05, **p<0.01.

CHAPTER 4

SHRUBS MODIFY SOIL C DYNAMICS IN ARCTIC TUNDRA THROUGH SOM QUALITY AND ROOT-INDUCED MICROBIAL ACTIVITY[3]

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Abstract

The response of arctic ecosystems to global change will have critical effects on future climate. Climate warming has already triggered the expansion of shrubs across tundra, raising questions about how shrubs will affect microbial activity and thus, ecosystem carbon (C) balance. We investigated whether shrubs promote microbial activity relative to other tundra vegetation by producing: 1) more labile SOM and/or 2) root systems with a greater stimulatory effect on microbial activity. We established a root in-growth core experiment, where soil cores from shrub and non-shrub areas were reciprocally-transplanted, and we quantified root length, soil C, heterotrophic respiration, and extracellular enzyme activity after two years. We found that shrub-derived SOM had higher microbial activity, and higher root-associated DOC concentrations, but only in upper organic soils. Further, we found that roots appeared to stimulate microbial activity across all horizons and both plant communities, but the relationships between microbial activity and soil C content were depth and community dependent. In organic horizons, heterotrophic activity was positively correlated with soil C content, and in the mineral horizon, it was negatively correlated to C content; however, shrub roots tempered this negative relationship. Our findings suggest that shrubs have differential effects on soil C loss and retention with soil depth. Shrubs may enhance microbial activity in the upper organic horizon by virtue of labile litter, but microbial byproducts could lead to greater C retention. In the mineral horizon, SOM appears vulnerable to root-induced microbial activity, but shrub roots may have weaker priming effects. Broadly, our results suggest that shrubs enhance soil C cycling and retention on a mass basis, but the long-term effects of this on the C balance remain unclear.

Keywords: shrub expansion; soil organic matter, arctic ecology, rhizosphere priming

Introduction

High latitude ecosystems store a majority of the world's soil carbon (C) (Hugelius et al. 2014; Tarnocai et al. 2009), but are experiencing an unprecedented rise in temperature (IPCC 2014). One consequence of warmer temperatures is an increase in the range and cover of deciduous shrub species (hereafter shrubs) (Beck et al. 2011; Loranty & Goetz 2012; Myers-Smith et al. 2011; Naito & Cairns 2011; Sturm et al. 2001; Tape et al. 2006; Tape et al. 2012), which may have profound effects on the C cycle of tundra ecosystems (Parker et al. 2015). Shrub expansion increases productivity (Shaver & Chapin 1991), but we know much less about the effect of shrubs on vast stores of soil C, despite the critical importance for global climate. While shrubs increase belowground productivity (Iversen et al. 2015; Sistla et al. 2013), and hence input rates of C to soils, shrubs are also linked to higher soil microbial activity and higher loss rates of soil C (Phillips Chapter 2, (Grogan & Chapin 1999; Grogan & Jonasson 2005)), making it critical to understand how shrubs modify belowground interactions to determine soil C stocks.

A key challenge is to resolve the mechanisms by which arctic plants, and shrubs in particular, influence soil microbial activity and the formation and loss of soil organic matter (SOM), as these relationships will determine the long-term magnitude and stability of soil C stocks. Most critically, there are two ways that shrubs could modify relationships in the soil system relative to other tundra vegetation. First, shrubs may change the quality of SOM (Mack et al. 2004; Sistla et al. 2013), thereby affecting its susceptibility to microbial decomposition and loss. Second, shrubs, may modify interactions with soil microbial communities in the rhizosphere (Iversen et al. 2015; Myers-Smith et al. 2011; Sullivan et al. 2007), and determine the rate at which both recent plant-derived C and existing SOM are decomposed. Interestingly, if these phenomena interact, shrub effects may further amplify loss rates of SOM. There is a

pressing need to understand these individual drivers in order to best predict over what time scales and by what mechanisms arctic soil C stores may change in the future.

Shrubs may produce higher quality SOM relative to non-shrub species as a result of the long-term interactions of shrub litter, microbial activity and processes that stabilize C (DeMarco et al. 2011). Such differences in SOM may be due to the composition of plant C inputs (Buckeridge et al. 2010) and the microbial assemblages that comprise shrub soils and drive decomposition processes (Wallenstein et al. 2007). Indeed, the composition of the vegetative community is a primary determinant of SOM decomposability in tundra (Hobbie 1996; Shaver et al. 2006). Specifically, shrubs are highly productive and thus support higher rates of leaf litter inputs (McLaren et al. 2017), thereby increasing the rate of C inputs to the soil system. Shrubs also produce labile litter (Weintraub & Schimel 2005), which may enhance decomposability of SOM by reducing the soil C:N ratio and increasing nitrogen (N) mineralization (Weintraub & Schimel 2003), and thus promote long term losses of C (Mack et al. 2004; Parker et al. 2015). Shrubs may further facilitate changes to SOM quality by trapping snow and insulating soils during the winter (Sturm et al. 2005b), which may maintain microbial activity and C mineralization. Together, shrubs appear to modify biophysical conditions for SOM formation and decay, to favor heterotrophic activity which may stimulate soil C loss.

The second pathway by which shrubs may enhance soil C loss is by their direct interactions with microbes in the rhizosphere. Arctic plants store a large proportion of their biomass belowground (Chapin et al. 1980; Iversen et al. 2015), and shrubs in particular represent between 30 % - 98 % total vascular, belowground biomass (Shaver & Chapin 1991). Shrubs produce long-lived roots relative to the perennial roots of graminoids (Sullivan et al. 2007). While lower rates of root turnover reduce rates of root C inputs to soils, these long-lived roots

may exude labile C to the rhizosphere (Iversen et al. 2015; Pries et al. 2013), counterbalancing the slow rate of biomass turnover. Arctic microbial communities appear to be limited by available C (Melle et al. 2015), and thus, may respond to such inputs of labile plant (litter and exudates) C to fuel their activity (Phillips Chapter 2). These same inputs also may stimulate C loss, via priming, whereby labile plant C inputs disproportionally stimulate microbial communities to decompose SOM (Blagodatskaya et al. 2011; Blagodatsky et al. 2010; Nottingham et al. 2009). Together, these direct interactions with shrub roots may stimulate microbial activity.

Further, shrubs may indirectly enhance soil C loss through their associations with root symbionts. Deciduous shrubs allocate plant C to ectomycorrhizal (ECM) fungi (Clemmensen & Michelsen 2006; Myers-Smith et al. 2011), which produce a suite of hydrolytic and oxidative enzymes that decompose SOM (Finlay 2008; Lindahl & Tunlid 2015), and may aid in depleting soil C stocks (Parker et al. 2015). However, shrubs in the genus *Alnus*, also associate with N-fixing bacteria (Tape et al. 2006; Tape et al. 2012), which can locally enrich soil N pools (Mitchell & Ruess 2009a) and as a result, may suppress decomposition (Frey et al. 2014). Thus, shrubs, via their symbionts, may either stimulate and suppress heterotrophic activity, providing an additional layer of complexity in our understanding of how shrubs may affect soil C dynamics.

Here, we examine two mechanisms by which shrubs may affect soil C cycling in tundra soils of arctic Alaska. We sought to understand if shrubs enhance soil C loss through an increase of SOM quality or by stimulating microbial activity in soil via changes to rhizosphere processes. In order to isolate the longer-term effects of shrubs on SOM decomposability from those on root – induced microbial activity, we designed a reciprocal transplant root in-growth experiment. We

collected soils under shrub and non-shrub vegetation to capture differences in SOM produced over a long period of time. We removed roots from these soils, repacked soils into root ingrowth cores and reciprocally-transplanted them into the opposite community type. First, we hypothesized that cores containing shrub-derived SOM (i.e., originating from shrub areas) would support higher rates of microbial activity, and thus a larger reduction of soil C when compared to those with non-shrub-derived SOM (i.e., originating from non-shrub areas). Secondly, we hypothesized that microbial activity would be positively related to root growth in all soils. However, we expected this relationship to be magnified in soils transplanted in shrub areas due to differences in root characteristics and growth rates, relative to non-shrub species. In both cases, we considered microbial activity as a function of heterotrophic respiration and extracellular enzyme activity, and that soil C loss would manifest as declines in soil % C and increases in DOC.

Materials and Methods

Experimental design

To understand how arctic shrub and non-shrub communities differentially affect soil C cycling, we established a reciprocal transplant experiment using root in-growth cores in moist acidic tundra on the North Slope of the Brooks Range in arctic Alaska. We established 16 10 x 10 m paired plots (shrub and non-shrub) within 20 m of each other, distributed across 7 sites within 50 km of Toolik Lake Field Station (68° 38' N, 149° 36' W) in July 2014. Shrub plots were dominated by deciduous shrubs (either *Betula nana* or *Alnus frusticosa*; hereafter birch and alder respectively), while non-shrub plots contained a variety of species including *Carex bigelowii, Eriophorum vaginatum, Empetrum nigrum, Vaccinium uliginosum, Vaccinium vitis-*

idaea and *Rhododendron lapponicum,* but were not dominated by any single species. For each shrub species, we established paired plots at 4 sites; one site included plots of both species. In each plot pair we sampled four soil cores (7 cm diameter, 17-30 cm depth, depending on thaw depth), two cores in the shrub plot and two cores in the non-shrub plot (Fig. 4.1), yielding a total of 64 experimental cores. We transported intact soil cores to the field station for processing, where they were stored at 4°C.

To process cores, we measured and recorded the length of each soil horizon (upper organic, lower organic, mineral). The upper organic was distinguished from lower by the degree of decomposition and compaction of the organic matter. Because of the spatial heterogeneity of tundra soils, not all cores contained all three horizons (all three n = 5, upper and lower organic n=13, upper organic and mineral =1, lower organic and mineral = 17, only upper organic = 2, only lower organic = 21, only mineral n= 4), leading to 21 upper organic samples, 56 lower organic samples, and 28 mineral samples. We separated each horizon, removed plant roots and repacked the soil into open top, 2 mm fiberglass mesh cores (7 cm diameter and variable depth). While root removal and repacking disturbed and mixed the soil, we sought to replicate the original depth and bulk density of each horizon as best as possible.

Once the cores from an individual site were repacked, we transported them back to the field where we created a 2 x 2 factorial reciprocal transplant experiment, crossing origin (shrub, non-shrub) and final location (shrub, non-shrub) within each paired plot (Fig. 4.1). One core from each shrub and non-shrub plot was replanted into its original sampling location, while the other two cores were transplanted into the opposite plot. Core installation occurred within 72 hours of soil sampling and continued over a 4-week period during the month of July.

Cores remained in the ground for 2 years, and were harvested in July 2016. As an additional comparison, we also sampled one un-manipulated soil core (hereafter control soil core) of similar diameter and depth in each plot (both shrub and non-shrub) on the same day as the experimental core removal (n=32). Due to the variable depths of our cores and thus our inability to accurately present our results on a per unit area basis, we expressed all our data per g dry soil.

Post-experiment processing

Following collection, we shipped cores on ice to the University of Georgia and immediately processed soils (all experimental and control cores) for a variety of measures. We visually separated cores into soil horizons (upper organic, lower organic, and mineral), subsampled a portion of each horizon, and removed roots by hand for respiration measurements, enzyme assays and nutrient analysis. We removed the remainder of the roots from each experimental core and used a subsample of each control core to quantify root length and biomass. We prepared soil microcosms for heterotrophic respiration immediately, and stored soil subsamples for enzyme assays at -20°C.

Heterotrophic respiration

We created 50 mL microcosms with 4 g of wet root-free soil to measure heterotrophic respiration. Soils were incubated at 10 °C for 7 days prior to gas sampling to reduce the impact of disturbance effects.

Prior to gas sampling, we briefly flushed microcosms with N_2 gas, and capped them with air tight lids outfitted with rubber septa. We then mixed the headspace vigorously, sampled 2 mL

of gas, and then analyzed CO_2 concentrations on an infrared gas analyzer (LiCor 6252, LiCor, Lincoln, NE). To determine a rate of CO_2 flux, we analyzed gas samples three times over a one-hour period. We accounted for differences in mass between microcosms by expressing flux rates as μmol $CO_2 \bullet g^{-1}$ dry soil day^{-1}.

Extracellular enzyme assays

To quantify the rate limiting step of SOM decomposition, we measured microbially derived potential extracellular enzyme activity for 6 hydrolytic enzymes using modified fluorometric assays. We measured three carbon cycling enzymes (β-glucosidase, β- xylosidase, and cellobiohydrolase) and three nitrogen and phosphorus cycling enzymes (N-acetyl-glucosaminidase, acid phosphatase, leucine-amino-peptidase) using both methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (AMC) linked substrates (Bell et al. 2013). To account for differences in initial soil mass, we expressed potential enzyme activity as nmol $\bullet g^{-1}$ dry soil h^{-1}.

Soil characteristics

We determined soil moisture by comparing weights before and after drying at 70 °C until stable. Dry weight was used to calculate bulk density using volumetric samples of wet soils. To determine concentrations of C and N as well as changes in the stoichiometry of our experimental soil, we dried samples at 70°C until stable and ground them to a fine powder in a ball mill grinder. We then weighed soils into tin capsules and analyzed samples by combustion (CHN Carlo-Erba Elemental Analyzer, NA 1500, Carlo-Erba Instruments, Milan Italy) to determine soil C and N content and C:N.

Our experimental manipulation of root growth, artificially decreased bulk density, such that any calculation of C or N stocks solely reflected this experimental artifact. As a result, we used % C and % N to best reflect overall C dynamics in our experimental design.

Root analyses

We directly quantified root biomass and length from all experimental cores, while we estimated root biomass and length from control cores by a subsampling procedure. For experimental cores, we removed and cleaned all roots from each soil horizon and core. We scanned roots (CanoScan LiDE210; Canon U.S.A., Inc., Melville, NY, USA) and quantified root length (cm) with WinRHIZO software (Regent Instruments Inc., Quebec, Canada; 2000). We then dried samples at 70°C to determine total biomass (g).

For control cores, because of the high root density, we subsampled 1/5 of each soil horizon by weight, and used a gridline intersection technique to estimate total root length. Soil and roots were dispersed on a Plexiglas tray with 1 cm^2 gridlines and placed on a light table to visualize roots and count root intersections. We used these measures to extrapolate root length (cm) for the whole core. For each sample, we dried a 10 cm root length to quantify specific root length, from which we estimated total core root biomass (g). We compared specific root lengths to ensure accuracy between the two methods and found no differences.

To normalize for the different depths, volumes, and bulk densities of both the experimental and control cores, we expressed root growth as root length (cm • g dry $soil^{-1}$). While we also calculated root biomass for each core, root length provided greater predictive power (lower AIC) in our models, and better represents the potential for fine roots to contribute to ecosystem processes.

Data Analysis

To understand how the identity of roots and organic matter modulate soil C cycling, we used linear mixed effect models to analyze our data with root length (cm • g dry soil^{-1}), origin, final location, and species as fixed effects, soil moisture (%) as a fixed covariate, and site as a random effect. For control cores, we used the same designation for both origin and final location. Although we considered using one model for all horizons, our high number of predictor variables and the complexity of their interactions led us to analyze each horizon separately. We natural log-transformed data when necessary to meet assumptions of normality and heteroscedasticity, and calculated p values using a Wald F-test and an α value of 0.05 (package nlme, R for Mac, version 3.1.2). For figures, we extracted model coefficients (package lsmeans, R for Mac, version 3.1.2). We initially included interactions among roots, origin, and final location, but removed them when $p > 0.2$.

Results

Soil Characteristics

% C

In accordance with our first hypothesis, we expected that soils originating from shrub areas to have lower soil C content compared to those from non-shrub areas, as a result of higher SOM decomposability and C loss over the course of the experiment. However, we observed no such differences in total soil % C in shrub vs. non-shrub-derived soils (all horizons, $p > 0.05$, Fig. 4.2).

Secondly, we expected that soil C content would decrease with increasing root length because of stimulated microbial activity in the rhizosphere, but only found evidence of this in mineral soil. Root length was negatively associated with % C ($F_{1,29}$ = 24.64, p < 0.0001), but roots in non-shrub areas had a stronger negative relationship with % C than did shrub roots (interaction of root length and final location ($F_{1,29}$ = 9.77, p = 0.004). In further support of the idea that shrub roots ameliorate soil C loss relative to non-shrub roots, mineral soils had greater % C in shrub vs. non-shrub areas ($F_{1,29}$ = 9.21, p = 0.005).

In upper and lower organic soils, we observed the opposite of our hypothesized pattern: total soil % C increased with increasing root length (UO- $F_{1,25}$ = 92.16, p < 0.0001; LO: $F_{1,70}$ = 13.07, p = 0.0006). In upper organic soils, and across all treatments, we also observed a species effect, with greater % C in birch than alder soils ($F_{1,25}$ = 15.07, p = 0.0007). Across all horizons, soil moisture was an important covariate, as % C increased with moisture (UO - ($F_{1,25}$ = 46.14, p < 0.0001), LO - ($F_{1,71}$ = 157.59, p < 0.0001), M - ($F_{1,30}$ = 71.93, p < 0.0001)).

% N

Across all horizons, soil % N followed similar patterns to % C. Soil % N increased with increasing root length in upper ($F_{1,25}$ = 26.58, p < 0.0001) and lower organic soils ($F_{1,70}$ = 13.84, p = 0.0004, Fig. 4.3). However, the extent of this response was greater in upper organic soils that originated from shrub areas (interaction of root length and origin $F_{1,25}$ = 4.31, p = 0.048, Fig. S4.1). Additionally, in upper organic soils, we observed greater soil N content in birch soils when compared to those of alder ($F_{1,25}$ = 4.60, p = 0.042, Fig. 4.3). Similar to our observations of mineral C, we found that root growth decreased % N ($F_{1,28}$ = 23.89, p < 0.0001). However, the magnitude of this response depended on the final location of cores (interaction of final location

and root length $F_{1,28} = 6.43$, $p = 0.017$) and the origin of SOM (interaction of origin and root length $F_{1,28} = 5.14$, $p = 0.031$).

C:N

In upper organic soils, we found that soil C:N increased as root length increased ($F_{1,25} = 44.70$, $p < 0.0001$), but found no relationship between C:N and root length in lower organic ($p > 0.05$) or mineral soil ($p > 0.05$).

Bulk density

Bulk density decreased with increasing root length across all horizons (UO – $F_{1,26} = 5.39$, $p = 0.028$, LO - $F_{1,70} = 13.28$, $p = 0.0005$, M - $F_{1,31} = 15.65$, $p = 0.0004$). In lower organic soils, soils sampled from shrub areas had lower bulk density than those from non-shrub areas ($F_{1,70} = 5.93$, $p = 0.018$).

Microbial function

Heterotrophic respiration

Based on our first hypothesis, we expected that soils originating from shrub areas would support greater microbial activity than those from non-shrub areas as a result of differences in SOM decomposability. We found support for this idea in upper organic soils, as respiration rates were higher on shrub vs. non-shrub derived SOM ($F_{1,25} = 7.05$, $p = 0.014$, Fig. 4.4). However, the effect of shrub-derived SOM did not extend to deeper soils ($p > 0.05$).

Secondly, we expected that microbial activity would increase with increasing root length, as roots provide labile C to rhizosphere microbes. We found support for this hypothesis across all

horizons (UO – $F_{1,25}$ = 4.35, p = 0.025, LO - $F_{1,69}$ = 9.13, p = 0.004, M - $F_{1,29}$ = 30.13, p < 0.0001). We also observed a species effect across all treatments, with greater respiration rates in alder vs. birch mineral soils ($F_{1,29}$ = 4.26, p = 0.048).

DOC

We found greater concentrations of DOC in shrub- vs. non-shrub-derived upper organic soils ($F_{1,26}$ = 17.42, p = 0.0003, Fig. 4.5), in support of our hypothesis that SOM quality of shrub soils promotes faster C loss. Further, we found evidence that shrub-derived SOM is more vulnerable to root-induced soil C loss—DOC concentrations increased more strongly with increasing root length when soils originated from shrub areas (interaction of origin and root length $F_{1,26}$ = 6.43, p = 0.018, Fig. 4.6). This interaction did not extend to deeper horizons (p > 0.05).

In support of our second hypothesis, we found that DOC concentrations increased as root length increased across all horizons (UO – $F_{1,26}$ = 14.16, p = 0.0009, $F_{1,71}$ = 14.36, p = 0.0003, $F_{1,31}$ = 12.90, p = 0.001, Fig. 4.5), indicating that roots either exude DOC or promote the dissolution of organic C in soils. However, we found no effect of the final location (i.e., effect of shrub vs. non-shrub roots) on DOC. Across all treatments, we observed a species effect, with greater concentrations of DOC in alder vs. birch soils ($F_{1,26}$ = 4.52, p = 0.043).

Extracellular enzyme activity

In upper organic soils, we observed higher BX ($F_{1,26}$ = 4.29, p = 0.049) and CB ($F_{1,26}$ = 5.49, p = 0.027) potential activity in shrub-originated soils, providing further evidence for the

stimulatory effect of shrub-derived SOM on microbial activity. We did not see this pattern for other enzymes in upper organic soils or in deeper horizons ($p > 0.05$).

We also observed a positive relationship between root length and all potential enzyme activity in lower organic and mineral soils (Table 4.1, Fig. 4.7). In upper organic soils, we observed the same pattern for BG ($F_{1,27} = 4.42$, $p = 0.045$), BX ($F_{1,26} = 7.99$, $p = 0.009$), NAG ($F_{1,26} = 13.67$, $p = 0.001$), and AP ($F_{1,26} = 16.56$, $p = 0.0004$) potential activity (Fig. 4.8). However, this relationship was not observed for CB and LAP potential activity ($p > 0.05$).

Discussion

We found that arctic plants and their expansive root systems appear to stimulate heterotrophic activity and regulate soil C dynamics, but that the magnitude and direction of these effects are both shrub and depth dependent. This finding lends support to the idea that root exudates or allocation to root-associated microorganisms stimulate microbial activity (Bais et al. 2006; Farrar et al. 2003; Jones et al. 2004). Alternatively, soil microbial activity could attract root growth (Giehl et al. 2014), leading to the observed relationship between root density and microbial processes. Both root growth and microbial activity appear to promote C retention in organic soils, while leading to C loss in mineral soils. Interestingly, our results suggest that shrub roots temper soil C loss in the mineral horizon. We also found that shrub-derived SOM supports higher microbial activity and resulted in greater C loss, however, we only observed this in upper organic soils (Fig. 4.2). Collectively, our findings suggest that SOM stabilization and retention are controlled by different factors in the organic and mineral soil horizons, and that shrubs have specific and contrasting effects on soil C dynamics with soil depth.

We found support for our hypothesis that shrub-derived SOM is more favorable for microbial activity. In upper organic soils, we found greater rates of heterotrophic respiration (Fig. 4.4) and concentrations of DOC in soils originating from shrub areas (Fig. 4.5). In further support of this idea, root length was more positively associated with DOC in shrub vs. non-shrub derived SOM (Fig. 4.6), and soils originating from shrub areas also supported greater CB and BX potential enzyme activity (Fig. S4.2). Such effects, and their isolation to upper horizons, suggests that shrub SOM may be intrinsically more susceptible to decomposition relative to SOM formed beneath other tundra vegetation because of high litter quality or the lateral structure of roots (Mack et al. 2004; Sistla et al. 2013). Moreover, these biotic differences may be compounded by environmental effects brought on by shrubs like winter warming (Sturm et al. 2005b), changes to permafrost thaw (Blok et al. 2010; Bonfils et al. 2012), and lower albedo (Juszak et al. 2014; Loranty et al. 2011; Sturm et al. 2005a), which may promote microbial activity. However, despite our strong evidence that shrub SOM stimulates microbial activity, this activity appears to either contribute to soil C and N retention (Cotrufo et al. 2013, Fig S4.1) or is offset by an increase in C and N inputs (Weintraub & Schimel 2005), as we observed no differences in overall soil C and N content between soil origins (Fig. 4.2).

We found saw a positive relationship between root growth and soil microbial activity (Figs. 4.4, 4.5, 4.6, 4.7), suggesting that roots and their inputs may stimulate microbial activity. This could also suggest that roots grow towards microbial hotspots to access liberated nutrients (Giehl et al. 2014). This heightened activity, however, appears to have divergent consequences on soil C content depending on horizon and sampling location (Fig. 4.2). In organic soils, our results suggest that higher root and microbial activity are associated with greater C retention (Fig. 4.2) in that increasing root length was positively correlated with both microbial activity and

soil C content. Indeed, a growing body of literature suggests that microbial activity and its byproducts may facilitate the creation and stabilization of SOM (Bradford et al. 2013; Cotrufo et al. 2015; Cotrufo et al. 2013; Lehmann & Kleber 2015), specifically in shrub-dominated tundra (Lynch et al. 2018; Weintraub & Schimel 2003). Further, the biotic features of shrubs (i.e., roots and leaf litter) may more strongly contribute to decomposition than shrub-mediated changes to the environment (DeMarco et al. 2014; Loya et al. 2004; Myers-Smith & Hik 2013). Thus, while shrubs may stimulate microbial activity, this may lead to greater C storage in organic soils.

In contrast, we found that greater root length in mineral soils was associated with both greater microbial activity and lower soil C content (Fig. 4.2, 4.5, 4.7). The divergence from patterns we observed in organic soils may be due to differences in the physicochemical properties of soil minerals, such as greater physical occlusion of SOM (Dungait et al. 2012; Schimel & Schaeffer 2012; Yoo et al. 2011), or increasing C limitation of microbial activity at depth (Karhu et al. 2016). Our results also corroborate evidence of soil priming across arctic soil profiles (Karhu et al. 2016; Wild et al. 2014), and specifically point to roots as the source of labile C inputs that triggers microbial activity and reduces soil C content.

Interestingly, the intensity of mineral soil C reductions depended on the final location of the core, where non-shrub roots appear to stimulate C and N loss to a greater extent than do shrub roots (Fig. 4.2). This pattern may explain the greater soil C and N content we observed in mineral shrub soils, and further suggests that root identity and thus, functional differences in roots, may be driving these patterns (Table 4.1). Large deciduous shrubs produce highly branched roots that grow laterally, and associate with ECM fungi (Iversen et al. 2015; Wang et al. 2016). These associations distribute C through hyphal networks, and may limit the amount of exudates released into the soil matrix (Jones et al. 2004). In contrast, graminoids are typically

non-mycorrhizal and have long, tubular roots with little branching (Iversen et al. 2015), that extend deep into the thawed soil profile (Wang et al. 2016), and may exude greater amounts of labile C. As such, non-shrub areas in our study may have received greater C inputs at depth than areas dominated by shrubs, leading to the observed patterns in C decline. Although all root growth stimulated reductions of C in mineral soil, root characteristics may explain the difference in the strength of this relationship between shrub and non-shrub areas. The ecosystem level effects of these responses will depend on the extent of shrub abiotic effects. For instance, if shrubs enhance the depth of permafrost thaw relative to other vegetation (Bonfils et al. 2012, but see Blok et al. 2010), they may indeed promote greater C loss at depth even if root-induced priming effects are weaker.

In our study, we did not observe consistent differences in C dynamics between our two shrub species. We expected greater soil C and N in alder areas due to their symbiotic association with N-fixing bacteria (Mitchell & Ruess 2009b; Ruess et al. 2013) and resulting suppression of soil decomposition (Frey et al. 2014). However, we observed greater % C and % N in upper organic soils in birch areas (Figs. 4.2, 4.3). We observed greater AP potential activity in birch soils (Fig. 4.7), suggesting greater P limitation (Sinsabaugh et al. 2008). However, we also observed greater DOC in upper organic alder soils (Fig. 4.5), suggesting greater lability of SOM. In lower organic soils, our only observed species difference was greater LAP potential activity in alder soils (Fig. 4.7), which may indicate greater N limitation, despite the process of N-fixation. Species-specific differences also extended to the mineral horizon, where we observed greater respiration from alder soils (Fig. 4.4) and greater CB potential activity (Fig. 4.7). Together our results suggest that variance between species contribute to changes in soil C and N cycling, but it remains unclear what species-specific traits might be driving these differences.

Higher root growth appeared to increase DOC concentrations (Fig. 4.5) across all three horizons, suggesting that roots either exude C or stimulate C loss from SOM. We observed similar concentrations of DOC in shrub vs. non-shrub areas (Table 4.1), despite differences in the phenology and distribution of roots between these plant communities (Iversen et al. 2015; Mack et al. 2004; Wang et al. 2016). Any such differences may be masked by the timing of our sampling as DOC concentrations are likely to peak during thaw, when lateral losses to streams and rivers are highest (McClelland et al. 2014; McNamara et al. 2008). We considered the possibility that greater DOC concentrations may be due to vertical transfer of C (i.e., leaching), despite high seasonal variability in estimates of soil leaching losses (Laodong et al. 2007; M. et al. 2008). Even so, the consistent positive relationship between DOC and root length suggests that roots are a main driver of DOC, and that any change to root productivity will affect dissolved C losses from the soil system.

Our findings reveal key ways that the expansion of shrubs could modulate soil C dynamics. While roots appear to be the driver of microbial activity in our study, the consequence of this activity strongly depended on soil depth and the presence of shrubs. Shrubs appear to produce labile SOM in the upper organic horizon; however, the higher microbial activity induced by SOM and root production appears to increase the stabilization of C. Further, our results suggest that shrubs ameliorate the negative effect of root growth on soil C loss (i.e. rhizosphere priming) in the mineral soil horizon (Karhu et al. 2016). Translating our findings to the ecosystem scale requires additional knowledge about C inputs to, and losses from, the soil system, as well as the extent of abiotic effects on rooting depth, production and associated microbial activity. Nevertheless, our experimental study isolates mechanisms by which shrubs

modulate soil C cycling, and our findings suggest that stimulated soil C cycling by shrubs enhances the retention of soil C.

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Table 4.1: Results from linear mixed effects models showing responses of soil characteristics, heterotrophic respiration, and enzyme activity to root length and experimental manipulation. F values are shown for main factor effects and their interaction. Marginal and conditional R^2 values represent the explanatory power of fixed effects alone and the combination of fixed and random effects respectively. Significant effects are indicated in bold and by +p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.0001.

		Root length (cm/g dry soil)	Origin	Final location	Species	% Moisture	Initial*Final	Origin*RLD	Final*RLD	Marginal R^2	Conditional R^2
Bulk density (g • cm^{-3})	UO	**5.39***	0.09	0.12	**3.03+**	NA	**4.12+**	———	———	0.157	0.716
	LO	**13.28****	0.08	**5.93***	**2.82+**	NA	**4.59***	———	———	0.197	0.483
	M	**15.65****	0.48	2.52	0.19	NA	———	———	———	0.315	0.315
C:N	UO	**44.70*****	2.51	0.93	2.03	**20.48****	2.45	———	———	0.647	0.719
	LO	0.21	1.79	0.36	0.003	**29.26*****	**2.89+**	———	———	0.288	0.362
	M	1.01	1.64	**3.40+**	0.08	———	———	———	———	0.129	0.129
% Carbon	UO	**92.16*****	0.15	0.17	**15.07****	**46.14*****	———	**4.12+**	———	0.810	0.817
	LO	**13.07****	0.0002	0.93	0.16	**155.79*****	———	———	———	0.672	0.693
	M	**24.64*****	2.21	**9.21****	1.75	**71.93*****	———	———	**9.77****	0.745	0.745
% Nitrogen	UO	**26.58*****	2.49	0.06	**4.60***	**24.78*****	———	**4.31***	———	0.627	0.648
	LO	**13.84****	0.007	0.02	**2.95+**	**104.69*****	———	———	———	0.600	0.600
	M	**23.89*****	2.87	**4.47***	0.64	**64.99*****	———	**5.14***	**6.43***	0.724	0.728
Soil respiration (µmol CO_2 • g dry soil^{-1} h^{-1})	UO	**4.35***	**7.05***	0.66	**3.95+**	———	2.66	———	2.34	0.371	0.482
	LO	**9.13****	0.02	0.55	0.002	———	**3.51+**	———	———	0.192	0.261
	M	**30.13*****	1.24	0.83	**4.26***	**21.59****	———	———	2.43	0.596	0.596
DOC (µg C• g dry soil^{-1})	UO	**14.16****	**17.42****	1.12	**4.52***	———	———	**6.43***	———	0.419	0.739
	LO	**14.36****	0.41	1.71	1.84	———	———	———	———	0.162	0.364
	M	**12.90****	0.01	0.77	1.19	———	———	———	———	0.271	0.349
BG potential activity (nmol • g dry soil^{-1} h^{-1})	UO	**4.42***	1.19	2.39	1.38	———	———	———	———	0.168	0.509
	LO	**5.59***	2.49	0.20	0.52	———	**5.59***	———	———	0.151	0.151
	M	**22.17****	**3.16+**	0.09	**3.66+**	**5.64***	———	———	———	0.468	0.477
BX potential activity (nmol • g dry soil^{-1} h^{-1})	UO	**7.99****	**4.29***	1.82	1.47	———	———	———	**2.93+**	0.262	0.607
	LO	**7.15****	**3.72+**	0.62	0.05	———	**3.19+**	———	———	0.153	0.157
	M	**10.43****	1.37	0.08	2.01	2.70	———	———	———	0.288	0.288
CB potential activity (nmol • g dry soil^{-1} h^{-1})	UO	0.49	**5.49***	2.60	0.005	———	———	———	2.65	0.147	0.551
	LO	**2.89+**	2.43	0.30	**3.45+**	———	**6.91***	———	———	0.167	0.211
	M	**19.52****	0.74	1.30	**5.86***	**7.48***	———	———	———	0.459	0.459
NAG potential activity (nmol • g dry soil^{-1} h^{-1})	UO	**13.67****	1.85	0.90	2.79	———	2.87	———	———	0.345	0.587
	LO	**6.85***	1.53	0.05	0.16	———	**3.68+**	———	———	0.130	0.145
	M	**24.35*****	1.22	0.03	**2.93+**	**3.63+**	———	———	———	0.445	0.449
AP potential activity (nmol • g dry soil^{-1} h^{-1})	UO	**16.56****	2.71	0.19	**8.84****	**4.62***	———	———	———	0.482	0.566
	LO	**9.74****	0.38	0.44	0.006	———	———	———	———	0.103	0.218
	M	**10.54****	0.88	0.26	0.006	1.83	———	———	———	0.249	0.342
LAP potential activity (nmol • g dry soil^{-1} h^{-1})	UO	0.34	1.03	0.32	1.98	———	———	———	———	0.096	0.161
	LO	**6.30***	0.04	0.01	**6.12***	———	———	———	———	0.133	0.133
	M	**17.45****	**4.57***	0.02	0.03	**4.25***	———	———	———	0.357	0.582

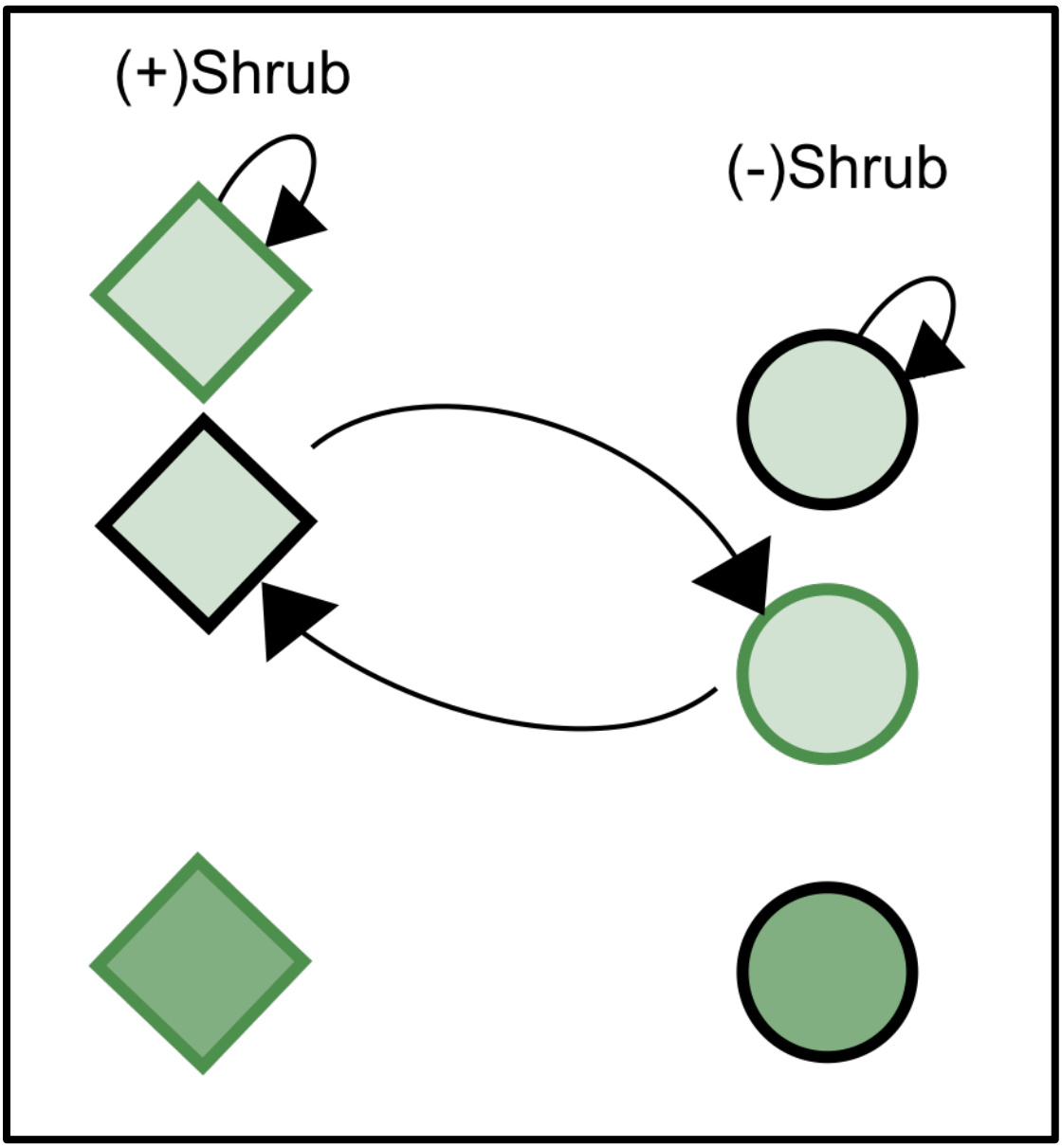

Figure 4.1: Design of reciprocal transplant experiment, indicating soil origin (outline: dark green – shrub, black – non-shrub), final location (shape: diamond – shrub, circle- non-shrub), experimental and control cores (color: light green - experimental, green - control).

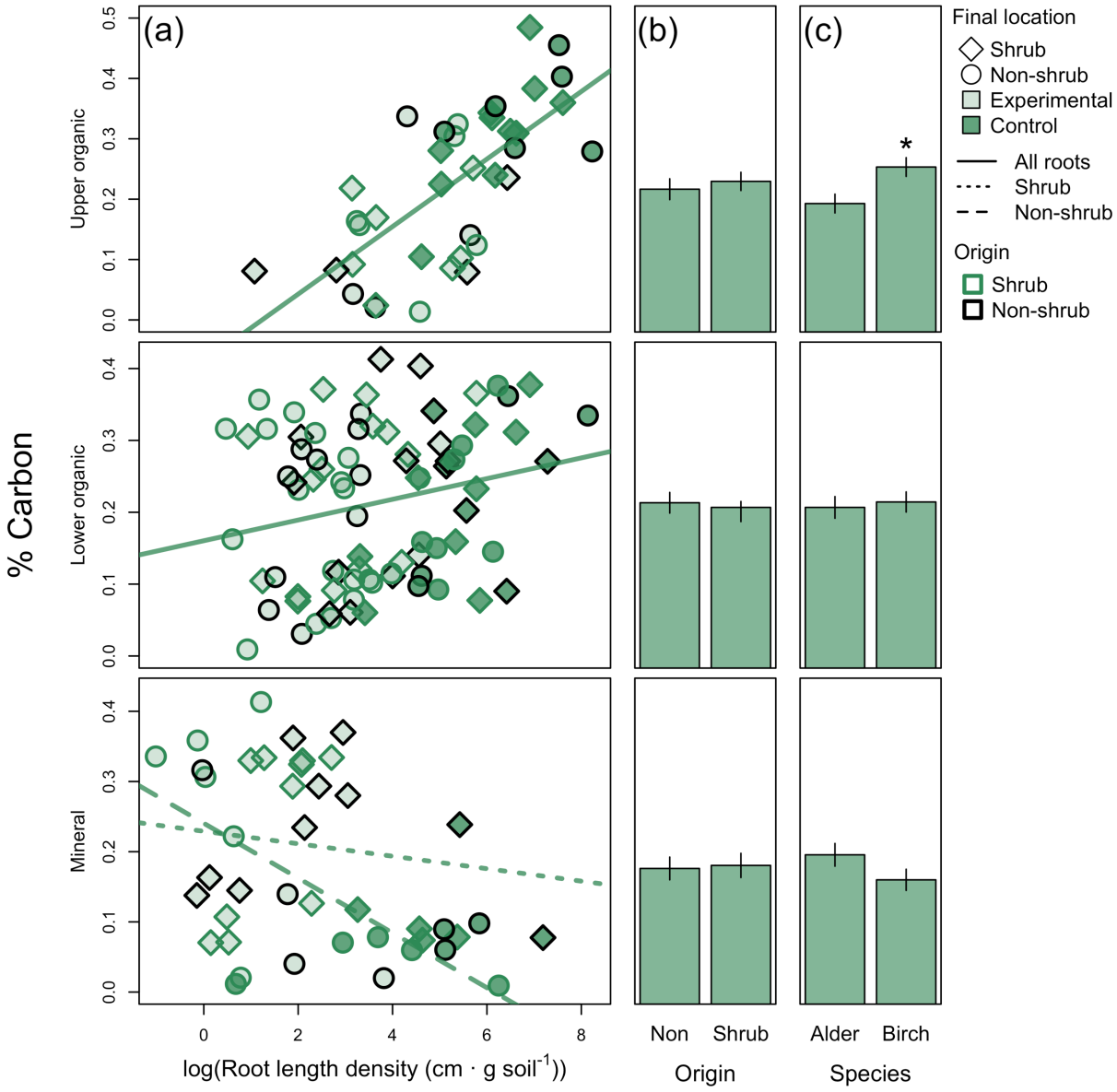

Figure 4.2: Soil % C at the end of the experiment, shown by columns of a) final location and root length, b) soil origin and c) species, across three soil horizons. Scatterplot points indicate final location and experimental status of soil cores. Lines represent significant relationship between soil % C and root length ($p < 0.05$). In the mineral horizon, lines represent a significant interaction between root length and final soil location ($p < 0.05$). Bars represent model coefficients ± standard error. Significant effects are indicated by *$p < 0.05$.

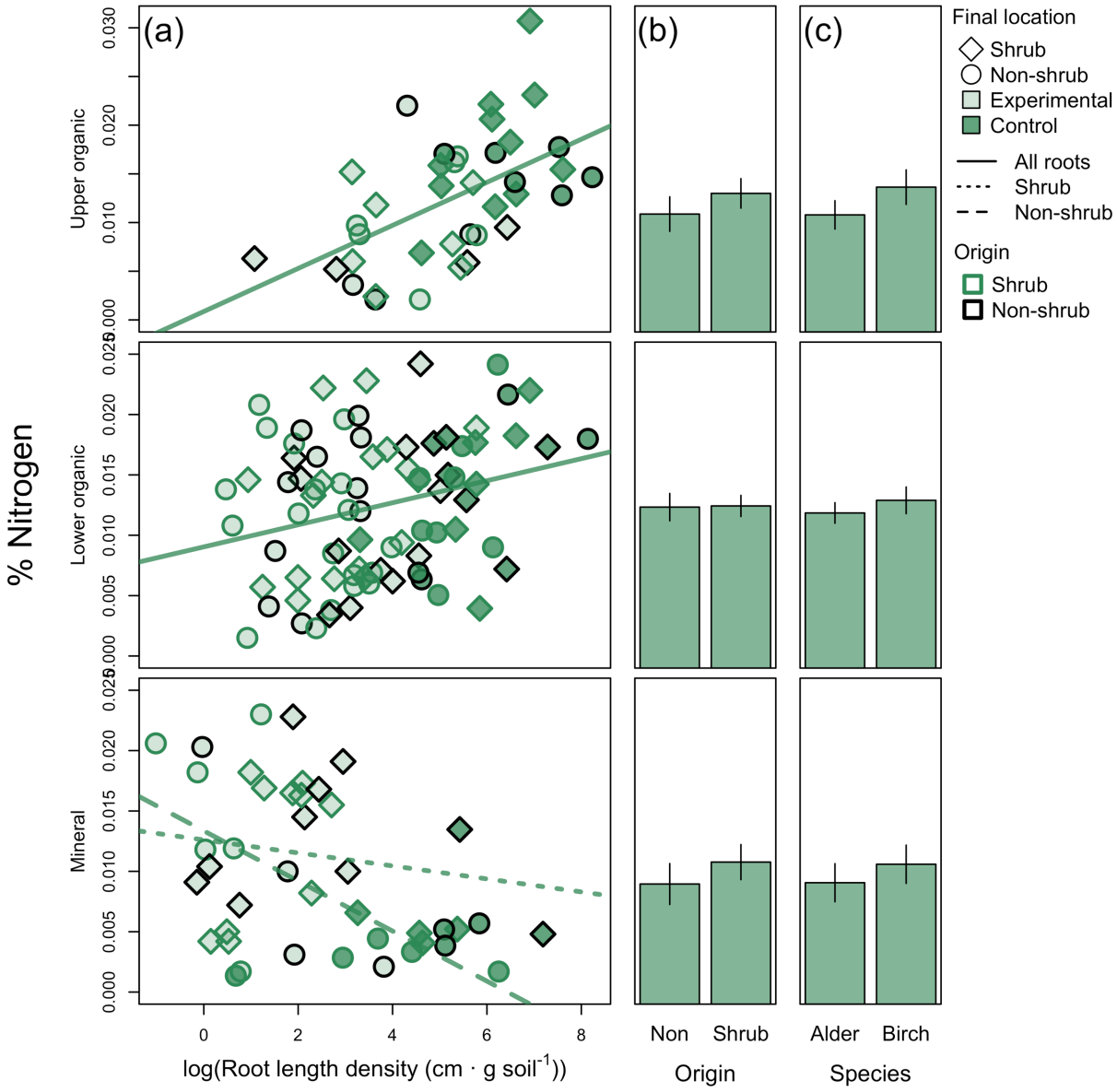

Figure 4.3: Soil % N at the end of the experiment, shown by columns of a) final location and root length, b) soil origin and c) species, across three soil horizons. Scatterplot points indicate final location and experimental status of soil cores. Lines represent significant relationship between soil % N and root length ($p < 0.05$). In mineral soils, lines represent significant interactions between root length and final soil location ($p < 0.05$). Bars represent model coefficients ± standard error. Significant effects are indicated by *$p < 0.05$.

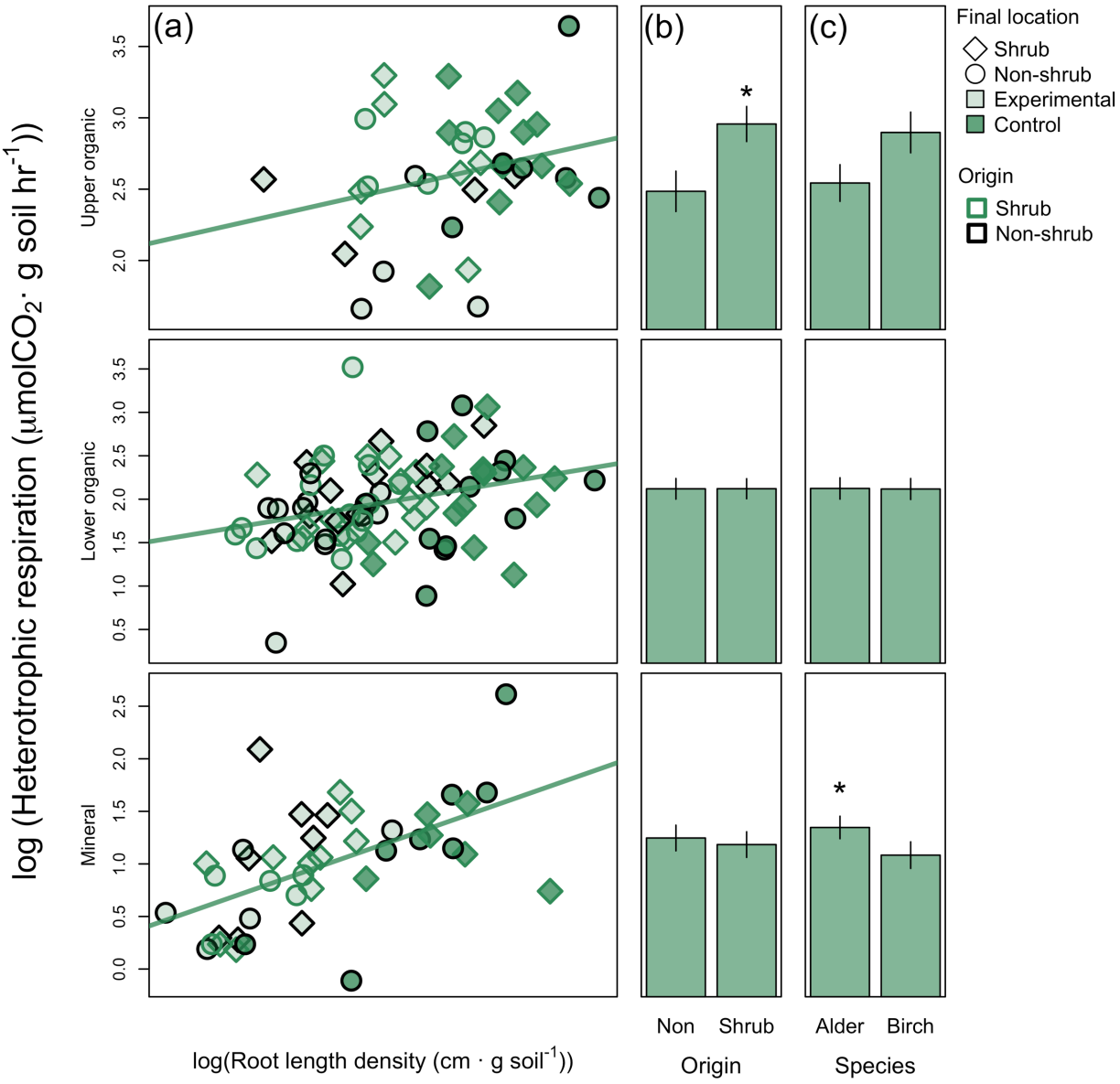

Figure 4.4: Heterotrophic respiration at the end of the experiment, shown by columns of a) final location and root length, b) soil origin and c) species, across three soil horizons. Data are natural log transformed. Scatterplot points indicate final location and experimental status of soil cores. Lines represent significant positive relationship between respiration and root length ($p < 0.05$). Bars represent model coefficients ± standard error. Significant effects are indicated by *$p < 0.05$.

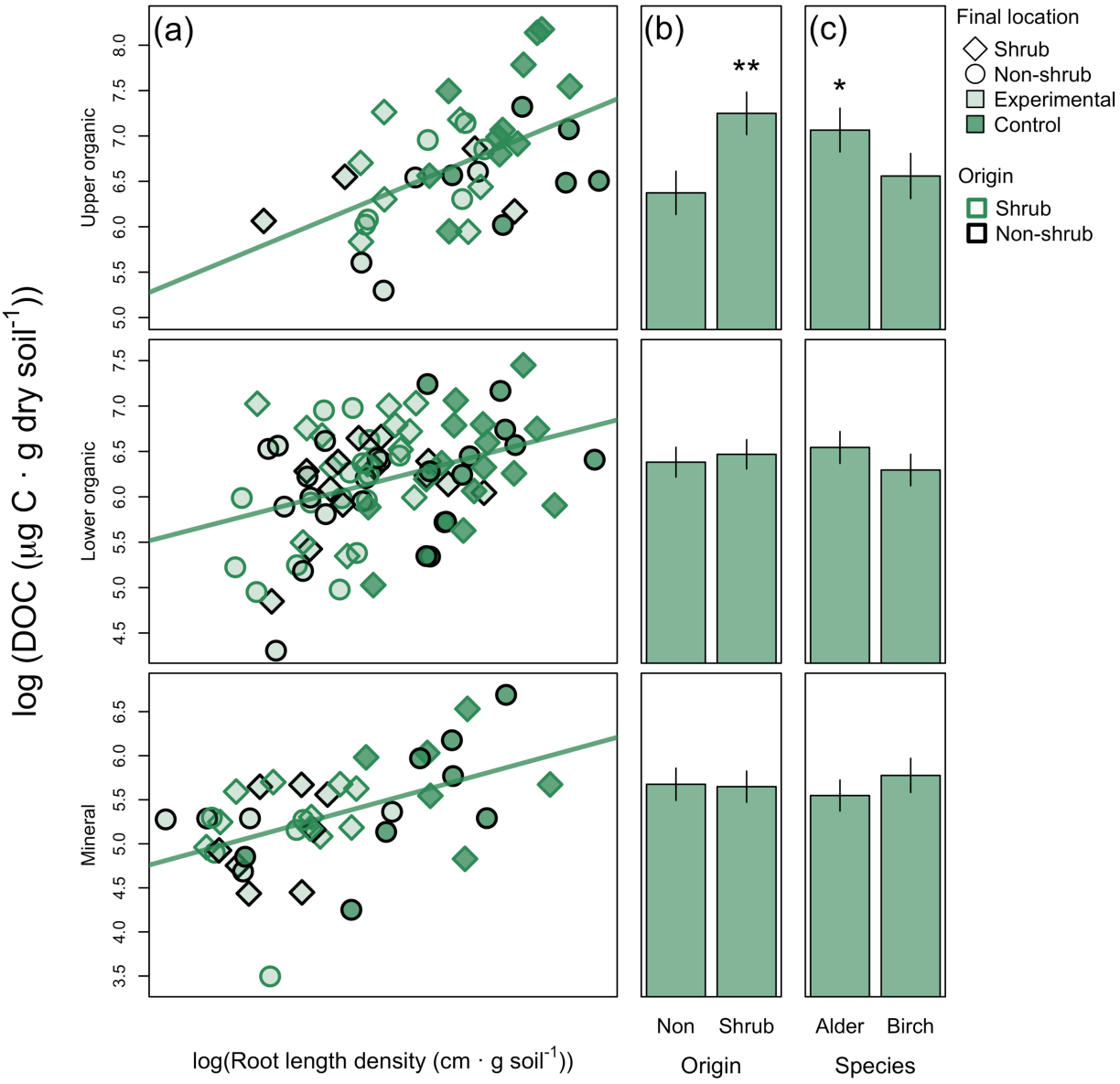

Figure 4.5: Dissolved organic carbon (DOC) at the end of the experiment, shown by columns of a) final location and root length, b) soil origin and c) species, across three soil horizons. Data are natural log transformed. Scatterplot points indicate final location and experimental status of soil cores. Lines represent significant relationship between DOC and root length ($p < 0.05$). Bars represent model coefficients ± standard error. Significant effects are indicated by *$p < 0.05$, **$p < 0.01$.

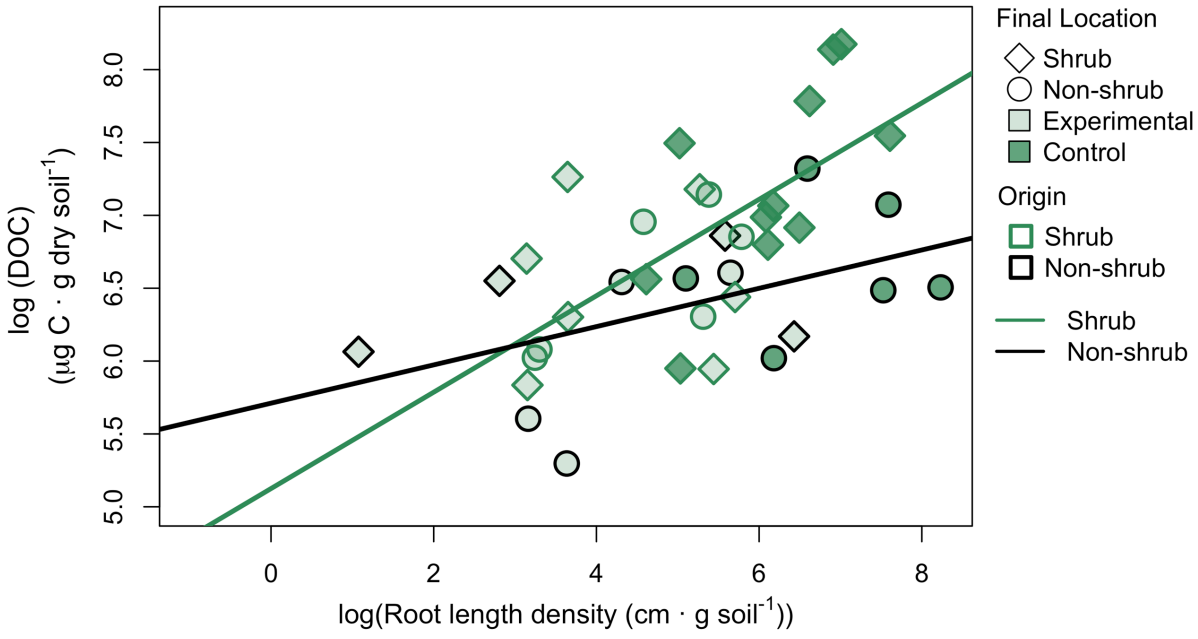

Figure 4.6: Dissolved organic carbon (DOC) in the upper organic horizon shown by root length and soil origin. Data are natural log transformed. Scatterplot points indicate origin and experimental status of soil cores. Lines represent significant interactions between root length and soil origin ($p < 0.05$).

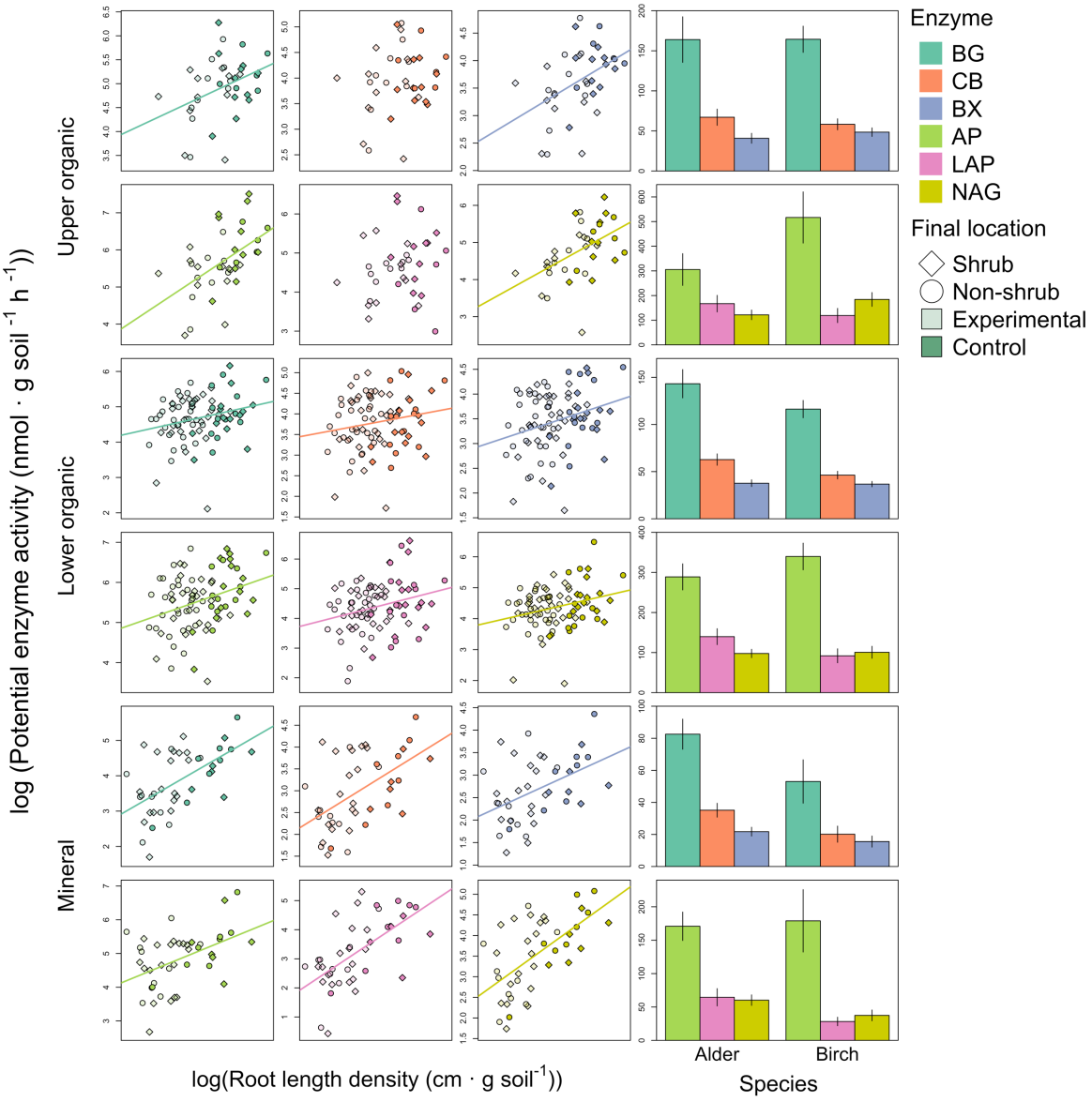

Figure 4.7: Potential enzyme activity shown by root length and species across three horizons. Data are natural log transformed. Scatterplot points indicate final location and experimental status of soil cores. Lines represent significant relationship between potential enzyme activity and root length ($p < 0.05$). Bars represent model coefficients ± standard error. Significant effects are indicated by *$p < 0.05$, **$p < 0.01$.

CHAPTER 5

CONCLUSIONS

The goal of this dissertation was to investigate the consequences of shrub expansion on interactions between plants, microbes, and soil organic matter (SOM), and thus carbon (C) dynamics in arctic ecosystems. I examined four pathways through which shrubs might disrupt historic belowground interactions. Relative to other tundra vegetation, shrubs may 1) increase rates of litter production and increase microbial response to shrub litter, 2) alleviate nutrient limitation, 3) create high quality SOM, and 4) produce root systems with stronger stimulatory effects on microbial activity. In each of my dissertation chapters, I examine these pathways to understand how heterotrophs respond to shrub expansion in arctic ecosystems, and the consequences of their response on soil C dynamics.

In Chapter 2, I examined the first of these three pathways to understand how shrubs modify heterotrophic soil C loss. I used microcosms and experimentally added litter and nutrients to mimic natural organic inputs and elevated concentrations of nitrogen (N) and phosphorus (P). I found greater rates of heterotrophic respiration from soils beneath shrubs relative to non-shrub soils, corroborating field measurements (Grogan and Chapin 1999). This may be due to greater microbial biomass or greater C inputs (Weintraub et al. 2005). However, my results provide no evidence to suggest that shrubs select microbial communities that can better break down their litter, despite litter stimulating microbial activity in both soil origins. Further, I found that shrubs do not alleviate N and/or P limitation to soil microbial communities, and instead, found evidence of mild P limitation across all soils. Most interestingly, my results

highlight the potential for shrubs to stimulate heterotrophic C losses, while also providing evidence of C limitation in highly organic soils.

In Chapter 3, I investigated the effects of direct and indirect effects of climate warming (i.e., litter addition) using a long-term experimental manipulation in sub-arctic Sweden. The long-term experiment employed a factorial design that crossed annual litter addition with growing season warming treatments. After sampling soils, I measured soil C and N stocks, microbial biomass, and heterotrophic activity. I found that warming reduced soil C and N stocks, while litter addition did not change overall C or N content. These results add to a growing body of literature that detail how the arctic will respond to a changing climate. A recent synthesis (Crowther et al. 2016) suggests that C losses may be on the order of 0-0.3 kg • m^{-2} yr^{-1} per degree warming depending on the initial C stock, but this study only focused on the upper 10 cm of soil (van Gestel et al. 2018). While this study includes only a few arctic sites, the loss rates I quantified (approximately 0.05 kg • m^{-2} yr^{-1}) fall within the lower end of this range, which may reflect a smaller initial C stock at our sub-arctic site. My results, however, did not identify a clear mechanism by which C was leaving the ecosystem, although warming did intensify respiratory and lateral C losses. Overall, these findings suggest that arctic SOM may be vulnerable to decomposition under rising global temperature, however, enhanced productivity of shrubs (simulated via litter inputs) had no effect on soil C stocks, despite the strong evidence for C loss we observed in Chapter 2.

In Chapter 4, I examined how shrub-induced changes to SOM and root growth regulate soil C dynamics by implementing a reciprocal transplant root in-growth core experiment. I found evidence that shrubs modify organic matter quality, suggesting that the accumulation of shrub litter (both leaf and root) increases the susceptibility of SOM to decomposition. Across the

board, I found that roots stimulate microbial activity, including potential enzyme activity and heterotrophic respiration. However, the relationship between root length and soil C content was strongly dependent on the presence of shrubs and depth. In organic soils, higher root length was associated with increasing soil C content, suggesting that microbial processing of fresh, plant inputs may lead to C stabilization (Cotrufo et al. 2013), in line with other arctic studies (Lynch et al. 2018). In contrast, higher root length was associated with a reduction of soil C content in mineral soil, suggesting that soil priming occurs at depth (Karhu et al. 2016), and the possibility that differences in the chemical and physical relationships of mineral soils affect C dynamics and loss of SOM (Dungait et al. 2012; Schimel & Schaeffer 2012; Yoo et al. 2011). Interestingly, the negative relationship between root length and soil C content depended on the final location of each core, where shrub roots appeared to temper this pattern of C loss. This suggests that functional differences between roots of shrubs and other tundra vegetation may regulate the nature of root-C inputs, and thus how these inputs stimulate microbial activity and loss of SOM. My findings from Chapter 4 suggest that while shrub-induced changes may stimulate heterotrophic activity, such activity may be offset by stabilizing C in organic soils or tempering C loss rates in mineral soils.

Collectively, the results of my dissertation demonstrate that shrubs stimulate microbial activity and increase rates of C cycling. The first two experiments of my dissertation provide convincing evidence to suggest that shrubs stimulate microbial activity, but the mechanism by which shrubs affect this response remains unclear. Shrub root and leaf litter do not appear to be driving this increase in activity, although the response of all soils to litter suggests C limitation as a potential mechanism. Further, heterotrophic activity, in both types of soils, appears to be limited by P, contrary to our expectations that shrubs would alleviate nutrient constraints on

heterotrophs. In Chapter 3, I observed that warming reduced C stocks. I did not find evidence in this long-term experiment to corroborate the findings of my incubations in Chapter 2, where shrub litter stimulated heterotrophic respiration. This suggests that our observed response to litter represents was a transient one. My *in situ* manipulation of root growth in Chapter 4, provides compelling evidence to suggest that roots are a driver of C cycling in arctic soils. My results also suggest that shrub-mediated changes to SOM, stimulate microbial activity in organic soils, providing a mechanism for my findings in Chapter 2.

These results add to a growing body of literature that investigates the consequences of global change on soil C cycling in arctic ecosystems (Blok et al. 2010; Bonfils et al. 2012; Lynch et al. 2018;. Myers-Smith et al. 2011; Parker et al. 2015). Specifically, my findings reveal key ways that shrub expansion may modify C dynamics, specifically, by stimulating heterotrophic activity. My results also suggest that the consequences of this activity are vegetation and depth dependent. The ecosystem level effects of these responses, however, will depend on shrub-induced abiotic changes. For instance, if shrubs enhance the depth of permafrost thaw relative to other vegetation (Bonfils et al. 2012, but see Blok et al. 2010), they may indeed promote greater C loss at depth. Further, the acceleration of winter soil processes (Sturm et al. 2001) by increased snow trapping (Sturm et al. 2005) may also modify these observed patterns in the long-term.

In conclusion, my dissertation demonstrates that shrubs fundamentally modify C cycling in arctic tundra, but their effect on ecosystem C storage still remains unclear. In shrub areas, C loss due to an increase in microbial activity may be offset by an increase in aboveground C fixation and greater C stabilization in organic soils, or exacerbated by C reductions in mineral soils. Although the long-term balance of inputs and outputs will ultimately determine the

consequences of shrub expansion on ecosystem level C storage, my research identifies roots and changes to SOM as the main pathways by which shrub modify the C cycle in arctic soils.

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APPENDIX A

CHAPTER 2 SUPPLEMENTAL INFORMATION

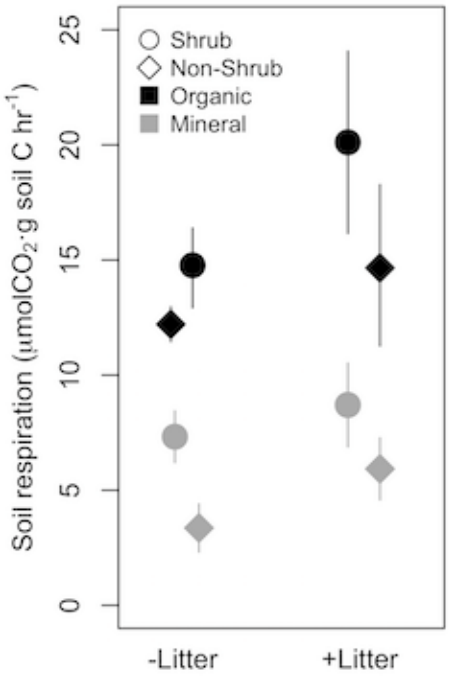

Fig. S2.1 Heterotrophic respiration shown for each experimental treatment combination. Points represent means ± standard error

APPENDIX B

CHAPTER 3 SUPPLEMENTAL INFORMATION

Table S3.1: Soil bulk density for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). Values represent mean treatment values ± standard error.

Depth	Treatment	Bulk density ($g \cdot cm^{-3}$)
0-5 cm	C	0.051 ± 0.008
	L	0.048 ± 0.006
	W	0.049 ± 0.008
	LW	0.049 ± 0.008
5-10 cm	C	0.087 ± 0.016
	L	0.070 ± 0.007
	W	0.052 ± 0.007
	LW	0.063 ± 0.016
10-15 cm	C	0.139 ± 0.027
	L	0.130 ± 0.028
	W	0.127 ± 0.032
	LW	0.135 ± 0.046
15-20 cm	C	0.176 ± 0.040
	L	0.228 ± 0.040
	W	0.215 ± 0.041
	LW	0.249 ± 0.059

Table S3.2: 3-way ANOVA results of responses of soil properties, microbial biomass, heterotrophic respiration, and enzyme activity to experimental warming and litter addition. F values are shown for main factor effects (warming, litter addition, and depth) and their interaction in 3 way ANOVAs. We crossed depth, warming, and litter addition, but removed interactions if $p > 0.2$. Block remained in the model if $p < 0.02$. Significant effects are indicated in bold and by +p < 0.1, *p<0.05, **p<0.01, ***p<0.001.

F values and significance levels							
Variable	**Warming**	**Litter**	**Depth**	**L x W**	**W x D**	**L x D**	**L x W x D**
Soil C ($g \cdot m^{-2}$)	**6.323***	1.504	**6.638*****	----------	----------	----------	----------
Soil N ($g \cdot m^{-2}$)	**4.183***	2.697	**24.2*****	----------	----------	----------	----------
% Carbon	0.099	0.059	**59.432*****	----------	----------	----------	----------
% Nitrogen	0.01	1.868	**13.741*****	----------	----------	----------	----------
Soil C:N	0.116	0.481	**64.727*****	**2.891+**	----------	----------	----------
Bulk density ($g \cdot cm^{-3}$)	0.002	1.099	**45.523*****	----------	----------	----------	----------
Soil moisture (%)	0.022	2.633	**7.989*****	----------	----------	----------	----------
Fine root biomass	0.998	0.00	**14.748*****	----------	----------	----------	----------
Soil respiration ($\mu mol\ CO_2 \cdot g\ soil\ C^{-1}\ day^{-1}$)	2.224	0.766	**3.066***	----------	----------	----------	----------
NH_4^+ ($\mu g \cdot g\ soil\ C^{-1}$)	0.232	1.534	**6.029****	----------	----------	----------	----------
NO_3^- ($ug \cdot g\ soil\ C^{-1}$)	1.7	**7.391****	**5.439****	**4.525***	1.889	1.044	**4.901***
PO_4^{3-} ($ug \cdot g\ soil\ C^{-1}$)	0.572	0.069	**1.54+**	**3.503+**	----------	----------	----------
DOC ($\mu g \cdot g\ soil\ C^{-1}$)	2.548	**3.461+**	**15.023*****	----------	----------	----------	----------
DON ($\mu g \cdot g\ soil\ C^{-1}$)	**7.27****	**5.622***	0.501	1.717	0.874	1.931	**5.406*****
Microbial C ($\mu g \cdot g\ soil\ C^{-1}$)	0.012	0.495	**4.969***	**3.287+**	----------	----------	----------
Microbial N ($\mu g \cdot g\ soil\ C^{-1}$)	2.288	0.002	0.963	0.577	0.282	0.001	**3.374***
Microbial P ($\mu g \cdot g\ soil\ C^{-1}$)	**14.092*****	**5.955***	**15.54*****	0.649	0.195	1.609	**3.191***
Microbial C:N	1.857	0.119	**2.495+**	----------	----------	----------	----------
Microbial C:P	**7.950****	0.013	**3.941***	----------	----------	----------	----------
Microbial N:P	**3.071+**	0.00	**6.906****	----------	----------	----------	----------
Microbial C:N:P	**2.987+**	0.014	**5.496****	----------	----------	----------	----------
BX potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	0.093	**6.524***	0.971	----------	----------	----------	----------
BG potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	0.298	1.68	1.556	----------	----------	----------	----------
CB potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	0.718	1.306	1.895	----------	----------	----------	----------
NAG potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	1.17	0.967	**18.882*****	**11.990*****	**4.250****	**2.440+**	----------
AP potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	2.72	**5.816***	**4.250****	----------	**3.680***	----------	----------
LAP potential activity ($nmol \cdot g\ soil\ C^{-1}\ hr^{-1}$)	**3.507+**	0.707	**2.672+**	----------	----------	----------	----------

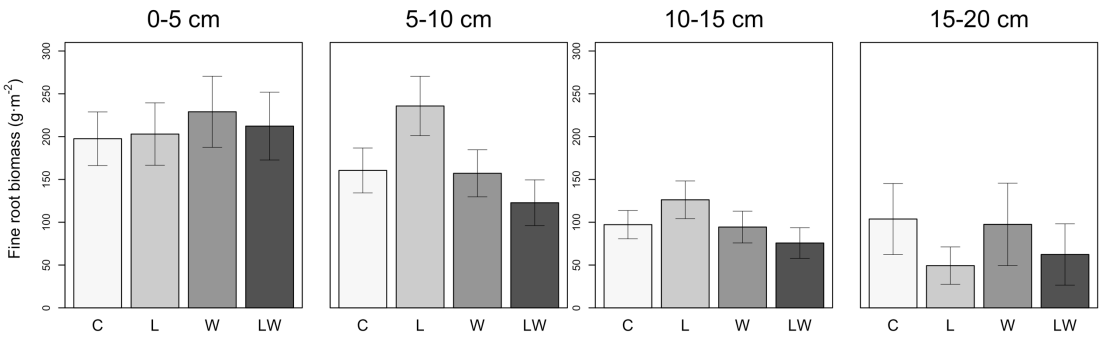

Figure S3.1: Fine root biomass in four 5 cm depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). Bars represent means ± SE. Levels of significance determined by a two way ANOVA for each individual depth crossing litter and warming with a block. We found no significant differences among treatments.

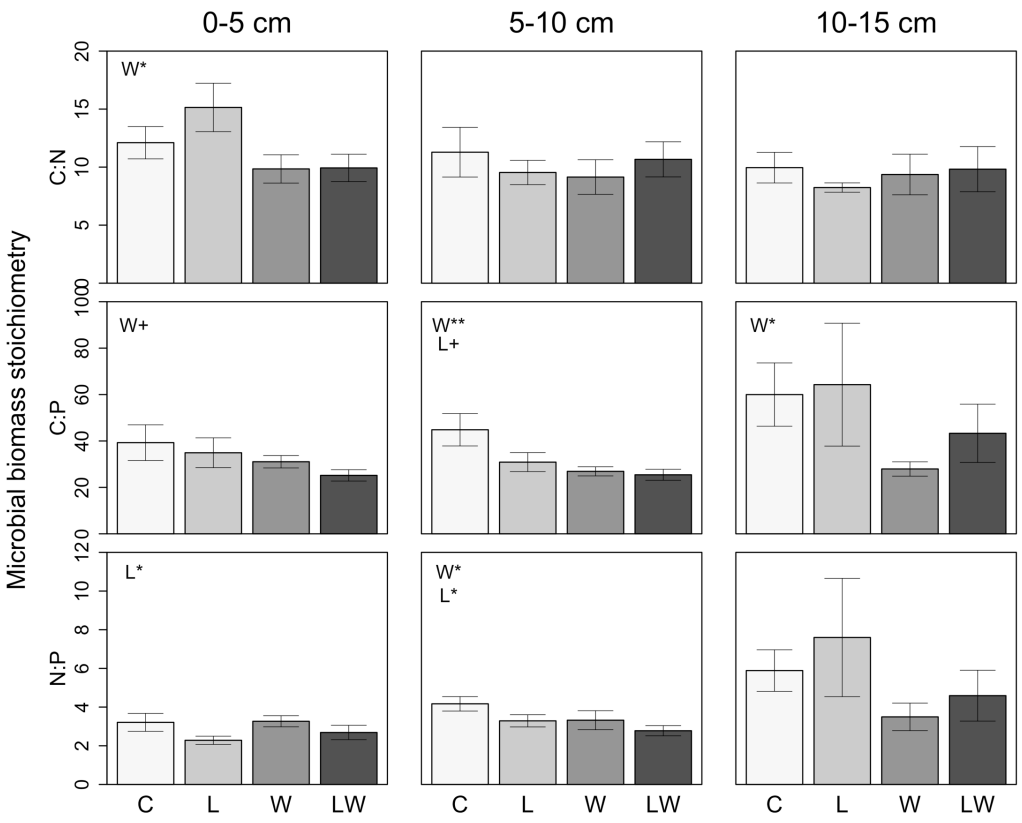

Figure S3.2: Microbial biomass stoichiometry at 3 depths for control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW). Bars represent means ± SE. Warming significantly decreased microbial C:P. Significant effects are indicated by +p < 0.1, *p<0.05, **p<0.01.

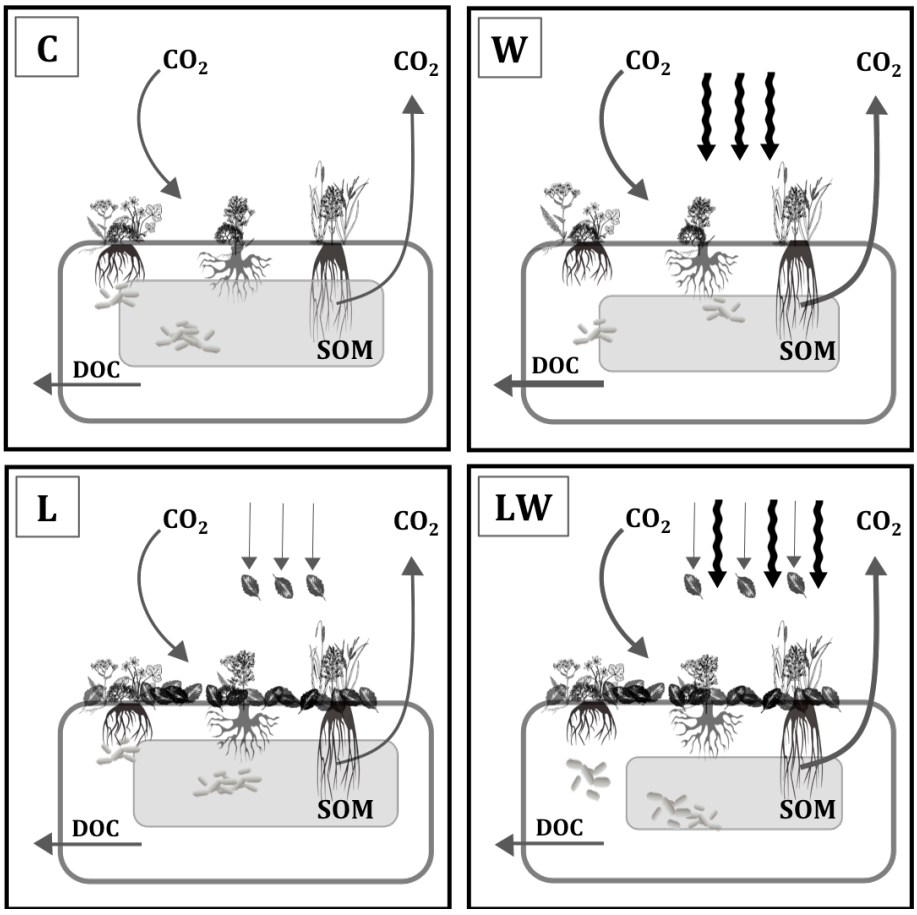

Figure S3.3: Summary of main findings in control plots (C), birch litter addition plots (L), warmed plots (W), and combined litter and warming (LW).

APPENDIX C

CHAPTER 4 SUPPLEMENTAL INFORMATION

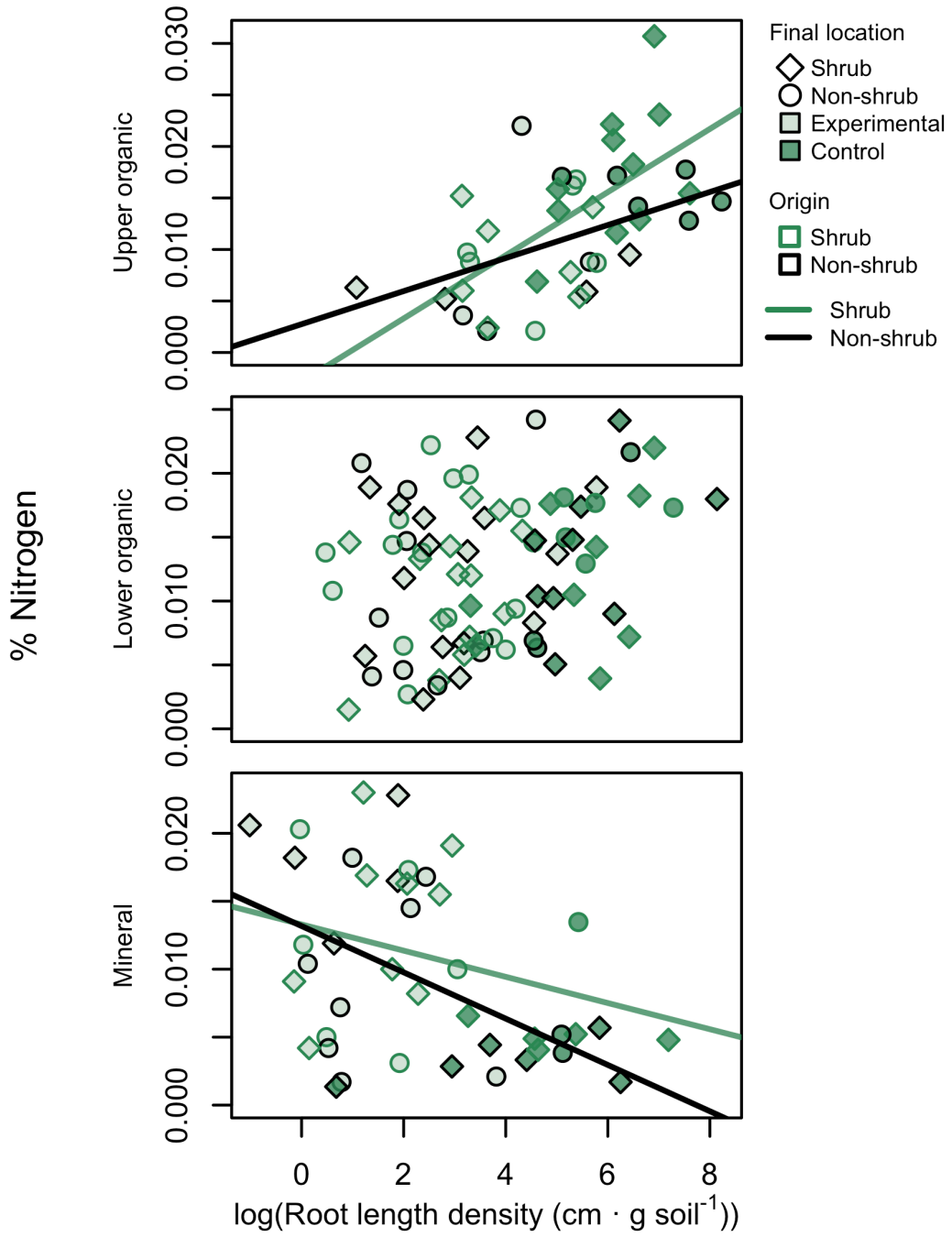

Figure S4.1: Soil % N shown by root length density. Scatterplot points indicate final location, origin and experimental status of soil cores. Lines represent significant interactions between root length and soil origin ($p < 0.05$).

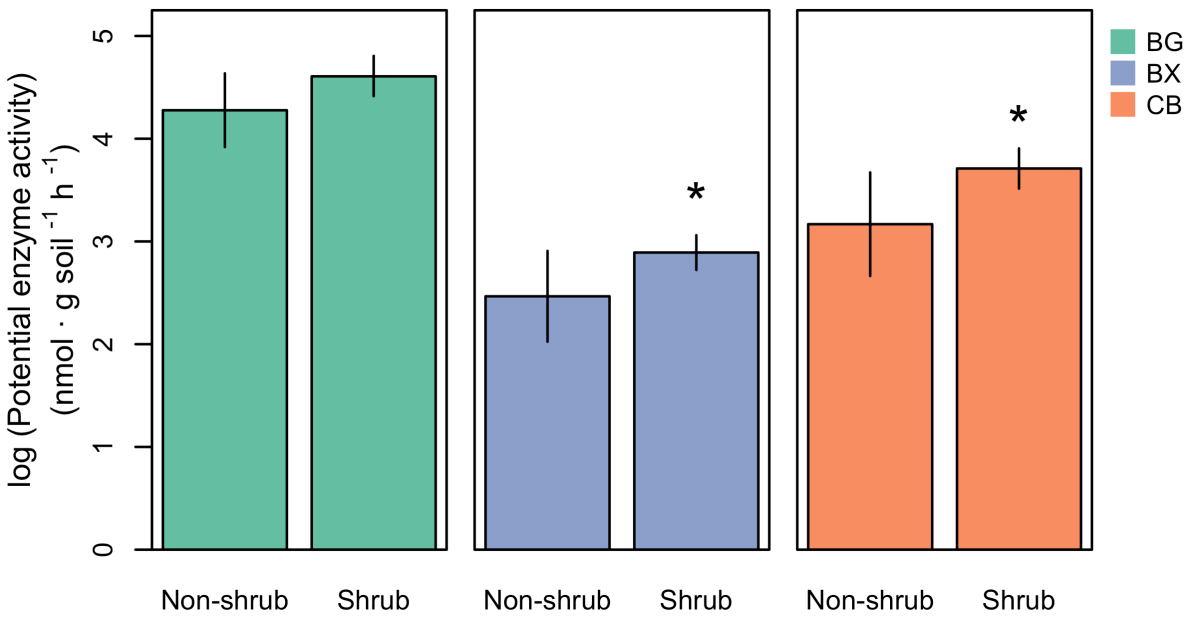

Figure S4.2: Potential enzyme activity shown by soil origin. Bars represent model coefficients ± standard error. Significant effects are indicated by *p < 0.05