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DECIDUOUS SHRUB ENCROACHMENT EFFECTS ON TUNDRA SOIL PROPERTIES

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DEDICATION

I would like to dedicate this paper to all the people who have supported me in its making.

Namely; My parents, my siblings, my advisor and my friends.

DECIDUOUS SHRUB ENCROACHMENT EFFECTS ON TUNDRA SOIL PROPERTIES

by

DANIELA AGUIRRE, BS

THESIS

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ABSTRACT

Deciduous shrub abundance is increasing in tundra ecosystems as an effect of rising temperatures which may change tundra physical properties and, in turn, microbial communities and biogeochemical processes. Two mechanisms through which shrub presence may affect tundra ecosystems were examined in this study; the physical presence of the shrubs and effects of increasing shrub litter inputs. In a sub-arctic alpine tundra ecosystem, dominated by the deciduous shrub *Betula glandulosa,* both shrub presence (shrub present and removed) and litter quantity (no litter/litter removed, ambient litter, and twice ambient litter) were manipulated; multiple ecosystem properties where measured within the treatment plots over four years. In Chapter 2, examining the effects of the treatments on physical properties of the environment, soil nutrients, and microbial processes, shrub presence is described to be the main mechanism by which shrubs encroachment caused changes in tundra ecosystems. Shrub presence increased soil temperatures in winter and decreased them in summer, decreased summer soil moisture, and increased soil C:N ratios. There were many fewer direct effects of increasing quantity of shrub litter, although increasing litter did increase microbial biomass, and soil C:N ratios. In Chapter 3 the effects of shrub removal and litter addition on litter decomposition rates were examined, describing impacts of both changes in the decomposition environment and changing litter quality. When compared to other litter types on the tundra *B. glandulosa* litter was of higher quality (lower C:N ratios) and decomposed faster. However, the presence of shrubs decreased decomposition rates through their effects on the decomposition environment. In conclusion, shrub encroachment is likely to have large effects on tundra ecosystem properties, although the opposing direction of these effects through different mechanisms hinder the ability to predict the net effect of these changes.

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1. INTRODUCTION: EFFECTS OF SHRUB ENCROACHMENT ON THE TUNDRA

Tundra ecosystems

Atmospheric carbon increases due to anthropogenic activities have caused worldwide temperature increases (IPCC 2014). However, changes caused by temperature increases are not equally distributed and some areas are experiencing changes faster than others. It has been estimated that arctic and sub-arctic areas (areas north of 60° N) have been experiencing temperature increases that are about twice as fast as other parts of the world (Böhm et al. 2001; Osborne et al. 2018). Tundra ecosystems occur in arctic and alpine areas, which are especially vulnerable to climate change due to their sensitivity to temperature (Grabherr et al. 2010; Gottfried et al. 2012). Although lower latitude alpine areas may not be experiencing temperature increases to the same extent, encroachment of warm conditioned plant species in to these increasingly warming areas are being seen in many mountain ranges around the world (Gottfried et al. 2012; Petitpierre et al. 2016; Vanneste et al. 2017). Due to this temperature sensitivity, alpine and arctic areas have become early indicators to what effects climate change will have in the future (Grabherr et al 2010; Gottfried et al. 2012).

The tundra is an important ecosystem in the global carbon cycle, holding over a third of the world's soil carbon (Ping et.al. 2008; McGuire et al. 2009; Chowdhury et al. 2015) with most of this store being held within the permafrost (Mack et al. 2004; Ping et.al. 2008). Historically the tundra has been a sink for carbon (Marion & Oechel 1993; Chowdhury et al. 2015), because low temperatures slowed the decomposition of organic material (Oechel et al. 1993). Temperature increases could affect the direction of carbon flux in the tundra, and because of the magnitude of the tundra's carbon stores, this could have large implications for the world's carbon cycle.

Rising temperatures are already affecting the tundra in ways that could affect carbon storage or release. Temperature increases have been accompanied by extended summers (Sharratt 1992; Hinzman et al. 2005; Cannone et al. 2007; Post & Forchhammer 2008; Wilson & Nilsson 2009), changes (primarily increases) in precipitation (IPCC 2014), increasing thickness of the active layer depth (the layer of the soil which thaws during the summer) (Schuur et al. 2008; IPCC 2014), and an increase in shrub abundance (Myers-Smith et al. 2011). Understanding the effects temperature increases are having on factors that control carbon flux can be complex due to the intertwined nature of positive and negative feedbacks to temperature increases caused by interactions between biotic and abiotic factors in the environment. In this project we will explore the effects global change is having on tundra ecosystem properties through the mechanism of shrub encroachment.

Shrub encroachment

Shrub encroachment is the increase in size, cover and/or abundance of shrubs in an area (Myers-Smith et al. 2011). Satellite and aerial imagery have recorded this increase of shrubs in many areas of the tundra (Silapaswan et al. 2001; Zhou et al. 2001; Jia et al. 2003; Tape et al. 2006). Arctic tundra shrubs have been reported to be increasing in cover by 1.2% per decade since the 1950's (Tape et al. 2006), and in the alpine tundra environment of the European Alps by 1.9% per decade since 1953 (Cannone et. al. 2007). The type of shrubs that are encroaching the tundra are primarily deciduous (herein, simply termed shrubs) (Myers-Smith et al. 2011; Beck et al. 2011) of the genera birch (*Betula* spp.), willow (*Salix* spp.) and alder (*Alnus* spp.) (Bret-Harte et al. 2002; Tape et al. 2006; Myers-Smith et al. 2011).

The mechanisms driving the increases in shrub productivity and encroachment have been linked to climate change (Bret-Harte et al. 2001; Myers-Smith 2011). Deeper soil thaw resulting from warming temperatures is expected to increase nutrient availability in the tundra because of increasing decomposition and mineralization rates (Nadelhoffer et al. 1991; Hinzman et al. 2005). Shrubs have been shown to be one of the strongest competitors for N in arctic tundra (Bret-Harte et al. 2008) perhaps as a result of their larger root biomass than other tundra species (Chapin et al. 1980) or their symbiotic relationship with mycorrhizal fungi (Orson 1982; Wallenstein et al. 2007). Long-term fertilization and warming experiments show that shrubs tend to outcompete other species when there are more nutrients available, higher temperatures or the combination of both (Jonasson et al. 1999; Shaver et al. 2001; Bret-Harte et al. 2002; Mack et al. 2004; Walker et al. 2005; Euskirchen et al. 2009; Sistla et al. 2013; DeMarco et al. 2014). This increase in shrub cover and size could have impacts in many tundra environmental properties.

Overall impacts of shrub encroachment

Effects on physical properties of the tundra

Shrub encroachment is affecting tundra snow cover and retention with their canopies. Snowfall is a big component of the water budget of tundra, as many of them get at least half of their yearly water supply as snow (Woo et al. 1983). Shrubs have been shown to accumulate more snow than other tundra plants, resulting in a thicker snow layer that insulates the soil from cold winter weather, causing higher soil temperatures in winter, a phenomena known as the snow-shrub hypothesis (Sturm et al. 2001). Several studies have also shown decreases in sublimation with increasing shrub abundance and height (Sturm et al. 2001; Sturm et al. 2005; Pomeroy et al. 2006). This is important to the hydrology of the tundra because approximately 10-25% of snowfall gets lost through sublimation during the winter in open tundra (Liston & Sturm

1998; Sturm et al. 2001). In the spring, shrubs tend to accelerate the melting of snow as their dark colored bark, compared to the snow, reduces albedo (Sturm et al. 2005) absorbing heat and melting the snow around it; lengthening the growing season and increasing the active layer depth during the summer (Pomeroy et al. 2006).

Shrubs could also affect temperatures and hydrology during the summer through the physical presence of the shrub canopy and water uptake. Shrubs have been shown to decrease soil temperatures beneath their canopy, likely through solar energy interception by their canopies (Block et al. 2010; Myers-Smith & Hik 2013), which could indirectly result in decreased soil moisture loss. Winter snow catchment and retention may also lead to shrub areas having higher moisture inputs during melt, causing higher soil moisture under shrubs very early in the summer. In contrast, shrub presence has been shown to decrease soil moisture (Myers-Smith & Hik 2013; Crofts et al. 2018), likely due to their high water uptake. During rainfall events shrub canopies could also potentially intercept more rain and reduce the amount of water that makes it to the soil (Tromble 1988; although see McLaren et al. 2004). Alternatively, shrubs may increase the water content directly beneath their canopies during rain events by funneling the rain along their stems down to where their stems meet the soil (Bhark & Small 2003).

Effects on carbon and nutrient cycling

Shrub effects on temperature could subsequently affect microbial activity. Previous studies have shown mixed effects of shrub winter temperatures on the productivity of microbial communities. Studies simulating shrub snow catchment with snow fences saw increases in winter microbial respiration rates (Welker et al. 2000; Schimel et al. 2003; Nobrega & Grogan 2007), increases in winter N mineralization (Schimel et al. 2003), decreased summer microbial respiration (Welker et al. 2000), and increases in summer N mineralization (DeMarco et al.

2011) although other studies have shown no significant effects of snow insulation on microbial activity (Myers-Smith & Hik 2013). Shrub encroached areas can keep soil temperatures higher than – 10° C for more days than open tundra (Sturm et al. 2001), which could allow for soil water to remain unfrozen and available for micro-organismal activity (Sturm et al. 2001). Some studies have shown that microbial respiration begins to rapidly decrease below 0° C but continues up to – 10° C (Mikan et al. 2002), signifying their continued activity at lower temperatures than those at which water freezes. With the contrasting effects of shrubs on temperature and hydrology during summer this may also lead to contrasting effects on microbial activity due to the sensitivity of microbial activity to temperature (Cornelissen et al. 2007) and soil moisture (Makkonen et al. 2012).

Higher amounts of litter fall by shrubs could also affect decomposition and nutrient movement. Shrubs have shown to have higher leaf litter inputs relative to other tundra plants (McLaren unpublished data), causing a thicker litter layer (Crofts et al. 2018). Shrubs also produce more woody tissue than most other tundra plants through the creation of their stems, rhizomes and roots (Chapin et al. 1980). Increasing shrub biomass will likely result in increases in carbon stocks within the shrubs biomass (Buckeridge et al. 2010) and increases in wood production could potentially become an important sink for carbon in the tundra because of woods recalcitrance (Sturm et al. 2005). Some studies have shown shrubs to have higher quality litter (lower C:N ratios) (McLaren et al. 2017) that decomposes faster than other litter types (Hobbie & Gough 2004; McLaren et al. 2017). Conversely, other studies have shown that shrub litter decomposes slower relative to other tundra plants (Cornelissen et al. 2007), which could increase immobilization of nutrients. A more labile litter and plant rhizodeposition can have a priming effect (PE) on organic matter reserves; which encourage increased mineralization or immobilization of soil carbon (C), nitrogen (N) and phosphorus (P) (Kuzyakov et al. 2000). Shrubs have a larger root biomass than other open tundra species (Chapin et al. 1980) and have a symbiotic relationship with mycorrhizal fungi (Orson 1982; Wallenstein et al. 2007), which could potentially mean a higher surface area for nutrient uptake. The increased surface area of their roots may mean that shrubs release more C-rich exudates to the soil (Nannipieri et al. 2008).

Shrub encroachment could also impact the microbial community through changes to the plant community composition. Shrubs are affecting plant community composition; in the understories of shrubs, forb cover increases and cryptogram and dwarf shrub cover decreases compared to shrub free areas (Pajunen et al. 2011; Crofts et al. 2018). Microbial communities can adapt to repeated exposure from a certain litter type (Ayres et al. 2009; Strickland et al. 2009). This adaptation of microbial communities to better decompose litter which they are repeatedly exposed to would increase the decomposition rate of the litter (Wallenstein et al. 2013) and carbon and nutrient cycling. This effect is commonly known as home field advantage (HFA) (Gholz et al. 2000), where litter decomposes faster in its native environment due to the composition of the microbial community which is adapted to decompose the litter from that environment (Ayres et al. 2009). HFA tends to have a stronger effect in microbe communities that are repeatedly exposed to recalcitrant litter (Wallenstein et al. 2013). If shrub litter is more recalcitrant than other litter type in the tundra (Cornelissen et al. 2007) then this could be a bigger driver for the microbial community to change. Already, some studies have seen differences in microbial community composition between shrubs and open tundra (Wallenstein et al. 2007; Chu et al. 2011). However, it is not clear how this change is affecting decomposition in these two areas, as studies have seen shrub areas to reduce (Lynch et al. 2018) and increase (Phillips et al. 2019) microbial respiration.

Synthesis

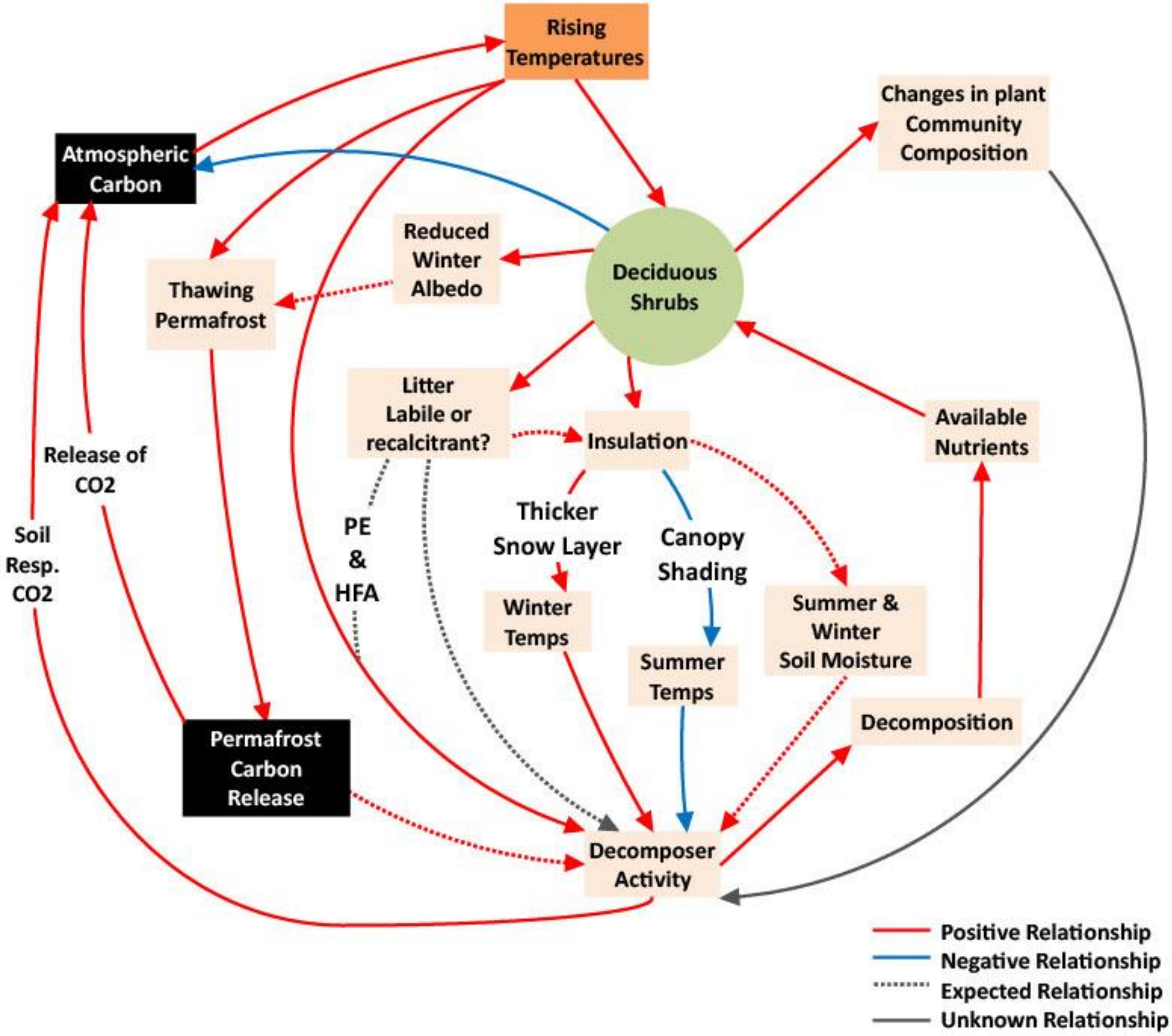

Figure 1.1. Diagram of supported (solid lines) and expected (dashed lines) shrub effects in the tundra.

Shrub encroachment has already been shown to affect many tundra environmental properties including; winter (Sturm et al. 2001) and summer (Block et al. 2010; Myers-Smith and Hik 2013) soil temperature, summer soil moisture (Myers-Smith & Hik 2013; Crofts et al. 2018), albedo in winter (Sturm et al. 2005), soil nutrients (Schimel et al. 2003; Nobrega & Grogan 2007), mycorrhizal composition (Orson 1982; Wallenstein et al. 2007), and plant composition (Pajunen et al. 2011; Crofts et al. 2018) to name a few. This results in a complex set of potential changes to tundra plant and microbial composition and ecosystem function (Figure 1.1). In this study it is hypothesized that shrubs affect tundra soil properties both through their physical presence but also by changing the quantity and quality of litter production of the plant community (see dashed and solid lines in Figure 1.1). Understanding the primary mechanism for changing ecosystem properties will help researchers better understand the direction of the changes and their potential ramifications. No studies have separated the effects of the physical presence of shrubs from that of their increased litter production. Therefore, this study will experimentally manipulate both the presence of shrubs and the quantity of litter inputs in a sub-arctic alpine tundra ecosystem near Kluane Lake, Yukon Territory, Canada to examine their effects on ecosystems properties and processes (Chapter 2) and litter decomposition rates (Chapter 3).

2. SHRUB ENCROACHMENT EFFECTS ON TUNDRA PROPERTIES

Introduction

Atmospheric carbon increases due to anthropogenic activities have caused worldwide temperature increases (IPCC 2014). However, changes caused by temperature increases are not equally distributed and some areas are experiencing changes faster than others. It has been estimated that arctic and sub-arctic regions (areas north of 60° N) have been experiencing temperature increases that are about twice as fast as other parts of the world (Böhm et al. 2001; Osborne et al. 2018). Tundra ecosystems which occur in arctic and also in alpine regions are especially vulnerable to this accelerated climate change due to their sensitivity to temperature (Grabherr et al. 2010; Gottfried et al. 2012). Because of their temperature sensitivity, these areas have become early indicators to what effects climate change will have in the future (Grabherr et al. 2010; Gottfried et al. 2012).

Rising temperatures have been linked to shifting plant communities in many tundra ecosystems (Zhou et al. 2001; Jia et al. 2003). Although lower latitude alpine areas may not be experiencing temperature increases to the same extent as their northern counterparts, encroachment of warm conditioned plant species in to these increasingly warming areas are being seen in many mountain ranges around the world (Gottfried et al. 2012; Petitpierre et al. 2016; Vanneste et al. 2017). Both arctic and alpine tundra are experiencing a shift from a graminoid dominated tundra to one dominated by deciduous shrubs (henceforth simply called shrubs) (Silapaswan et al. 2001; Tape et al. 2006) primarily of the genera birch (*Betula* spp.), willow (*Salix* spp.) and alder (*Alnus* spp.) (Bret-Harte et al. 2002; Tape et al. 2006; Myers-Smith et al. 2011), all three of which were previously common in the tundra at low densities. Warming temperatures in the tundra are predicted to increase nutrient availability through increased decomposition of soil organic matter and increased nutrient mineralization rates (Oechel & Billings 1992; Sturm et al. 2005). Both warming temperatures and increased nutrients have been shown to promote shrub abundance in long-term fertilization and warming experiments (Jonasson et al. 1999; Shaver et al. 2001; Bret-Harte et al. 2002; Mack et al. 2004; Walker et al. 2005; Euskirchen et al. 2009; Sistla et al. 2013; DeMarco et al. 2014).

Shrub encroachment is already affecting soil physical properties in the tundra. For example, during the winter shrubs have been shown to accumulate more snow than other shorter-statured tundra plants, resulting in a thicker snow layer that insulates the soil from cold weather and causes higher soil temperatures in winter (i.e., the "snow-shrub effect", Sturm et al. 2001). Increased snow depth may also result from decreases in snow sublimation with increasing shrub abundance and height (Sturm et al. 2001; Sturm et al. 2005; Pomeroy et al. 2006). This is important because snowfall comprises of at least half of the yearly water supply of many tundra areas (Woo et al. 1983) and around 10-25% of snowfall usually gets lost through sublimation (Liston & Sturm 1998; Sturm et al. 2001). In contrast, during the summer shrubs have been shown to decrease soil temperatures, likely through solar energy interception with their canopies (Block et al. 2010; Myers-Smith & Hik 2013). Observational studies have also noted decreased soil moisture under shrub canopies (Myers-Smith & Hik 2013; Crofts et al. 2018), perhaps resulting from increased water uptake by shrubs.

Shrubs could also affect carbon and nutrient cycling in the tundra, both directly through uptake and indirectly through their effects on physical properties. Shrubs could increase nutrient movement from the soil by direct uptake; shrubs have large shallow root systems (Chapin et al. 1980; Wang et al. 2016) and symbiotic relationships with mycorrhizal fungi (Orson 1982; Wallenstein et al. 2007) that could extend their reach for nutrients. Studies simulating shrub snow catchment with snow fences saw increased in winter microbial respiration (Welker et al. 2000; Schimel et al. 2003; Nobrega & Grogan 2007), and winter (Schimel et al. 2003) and summer N mineralization (DeMarco et al. 2011) as well as decreased summer microbial respiration (Welker et al. 2000). Shrubs have also been linked to increased microbial biomass in summer (Buckeridge et al. 2010), both reduced (Lynch et al. 2018) and increased (Phillips et al. 2019) microbial respiration, and differences in microbial community composition (Wallenstein et al. 2007; Chu et al. 2011). The tundra holds over a third of the world's soil carbon (Ping et.al. 2008; McGuire et al. 2009; Chowdhury et al. 2015) with most of this store being held within the permafrost (Mack et al. 2004; Ping et.al. 2008). Shrub induced changes in the release and storage of carbon have the potential to not only affect tundra ecosystems but also climate change because of the tundra's vast reserves. Therefore, understanding the implications of these shrub effects on tundra carbon storage are particularly important because of the potential amplification of climate change from the increased rate of release of the large tundra carbon reserve.

In addition to changes resulting from the shrub canopy, differences in the quantity and quality of the litter produced by deciduous shrubs may also affect physical properties and carbon and nutrient cycling under shrubs. Shrubs produce higher litter inputs than other tundra plants (McLaren unpublished data) resulting in a thicker litter layer under shrubs than surrounding non-shrub tundra (Crofts et al. 2018). In other ecosystems litter plays a vital role in acting as an insulator for the soil (Sayer 2006; Song et al. 2018) and the thicker litter layer under shrubs could also insulate the soil in tundra ecosystems. In addition to changing the quantity of litter input, changes in the quality of litter with increasing shrub dominance may also affect tundra nutrient cycling. Shrubs have higher quality litter (lower C:N ratios) (McLaren et al. 2017) and have been shown to decompose faster than other tundra litter types (Hobbie & Gough 2004; McLaren et al.

2017), although other studies have found that shrub litter decomposes slowly relative to other tundra plants (Cornelissen et al. 2007). A combination of the changing quantity and quality of litter production by shrubs are likely to affect carbon and nutrient movement in the tundra.

Although many studies have shown ecosystem consequences of encroaching shrubs, the direction of these effects is not consistent between studies, and none have separated out the effects of the physical presence of shrubs from that of their increased litter production. This study experimentally manipulates both the presence of shrubs and the quantity of litter inputs in a sub-arctic alpine tundra ecosystem near Kluane Lake, Yukon Territory, Canada to examine the following objectives: to understand how deciduous shrubs (*B. glandulosa*) and their litter, both independently and in combination, affect (1) soil physical properties, (2) soil nutrient availability and (3) soil microbial processes.

Materials and Methods

Site description

This study is located in the sub-arctic alpine tundra of northern Canada, within the Kluane Lake region of the Yukon Territory. This region is considered semiarid as it receives less than 30 cm of precipitation annually, with about 40-50% of this precipitation falling as snow and the rest as rain (Krebs et al. 2001). The soil has two distinct layers, a strongly organic layer making up ca. the top 13 cm of the soil profile ("organic layer") and a layer below which is primarily clay with little organic material ("mineral layer"), collected up to ca. 35 cm in depth. In this alpine region, vegetation transitions with elevation, with lower elevations a *Picea glauca* dominated boreal forest, followed by shrub (*Salix* spp and *Betula glandulosa)* dominated alpine tundra and then open alpine tundra (Crofts et al. 2018). The experimental plots are in patchy shrub alpine tundra at 1,493 m in elevation on a north facing slope with shrub cover dominated by *B. glandulosa,* a deciduous dwarf shrub.

Experimental manipulations

The presence of shrubs (removed vs. present) and shrub leaf litter (0x (all litter removed), 1x (natural abundance litter), 2x (double natural abundance litter)) were experimentally manipulated in a fully factorial experiment, with each of the six different treatments combinations (2 shrub x 3 litter treatments) replicated six times, for a total of 36 plots. Plots are 1x1 m in area and are surrounded by ca. 20 cm high nylon fencing to prevent litter movement in or out of plots. Plots were arranged into 12 blocks, with each block consisting of a single patch of *B. glandulosa*. Shrub patches are ~12 m^2 areas of continuous *B. glandulosa* growth. Shrub treatment (absent or present) was applied at the block level and within each block (shrub patch) there is a single replicate of each litter treatment. Every plot within a block is separated by a minimum of 1 m and blocks were separated by a minimum of 8 m.

Shrub removals were done manually, clipping all shrub stems in the patch of *B. glandulosa* at the soil surface, with the removals maintained every year. The three litter treatments represent a range of litter quantity: all litter removed, natural litter abundance, and double litter abundance. Litter treatments were first applied in summer 2015 by collecting litter from all 36 plots and determining the average litter mass per plot; this was considered natural litter abundance and it averaged 470 g/m^2. Litter was homogenized and then re-distributed among plots, with the amount dependent on litter treatment (none, 1x natural abundance or 2x natural abundance). In subsequent years, natural litter fall was determined by averaging the mass of collected newly fallen or senesced litter from the litter-removed/shrubs-present plots in 2016 it was 171 g/m^2, and in 2017 it was 215 g/m^2. Additional litter was collected from the litter-removed plots and from neighboring plants to complete litter additions in the remaining plots (a mass equivalent to natural litter fall added to natural abundance plots without shrubs, and to 2x natural abundance plots with shrubs, and a mass equivalent to double natural litter fall added to 2x natural abundance plots without shrubs). Litter manipulations were maintained by hand each fall. All soil and ecosystem sampling occurred during the growing season (June – August) of 2015 – 2018 with the exception of soil temperature, which was recorded year round.

Response Variables

A number of responses were examined including; *physical properties* (soil temperatures, soil moisture, photosynthetically active radiation (PAR) and pH for both soil layers), *nutrients* (available nutrients (ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-})), extractable organic carbon (EOC), and extractable total nitrogen (ETN), soil total %C and %N), and *microbial processes* (microbial biomass (microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphate (MBP)), microbial extracellular enzyme (exo-enzyme) activity, nitrogen mineralization rates (N-Mineralization), and soil respiration (root + microbial respiration)).

Field sampling

Physical properties: Soil temperature was measured year-round (July 2015 – July 2018) at the soil surface and 5 cm below surface using Thermochron iButton temperature loggers (model DS1921G-F5, Maxim integrated, San Jose, CA, USA) in 8 of 12 blocks (randomly chosen with the constraint of 4 blocks with shrubs present and 4 with shrubs removed). During the growing season (June – August) temperature was logged every 90 minutes, and every 255 minutes during the remainder of the year. Soil moisture was measured at the top 12 cm of the soil layer once a month during the growing season, with three replicate measures per plot, using a soil moisture probe (HydroSense II Soil Moisture Measurement System, Campbell Scientific, Thuringowa Central, QLD, Australia). PAR was measured on a single cloudless day mid-summer each year (2015-2018) using a MQ-306: Quantum Sensor (Apogee Instruments, Logan, UT, USA) just above the shrub height (~1 m) and at soil surface to calculate interception of PAR by the vegetation.

Nutrients and Microbial processes:

In July of each year two soil cores were collected from both the organic and the mineral layer of each plot, soil was homogenized and rocks (larger than 2mm) removed, and then partitioned for the various analyses described below. Soil sub-samples were frozen at -20°C and shipped to UTEP for analyses except for %C, %N samples which were dried at 50°C prior to shipping. N-Mineralization rates for the organic layer only were determined in the 2015 and 2018 growing seasons using an *in situ* soil incubation (DiStefano & Gholz 1986; DeMarco et al. 2011). A single soil core was incubated in each plot in butyrate plastic cores (4.8 cm in diameter and 12 cm in length) which are open at the top and bottom to permit water flow and enclosed in a nylon stocking. Each core had a resin bag on the surface (to prevent inflow of N from above), a resin bag below the organic layer (to capture N leaching out of the core) and a second resin bag above the mineral layer (to prevent inflow of N from below). Resin bags contained 20 g wet weight of resin beads (IONAC NM-60 H+/OH- form, type I beads 16–50 mesh; J. T. Baker, Phillipsburg, NJ, USA) enclosed in a nylon stocking. An initial soil sample was collected adjacent to the core at the time of installation for determining bulk density and initial soil available nitrogen as below. Cores were incubated *in situ* for ca. 1 month (2015: 7/11-8/2, 2018: 06/25-7/29). Soil was removed and homogenized and then soil and resin bags frozen and sent to UTEP for available nitrogen analysis as below.

Soil respiration (root + microbial respiration) was measured in 2016-2018 every two weeks during the summer months using an EGM-5 Portable CO_2 Gas Analyzer (PP Systems, Amesbury, MA, USA). A PVC collar with a diameter of 10 cm was installed in every plot to 5 cm depth, and the vegetation removed from within each collar to exclude aboveground plant respiration from measurements. Soil respiration monthly mean, for each June and July, was used for statistical analysis.

Lab analysis

Physical Properties: For soil moisture only mid-July measurements were used because mid-June soil moisture measurements were not done every year. pH was measured using a pH meter (pHTestr 30, Oakton Instruments, Vernon Hills, IL, USA) on 15 g of soil mixed with 30 ml of DI water, after settling for 30 minutes to equilibrate with atmospheric CO_2.

Nutrients: Nutrients were extracted from 5 g of thawed soils using 25 ml of 0.5M K_2SO_4 shaken for 2 hours, and then filtered through glass filter paper. Available nutrients (NH_4^+, NO_3^-, & PO_4^{3-}) were analyzed on extracts using colorimetric microplate analysis (Synergy HT, BioTek Instruments, Winooski, VT, USA). NH_4^+ analysis was done using the Rhine et al. (1998) method, which uses a colorimetric reaction of ammonia to Berthelot reagent. NO_3^- analysis was done using the Doane and Horwáth (2003) method where nitrate is reduced to nitrite and reacts with Griess reagents. PO_4^{3-} analysis was done using the malachite green assay (D'Angelo et al. 2001). EOC and ETN, in the extracts were analyzed using a Schimadzu CN analyzer (TOC-VCPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). %C and %N were measured using a dry combustion C and N analyzer (ElementarPyroCube®) on subsamples of both soil layers which had been dried and ground.

Microbial processes:

MBC, MBN, and MBP were quantified using a modification of the chloroform fumigation-extraction technique (Brookes 1985). 5 g of thawed soil was incubated with 2 ml chloroform for 24 hours, extracted using 0.5M K_2SO_4 and analyzed for EOC, ETN and PO_4^{3-} as described above. MBC, MBN and MBP flushes were calculated as the difference between EOC, ETN and PO_4^{3-} in fumigated and non-fumigated extracts. No correction factor was applied for incomplete $CHCL_3$-release or sorption of P because these values are not known for K_2SO_4 extraction for this ecosystem.

Extracellular enzyme activity was measured using microplate assays (Saiya-Cork et al. 2002; McLaren et al. 2017) through the use of fluorescently tagged substrates. Activity was examined on a suite of hydrolytic enzymes that release carbon, nitrogen and phosphorous at the final stages of organic matter decomposition; cellulose-degrading β-glucosidase (BG) and cellobiohydrolase (CBH), hemicellulose-degrading β –xylosidase (BX), carbohydrate-degrading α-glucosidase (AG), chitin-degrading N-acetyl-glucosaminidase (NAG), an amino acid with N-terminal end degrading enzyme (LAP), phosphatase (Phos), and phosphodiesterase (Phos-D). A slurry was created by blending 1 g of soil thawed immediately before analysis with 125 ml of a modified universal buffer adjusted to the average soil pH (6.3). The slurry was pipetted into a black microplate, mixed with the fluorescently tagged substrates, and incubated for 3.5 hours with fluorometric measurements taken at 30 minute intervals (Synergy HT BioTek plate reader) at 360 nm excitation and 460 nm emission. For each substrate/soil combination fluorescence was adjusted for background fluorescence of soils and substrate and the quenching of MUB. A MUB standard curve was used to calculate μg of substrate hydrolyzed per hour per g of soil. Oxidative enzymes (phenol oxidase (phenol) and peroxidase (perox)) were quantified by looking at the degradation of a l-3,4-dihydroxyphenylalanine (l-DOPA) substrate. Color absorbance at 460 nm was measured after 24 hours of incubation at 10°C.

Analysis

N-mineralization rates: Inorganic N (N-NH_4^+ + N-NO_3^-) within the incubated soil core (N^{Final}) and from the initial soil sample ($N^{Initial}$) were calculated based of the bulk density (BD) and the dimensions of the organic layer core in each plot, these values were calculated as μg N per core, or equivalent soil volume for $N^{Initial}$.

N values for the middle resin bags were calculated as μg N per resin bag (N^{resin}). Total N mineralized (N^{Total}) was calculated as:

$$N^{Total} = (N^{Resin} + N^{Final}) - N^{Initial}$$

Mineralization rates were then converted to g N/organic layer m^2 based on the area of the core, and divided by incubation time.

Statistical Analysis: All data was analyzed as a 3 way ANOVA with year, shrub removals and litter treatments as the main factors. The organic and mineral layer soils were analyzed separately. Data that did not follow the ANOVA assumption of normality was either: transformed using a log transformation or analyzed using a Kruskal Wallis test if data could not be transformed. For the variables where a Kruskal-Wallis was used, only main treatment effects were examined and not their interactions. For most variables data from all 4 years of the study (2014-2018) were included in analyses. However, in some years the majority of a particular nutrient, potential exoenzyme activity or microbial biomass measurements were below the detectable range of the analysis and these years were excluded from analyses. Statistics for year-round data such as soil temperature were done by calculating mean monthly temperature within each year and then averaging the summer (June-August) and winter (November-April) seasons separately for each year and running separate analysis on the two seasons. When there was a significant effect of litter treatment or year, we used a post-hoc Tukey tests to test for differences between individual treatments or years. All statistics were done using R software version 3.5.3 and JMP 12 (SAS Institute, Cary, NC, USA).

Results

Physical Properties

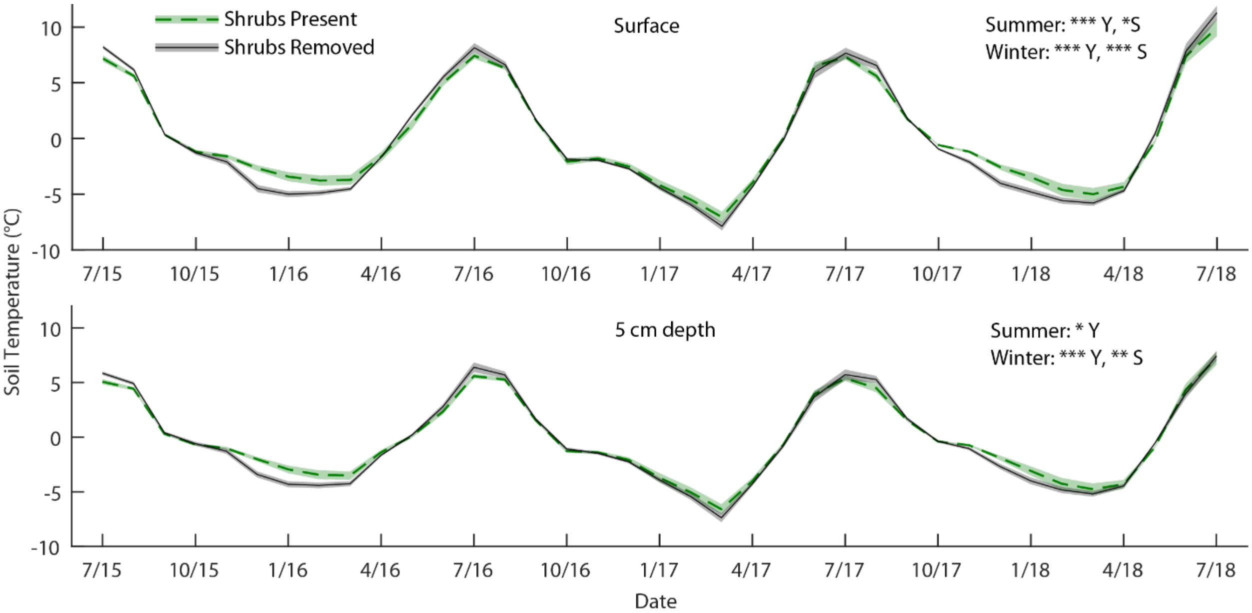

Figure 2.2. Monthly mean (line) (± SE (shaded area)) soil temperature at the soil surface (a) and 5 cm depth (b) for shrub (dashed green line) and shrub removal (solid black line) plots between July 2015 and June 2018 in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Statistical significance of Y (Year), S (Shrub), L (litter), and their interactions is presented as: ~ 0.1 > p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001. Analyses were run independently for summer (June – August) and winter (November-April) across all 4 years.

Table 2.1. Summary of three-way ANOVAs (or Kruskal-Wallis when data could not be normalized) for year, shrub, litter and their interactions on physical properties of the environment for a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Bold values are significant at $p < 0.05$.

Physical Properties		Year (Y)	Shrub (S)	Litter (L)	Y x S	Y x L	S x L	Y x S x L
Soil Temperature	df	3,72	1,72	2,72	3,72	6,72	2,72	6,72
Summer Temp 0cm	F	15.81	4.93	0.61	0.38	0.15	0.78	1.35
	p	**<0.001**	**0.030**	0.545	0.767	0.988	0.464	0.248
Winter Temp 0 cm	F	19.27	13.84	0.23	0.25	0.10	0.17	0.08
	p	**<0.001**	**<0.001**	0.792	0.860	0.997	0.843	0.998
Summer Temp 5 cm	F	3.75	1.96	0.23	0.41	0.26	0.08	0.27
	p	**0.015**	0.166	0.792	0.744	0.952	0.924	0.950
Winter Temp 5 cm	F	27.25	8.13	0.19	0.16	0.02	0.20	0.10
	p	**<0.001**	**0.006**	0.825	0.926	1.000	0.823	0.996
Soil Moisture	df	3,120	1,120	2,120	3,120	6,120	2,120	6,120
Mid July	F	39.53	5.95	0.83	1.67	0.65	0.12	0.66
	p	**<0.001**	**0.016**	0.441	0.177	0.693	0.889	0.684
PAR Interception	df	3, N=144	1, N=144	2, N=144	–	–	–	–
	γ^2	2.47	88.92	0.93	–	–	–	–
	p	0.481	**<0.001**	0.628	–	–	–	–
Organic Soil pH	df	3,120	1,120	2,120	3,120	6,120	2,120	6,120
	F	118.99	0.12	1.03	1.60	0.21	0.91	0.31
	p	**<0.001**	0.735	0.362	0.194	0.972	0.407	0.931
Mineral Soil pH	df	3, N=144	1, N=144	2, N=144	–	–	–	–
	γ^2	82.27	5.43	1.75	–	–	–	–
	p	**<0.001**	**0.020**	0.417	–	–	–	–

Note: Degrees of freedom differ by variable due to the type of statistical analysis

Soil temperatures at both depths (0 cm and 5 cm) were warmer in shrub than shrub removal plots during winter months (November-April) (Figure 2.1, Table 2.1). In summer months (June-August) there was a shrub effect at the soil surface where soil temperatures were colder in shrub plots, but there was no shrub effect at 5 cm depth (Figure 2.1, Table 2.1). Differences between shrub present plots and removal plots in winter at the soil surface average 0.77 °C and at 5cm depth average 0.56 °C, in summer at the soil surface they average 0.61 °C. There were no effects of litter treatment on soil temperature for either soil depth or season (Table 2.1). Shrub presence both reduced mid-July soil moisture and increased PAR interception (Figure S1). The mineral soil pH was lower in shrub plots although this effect was not seen in the organic layer (Table 2.1). There were no effects of litter treatments on any of these variables (Table 2.1). Mid-July soil moisture varied by year with the highest year soil moisture in 2017 and the lowest 2018 (Figure S2). pH for both the organic and mineral layer differed by years and was lowest in 2018 (Figure S2).

Nutrients

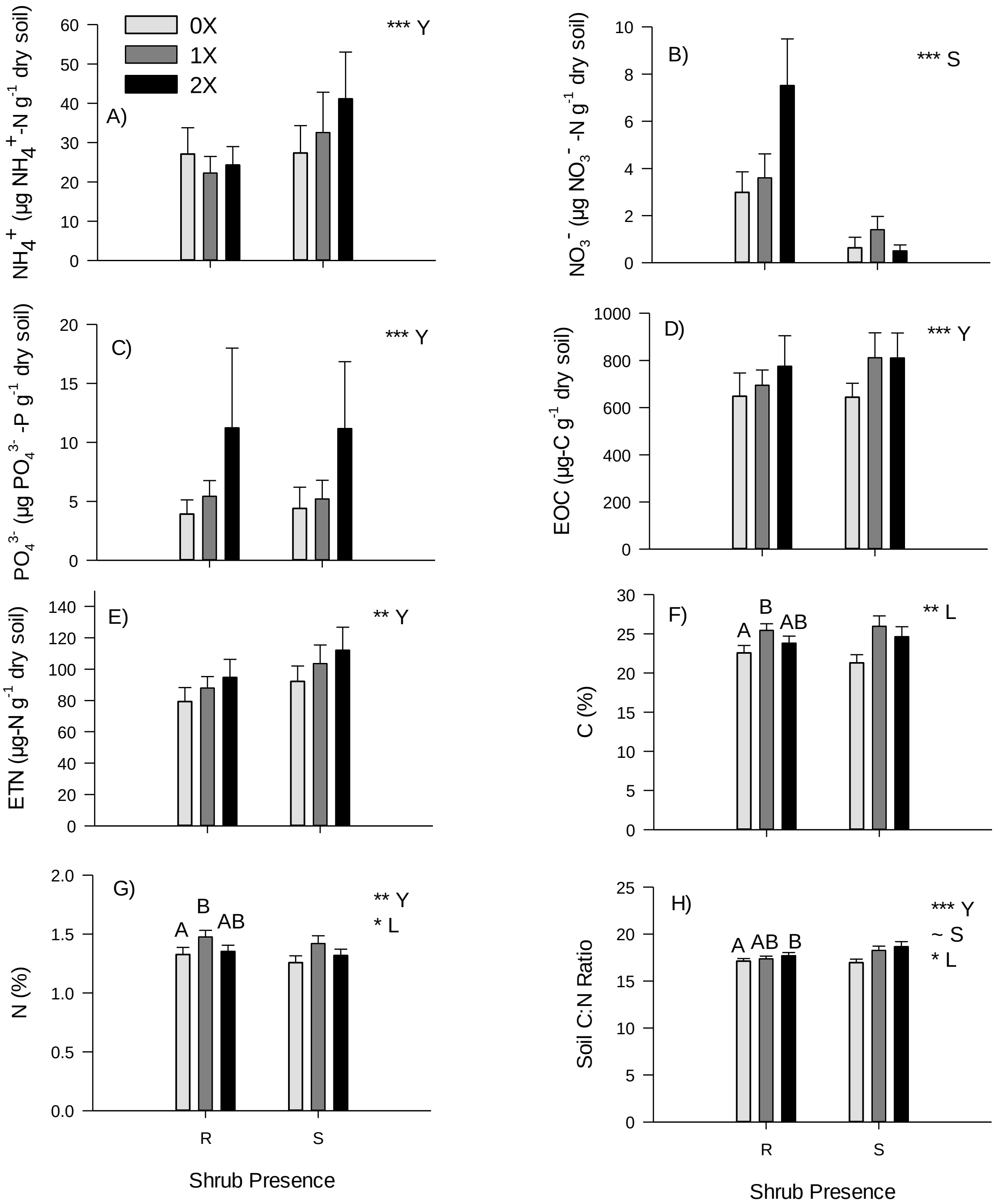

Figure 2.3. Mean (± SE) of soil nutrient measures (NO_3^-, NH_4^+, PO_4^{3-}, EOC, ETN, %C, and % N and C:N ratios) in the organic soil in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Means are across 4 years (2014-2018), for most variables, with a single measurement in July of each summer. Statistical significance for Y (Year), S (Shrub), L (litter), and their interactions is presented as: ~

0.1 > p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001. Letters above the bars present statistically significant differences in litter quantity.

Table 2.2. Summary of three-way ANOVAs (or Kruskal-Wallis when data could not be normalized) for year, shrub, litter and their interactions on organic layer soil nutrients in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Bold values are significant at $p < 0.05$.

Organic Layer		Year (Y)	Shrub (S)	Litter (L)	Y x S	Y x L	S x L	Y x S x L
	df	3,120	1,120	2,120	3,120	6,120	2,120	6,120
NH_4^+	F	81.50	0.26	0.57	1.00	1.59	0.16	1.03
	p	**<0.001**	0.609	0.566	0.395	0.155	0.853	0.408
EOC	F	21.77	1.06	1.40	1.96	0.72	0.10	0.83
	p	**<0.001**	0.305	0.250	0.124	0.635	0.908	0.550
EON	F	4.19	2.10	0.88	1.04	0.99	0.11	0.52
	p	**0.007**	0.150	0.417	0.377	0.432	0.892	0.794
Soil C%	F	0.93	0.00	5.81	0.01	0.30	0.51	0.78
	p	0.427	0.978	**0.004**	1.000	0.934	0.600	0.588
Soil N%	F	4.57	1.27	3.76	0.06	0.35	0.05	0.33
	p	**0.005**	0.262	**0.026**	0.979	0.908	0.956	0.918
CN Ratio	F	6.51	3.29	4.44	0.07	0.20	1.36	1.46
	p	**<0.001**	0.072	**0.014**	0.977	0.978	0.262	0.196
NO_3^-	df	—	1, N=36	2, N=36	—	—	—	—
	χ^2	—	15.93	1.28	—	—	—	—
	p	—	**<0.001**	0.529	—	—	—	—
PO_4^{3-}	df	2, 90	1, 90	2, 90	2, 90	4, 90	2, 90	4, 90
	F	15.30	0.02	1.41	1.19	1.07	0.16	0.62
	p	**<0.001**	0.877	0.250	0.309	0.377	0.854	0.648

Note: Degrees of freedom differ by variable depending on the number of years the measurement was repeated (or was usable) and/or due to the type of statistical analysis.

In the organic layer, NO_3^- was lower and CN ratios marginally higher in shrub plots than in shrub removal plots, while none of the other nutrients were affected by shrub removal treatments (Figure 2.2, Table 2.2). Soil % C, % N and C:N ratios were significantly affected by litter treatments (Figure 2.2, Table 2.2). The 0x litter and 2x litter plots both had lower %C and %N than 1x litter plots (Figure 2.2). The C:N ratios for the 0x litter pots were lower than 2x litter plots while neither differed from the 1x litter plots (Figure 2.2). Only some years of data were analyzed for NO_3^- (2017) and PO_4^{3-} (2016-2018) because most values in other years were below the detectable range of the analysis.

Few nutrient measures showed responses to shrub and litter treatments in the mineral layer (Figure S3, Table S1). The effect of shrubs on mineral layer NH_4^+ depended on year (Table S1) where 2015-16 shrub plots had lower NH_4^+ than shrub removal plots, in 2017 there was no difference between shrub treatments, and in 2018 the relationship seen in the 1st 2 years reversed so that shrub plots had higher NH_4^+ than shrub removal plots (Figure S14, Table S1). Only some years were analyzed for NO_3^- (2017-2018) because most values in other years were below the detectable range of the analysis. PO_4^{3-} was not analyzed because most values were below the detectable limit of the analysis.

Microbial Processes

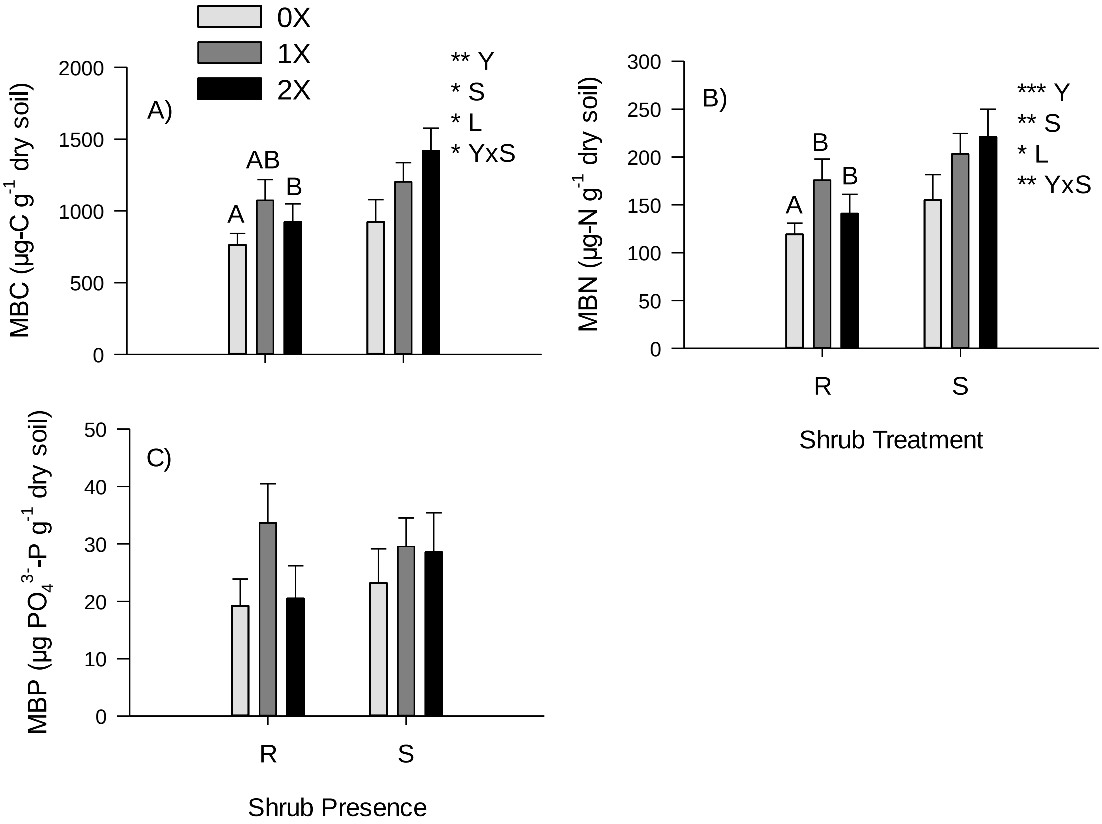

Figure 2.4. Mean (± SE) for organic layer microbial biomass (MBC, MBN, MBP) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Means are across 4 years (2014-2018) (2 years, 2017-2018, for MBP) with a single measurement in July of each summer. Statistical significance between Y (Year), S (Shrub), L (litter), and their interactions is presented as: $\sim 0.1 > p > 0.05$, *$p < 0.05$,

**p < 0.01, ***p < 0.001. Letters above the bars present statistically significant differences in litter quantity.

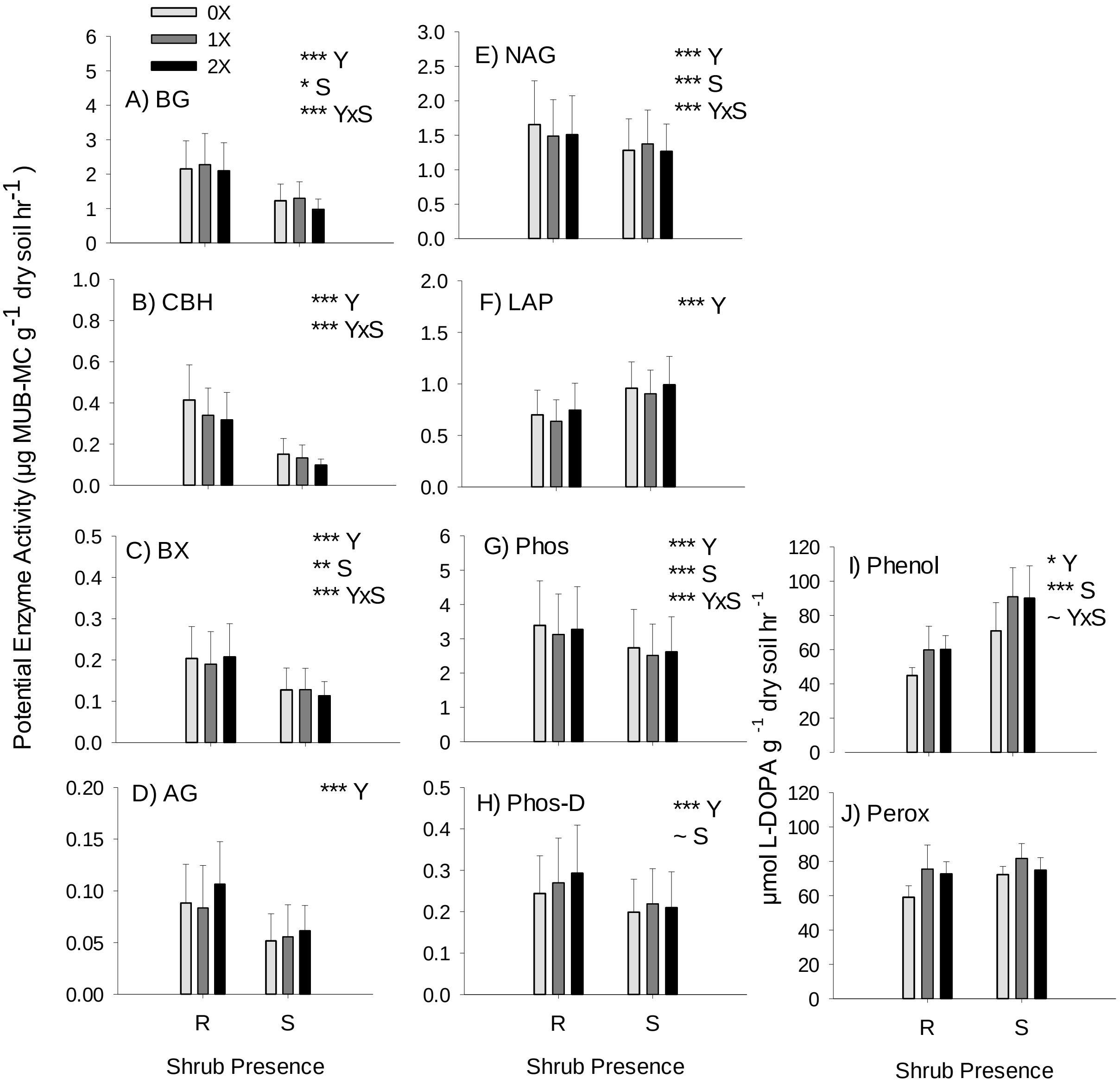

Figure 2.5. Mean (± SE) potential activity of hydrolytic enzymes (BG, CBH, BX, AG, NAG, LAP, Phos, and Phos-D) and oxidative enzymes (Phenol & Perox) in the soil organic layer of a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Means are across 4 years (2014-2018), for most variables, with a single measurement in July of each summer. Statistical significance for Y (Year), S (Shrub), L (litter), and their interactions is presented as: ~ 0.1 > p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.

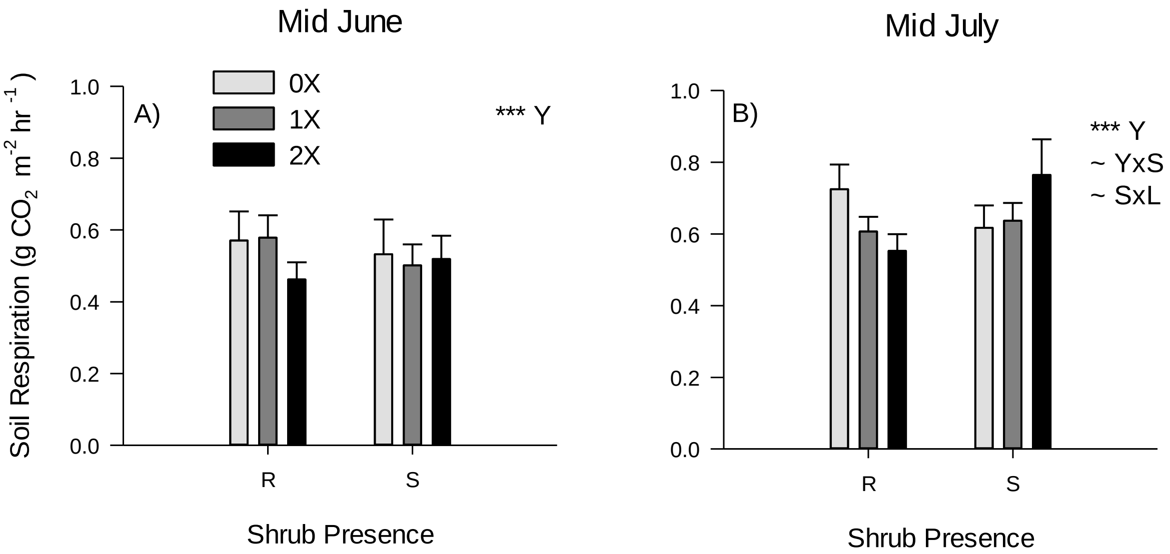

Figure 2.6. Mean (± SE) soil respiration (root + microbial respiration) in mid-June (A) and mid-July (B) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Means are across 4 years (2014-2018) with a single measurement in mid-June and mid-July of each summer. Statistical significance between Y (Year), S (Shrub), L (litter), and their interactions is presented as: ~ $0.1 > p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2.3. Summary of three-way ANOVAs (or Kruskal-Wallis when data could not be normalized) for year, shrub, litter and their interactions in organic layer soil microbial processes and properties in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Bold values are significant at $p < 0.05$.

Organic Layer		Year (Y)	Shrub (S)	Litter (L)	Y x S	Y x L	S x L	Y x S x L
	df	3,120	1,120	2,120	3,120	6,120	2,120	6,120
MBC	F	5.36	6.24	4.21	3.45	1.59	1.00	0.51
	p	**0.002**	**0.014**	**0.017**	**0.019**	0.157	0.371	0.804
MBN	F	18.39	9.96	4.80	5.38	1.53	1.02	0.68
	p	**<0.001**	**0.002**	**0.010**	**0.002**	0.173	0.363	0.663
BG	F	137.95	5.55	1.25	25.38	0.17	0.39	0.35
	p	**<0.001**	**0.020**	0.291	**<0.001**	0.984	0.676	0.910
CBH	F	129.04	0.02	0.44	14.92	0.36	0.14	0.67
	p	**<0.001**	0.887	0.647	**<0.001**	0.901	0.872	0.673
BX	F	156.93	9.55	0.13	26.03	0.11	0.09	0.21
	p	**<0.001**	**0.002**	0.877	**<0.001**	0.995	0.918	0.974
NAG	F	149.73	17.44	0.32	27.33	0.18	0.00	0.15
	p	**<0.001**	**<0.001**	0.727	**<0.001**	0.983	1.000	0.988
Phos	F	236.80	26.18	0.60	29.77	0.16	0.08	0.08
	p	**<0.001**	**<0.001**	0.553	**<0.001**	0.986	0.924	0.998
	df	1,60	1,60	2,60	1,60	2,60	2,60	2,60
MBP	F	0.57	0.15	2.01	0.08	0.53	0.00	0.67
	p	0.454	0.701	0.142	0.786	0.590	0.996	0.513
Phenol	F	4.28	7.37	1.38	3.37	0.13	0.16	0.01
	p	**0.043**	**0.009**	0.260	0.072	0.877	0.852	0.991
Perox	F	1.94	2.53	0.63	0.34	0.21	0.47	0.03
	p	0.169	0.117	0.539	0.562	0.811	0.626	0.975
	df	2, N=108	1, N=108	2, N=108	—	—	—	—
AG	γ^2	63.93	0.46	0.96	—	—	—	—
	p	**<0.001**	0.499	0.617	—	—	—	—
Phos-D	γ^2	75.25	3.35	0.23	—	—	—	—
	p	**<0.001**	0.067	0.893	—	—	—	—
	df	1, N=72	1, N=72	2, N=72	—	—	—	—
LAP	γ^2	52.28	2.52	0.07	—	—	—	—
	p	**<0.001**	0.112	0.964	—	—	—	—
2015 N Mineralization	df	—	1,30	2,30	—	—	2,30	—
	F	—	1.550	1.319	—	—	1.734	—
	p	—	0.223	0.282	—	—	0.194	—
2018 N Mineralization	df	—	1, N=36	2, N=36	—	—	—	—
	F	—	9.614	3.389	—	—	—	—
	p	—	**0.002**	0.184	—	—	—	—
Soil Respiration	df	3,90	1,90	2,90	3,90	6,90	2,90	6,90
Mid June	F	57.52	0.81	0.59	1.86	1.72	1.55	0.82
	p	**<0.001**	0.371	0.555	0.161	0.152	0.217	0.516
Mid July	F	26.36	0.33	0.01	2.79	0.49	3.01	0.15
	p	**<0.001**	0.565	0.993	0.067	0.741	0.054	0.964

Note: Degrees of freedom differ by variable depending on the number of years the measurement was repeated (or data was usable) and/or due to the type of statistical analysis.

In the organic layer, shrub plots had higher MBC and MBN only in 2016, while there was no difference between the shrub treatments in other years (year x shrub interaction, Figure S13,

Table 2.3). Effects of shrubs on potential enzyme activity of the carbon acquiring enzymes BG, CBH, and BX were also complex and varied by year (Table 2.3). Effects of shrubs were found in 2017 when there was lower activity of BG and CBH in shrub plots and in 2018 BG, CBH, and BX all showed higher activity in shrub plots than in shrub removal plots (Figure S13, Table 2.3). The nitrogen and phosphorus acquiring enzymes NAG and Phos both showed higher activity in shrub plots than in removal plots, but only in the final year of the experiment (shrub x year interaction, Figure S13, Table 2.3). N mineralization rates in 2018were lower in shrub present plots than in shrub removal plots, but there was no effect earlier in the experiment (Figure S6, Table 2.3).

For microbial responses to litter quantity, both MBC and MBN increased with increasing litter quantity, while MBP did not respond to litter treatments (Figure 2.3, Table 2.3). Neither enzyme activity nor N mineralization rates responded to litter treatment (Table 2.3). For AG, LAP, Phenol, Perox and MBP only 2017-18 data was analyzed and for Phos-D 2016-18 data was analyzed because most values in the other years were below the detectable range of the analysis. In the mineral layer there were few responses to shrub and litter treatment (Table S2). MBC and MBN did not responds to shrub removal (Table S2), and MBP was not analyzed as most values were below detection. CBH enzyme was lower in shrub present than removal plots, though no other hydrolytic enzyme responded to shrub removal treatments (Figure S8, Table S2). Perox was lower in the shrub plots (Figure S14, Table S2) in 2018 only. Only data from 2016-2017 was analyzed for BX and Phos-D, and 2017-2018 for AG, Perox and Phenol because most values in the other years were below the detectable range of the analysis. MBP and AG data was not analyzed because most values in all years were below the detectable limit of the analysis.

Although mid-June soil respiration did not respond to shrub or litter treatments, there was a marginal shrub x litter treatment interaction for mid-July measurements (Table 2.3) where increased litter quantity resulted in higher respiration in shrub present plots but lower soil respiration in shrub removal plots (Figure 2.5, Table 2.3). The mid-July measurements also showed a marginal year x shrub interaction where soil respiration was higher in shrub present plots form 2016-17 but lower in shrub present plots in 2018 (Figure S13, Table 2.3).

Discussion

Changes in soil physical properties caused by shrub encroachment

Warmer winter soil temperatures observed under shrubs plots than shrub removal plots provide support for the snow-shrub hypothesis (Sturm et al. 2001) and has been reported earlier by others (DeMarco et al. 2011; Myers-Smith & Hik 2013). This hypothesis suggests that shrubs catch and collect wind-blown snow with their canopies, creating a thicker snow layer than in non-shrub tundra which provides more insulation for the soil from the cold winter temperatures. The lower summer soil temperatures in shrub present plots found in this study are a lesser reported effect of shrubs on soil temperature (although see Block et al (2010) and Myers-Smith and Hik (2013)) and is likely caused by canopy shading of the soil surface. In this study shrub canopies intercepted ca. 60% more PAR than plants in the shrub removal plots, reducing the solar radiation reaching the soil surface. Soil temperature strongly affects microbial processes and thus nutrient cycling rates (Cornelissen et al. 2007) and the net effect of these contrasting effects on temperature will depend on the duration and intensity of the temperature effect across the seasons.

Shrubs may directly affect both the input of water into the soil and its use, a combination of which may have resulted in the decrease in soil moisture with shrub presence observed in this study. Decreases in soil moisture with shrub presence have also been seen in observational studies comparing shrub to non-shrub tundra (Myers-Smith & Hik 2013; Crofts et al. 2018). Shrub may transpire more water than other plant types, likely at least partially because of their larger biomass (Tardieu & Parent 2017) and higher productivity, in terms of recruitment and growth rate (DeMarco et al. 2014). Shrubs may also reduce water inputs if they intercept more rainfall than other plant types, reducing the amount of rainfall that reaches the soil before it evaporates from the shrub leaves (Tromble 1988, although see McLaren et al. 2004). Soil moisture was only monitored during summer in this study, yet the ground remains thawed late into fall and effects of shrubs on snow may results in effects on soil moisture early the following year. Sturm et al. (2001) and Myers-Smith and Hik (2013) found increases in snow catchment during the winter months in shrub stands, which may lead to shrub areas having higher moisture inputs during melt, causing higher soil moisture under shrubs very early in the growing season.

Effects of shrubs on summer nutrients and soil microbes

Although there were few effects of shrub presence on soil nutrients directly, there were numerous effects on microbial abundance and activity (extracellular enzyme activity and microbial biomass), suggesting overall effects on carbon and nutrient cycling rates. Most of the effects (of both shrub and litter) seen in this study happened in the organic layer of the soil and the mineral layer had few effects. Shrubs may have smaller effects on deeper soil layers in part due to their shallow root systems (Wang et al. 2016). Due to the lack of shrub and litter effects on the mineral layer, only the effects on the organic layer are discussed and effects on the mineral layer soils can be found in the appendix.

Although effects of shrubs on N-availability were not frequently found (nitrate was lower under shrubs in 2017, the only year which nitrate was detectable in this study), the soil extractions sampled only a single time point during each growing season. N availability in tundra is seasonally variable (McLaren et al. 2018), and this study may have missed other potentially short-lived differences during other times in the season. During the year in which effects on soil N were found, the decreased N availability observed under shrubs could be due to uptake of available N by shrubs. Shrubs have been shown to be one of the strongest competitors for N in arctic tundra (Bret-Harte et al. 2008) which could be partially due to their larger root biomass (Chapin et al 1980; Wang et al. 2016) and symbiotic relationship with mycorrhizal fungi (Orson 1982; Wallenstein et al. 2007). Decreased N availability in shrub plots may also be due to increased microbial uptake of N caused by higher microbial biomass seen in shrub plots, although the microbial biomass effect was also temporally variable (only significantly higher under shrubs in 2016). An observational study also saw higher microbial biomass in areas densely covered with *B. glandulosa* when compared to areas with patchy *B. glandulosa* cover (Buckeridge et al. 2010). Higher plant biomass, in combination with higher microbial biomass, may result in increasing uptake of available N from tundra soils and lower overall nutrient availability.

Despite few effects on the standing pools of nutrients in the soil and microbial biomass, there were effects of shrubs on microbial processes. In the final year of this study, after four years of treatments, shrub presence caused lower N mineralization rates, with N instead being immobilized by the microbial community, and higher exoenzyme activity. The results of this study suggest that increased carbon supply to the microbial community under shrubs may be driving these effects. Higher C:N ratios in shrub plots may be caused by increased plant carbon allocation by shrubs to the soil; Higher plant biomass and the corresponding larger root biomass may release more C-rich exudates, supporting carbon movement between shrub roots and the soil

(Nannipieri et al. 2008). Further, during times of low nutrient availability shrubs may allocate more carbon to their fungal symbionts to stimulate nutrient exchange (Hobbie and Hobbie 2008).

The effects of shrubs on microbial exoenzyme activity did not develop until the 3rd year of the experiment and were not consistent between years after this point. Results showed a lower enzyme activity under shrubs in the 3rd year of the experiment (2017), but a higher enzyme activity in 2018. The higher enzyme activity under shrubs in 2018 parallels the increased acquisition of N by microbes (N immobilization) in that year, suggesting available resources for enzyme production. The switch in direction of the shrub effect on enzymes between years could be due to a variety of factors. The degree of insulation of shrub canopies was much stronger in warmer winters (Fig 2.1, larger difference between shrub – no shrub plots winters of 2015/16 and 2017/18 and a smaller effect in 2016/17) which may driver higher microbial enzyme production under shrub soils the following summer. Other studies have reported increasing winter soil temperature caused increases in summer N mineralization (DeMarco et al. 2011). Because there was little temperature effect of shrubs during the cold winter (2016/2017), the enzyme effects in the following summer are likely due to other mechanisms.

Effects of litter additions on nutrients

There were fewer effects of litter quantity and quality on soil nutrients and microbial functioning. There is an increase in soil C:N ratios with added litter, likely because *B. glandulosa* litter has higher C:N ratios than soil (Figure 3.1; also seen in Buckeridge et al. 2010) and as the litter decomposed it increased the soils C:N ratios. Microbial biomass also increased with litter quantity likely because of the higher availability of organic matter to decompose. Increasing carbon inputs with litter additions have been shown to stimulate microbial activity; For example, in a soil incubation study, Phillips et al (2019) reported an increase in microbial respiration with deciduous shrub litter additions to tundra soils.

Conclusion

In conclusion, although there were some effects of litter quantity on soil and microbial properties, most of the effects of shrub encroachment are likely through their physical presence rather than their litter input. There was variability between years, and interactions between the variables that control microbial activity, but from this study shrub encroachment is likely to affect tundra properties in the following ways;

With increased winter temperatures microbial activity will increase in the winter (Welker et al. 2000; Schimel et al. 2003; Nobrega & Grogan 2007) but with decreasing summer temperatures microbial activity will likely decrease in the summer. Because of the opposing effects across seasons, it is difficult to predict the overall effects of shrub encroachment caused temperature changes on carbon storage. Increased soil C:N ratios and decreased N mineralization point towards higher shrub carbon allocation to the soil which suggests shrub productivity is increasing carbon storage in tundra soils. Further, because of the high interannual variability on microbial activities such as enzyme activity and N-mineralization, a longer time-series of data would be required to make predictions on overall effects of shrub presence on soil microbial functions. Litter quality will increase soil C:N ratios and quantity will increase microbial biomass, but the overall effects of litter were relatively minor with respect to shrub effects. Shrub presence, not shrub litter, will likely be the bigger driver of change in physical properties which consequently will affect microbial activity and nutrient availability.

3. SHRUB ENCROACHMENT EFFECTS ON DECOMPOSITION

Introduction

Tundra ecosystems are experiencing a shift in plant community composition from graminoid to deciduous shrub (henceforth simply called shrubs) dominated tundra, as indicated by satellite and aerial imagery (Silapaswan et al. 2001; Zhou et al. 2001; Jia et al. 2003; Tape et al. 2006). Manipulative experiments have demonstrated that shrubs outcompete other tundra plants when there are higher temperatures, and nutrient availability (Jonasson et al. 1999; Shaver et al. 2001; Bret-Harte et al. 2002; Mack et al. 2004; Walker et al. 2005; Euskirchen et al. 2009; Sistla et al. 2013; DeMarco et al. 2014). This change in plant community composition is expected to continue to expand with climate change and could affect many tundra ecosystem properties.

Shrub encroachment could affect carbon stocks in the tundra through a variety of mechanisms. Historically the tundra has been a sink for carbon (Marion & Oechel 1993; Chowdhury et al. 2015), because low temperatures slow the decomposition of organic material (Oechel et al. 1993). As a result, the tundra holds over a third of the world’s soil carbon within the permafrost (Ping et.al. 2008; McGuire et al. 2009; Chowdhury et al. 2015), but rising temperatures are causing the permafrost to thaw (Schuur et al. 2008; IPCC 2014) which leaves these stores vulnerable to decomposition (Chowdhury et al. 2015). Shrubs are one of the largest plant forms in tundra environments (Myers-Smith & Hik 2013) and have higher litter production than other tundra plants (McLaren unpublished data). Changes in the species composition and litter abundance caused by shrub increases could affect the tundra’s carbon retention abilities through effects on decomposition. If increasing shrubs do affect carbon flux in the tundra this could create a feedback loop with climate change due to the tundra's large carbon stocks.

Shrubs may affect litter decomposition rates through effects on the litter decomposition environment. Increased shrub coverage has been linked to deeper snow causing higher soil temperatures in the winter (Sturm et al. 2001; DeMarco et al. 2011) and to increased shade resulting in lower temperatures in the summer (Block et al. 2010; Myers-Smith & Hik 2013). The higher inputs of litter by shrubs compared with the surrounding tundra could result in a thicker litter layer, as seen in observational studies (Crofts et al. 2018), which may further insulate the soil. Although there has not been documentation of changing soil temperature due to increased shrub litter in tundra environments, studies in other environments have found litter to be an important insulator of the soil (Sayer 2006; Song et al. 2018). Changing soil temperatures may directly impact decomposition rates because of microbe's sensitivity to temperature (Cornelissen et al. 2007), with warmer soils resulting in faster decomposition. However, because of opposing effects of shrubs on temperature between winter and summer, the balance of the effects on decomposition will depend on the duration of the season, the size of the effect, and the importance of each season on yearly decomposition rates.

Changes in the bulk litter chemistry because of an increasing contribution of shrub litter could also affect decomposition rates. Some studies have found shrub litter to be slower to decompose (Cornelissen et al. 2007) than other tundra plants, perhaps as a result of the higher lignin:N ratio in some shrubs (Hobbie 1996). In contrast others have found shrub litter to be faster to decompose (Hobbie & Gough 2004; McLaren et al. 2017), perhaps resulting from its relatively high N content (Chapin & Shaver 1996; Aerts et al. 2006) or high specific leaf area (Cornelissen & Thompson 1997). The presence of shrub litter may also affect the decomposition rate of other species – the presence of *Betula* litter was shown to decrease the decomposition rate of nearby graminoid and evergreen shrub litter (McLaren et al. 2017). The rate of shrub litter decomposition in contrast with other tundra species, in addition to the proportional input of these species into the litter community, will determine the effects of changing litter communities on the overall carbon flux of the tundra.

The effects of increasing shrubs abundance, through the changing quality and quantity of litter produced, are not well understood. Using a manipulative experiment in a sub-arctic alpine tundra ecosystem near Kluane Lake, Yukon Territory, Canada, the following objectives were explored:

Objectives

1. To understand how deciduous shrubs (*B. glandulosa*) presence and their litter quantity affect the decomposition rate of *B. glandulosa* litter through their effects on the decomposition environment.
2. To understand how changing litter quality resulting from deciduous shrubs (*B. glandulosa*) encroachment may affect decomposition by comparing the decomposition of several litter types (*B. glandulosa, S. reticulata, C. consimilis*).

Materials and Methods

Site description

This study is located in the sub-arctic alpine tundra of northern Canada, within the Kluane Lake region of the Yukon Territory. This region is considered semiarid as it receives less than 30 cm of precipitation annually, with about 40-50% of this precipitation falling as snow and the rest as rain (Krebs et al. 2001). The soil has two distinct layers, a strongly organic layer making up ca. the top 13 cm of the soil profile ("organic layer") and a layer below which is primarily clay with little organic material ("mineral layer"), collected up to ca. 35 cm in depth. In this alpine region, vegetation transitions with elevation, with lower elevations a *Picea glauca* dominated boreal forest, followed by shrub (*Salix* spp and *Betula glandulosa)* dominated alpine tundra and then open alpine tundra (Crofts et al. 2018). The experimental plots are in patchy shrub alpine tundra at 1,493 m in elevation on a north facing slope with shrub cover dominated by *B. glandulosa,* a deciduous dwarf shrub.

Experimental manipulations

The presence of shrubs (removed vs. present) and shrub leaf litter (0x (all litter removed), 1x (natural abundance litter), 2x (double natural abundance litter)) were experimentally manipulated in a fully factorial experiment, with each of the six different treatments combinations (2 shrub x 3 litter treatments) replicated six times, for a total of 36 plots. Plots are 1x1 m in area and are surrounded by ca. 20 cm high nylon fencing to prevent litter movement in or out of plots. Plots were arranged into 12 blocks, with each block consisting of a single patch of *B. glandulosa*. Shrub patches are ~12 m^2 areas of continuous *B. glandulosa* growth. Shrub treatment (absent or present) was applied at the block level and within each block (shrub patch) there is a single replicate of each litter treatment. Every plot within a block is separated by a minimum of 1 m and blocks were separated by a minimum of 8 m.

Shrub removals were done manually, clipping all shrub stems in the patch of *B. glandulosa* at the soil surface, with the removals maintained every year. The three litter treatments represent a range of litter quantity: all litter removed, natural litter abundance, and double litter abundance. Litter treatments were first applied in summer 2015 by collecting litter from all 36 plots and determining the average litter mass per plot; this was considered natural litter abundance and it averaged 470 g/m^2. Litter was homogenized and then re-distributed among plots, with the amount dependent on litter treatment (none, 1x natural abundance or 2x natural abundance). In subsequent years, natural litter fall was determined by averaging the mass of collected newly fallen or senesced litter from the litter-removed/shrubs-present plots in 2016 it was 171 g/m^2, and in 2017 it was 215 g/m^2. Additional litter was collected from the litter-removed plots and from neighboring plants to complete litter additions in the remaining plots (a mass equivalent to natural litter fall added to natural abundance plots without shrubs, and to 2x natural abundance plots with shrubs, and a mass equivalent to double natural litter fall added to 2x natural abundance plots without shrubs). Litter manipulations were maintained by hand each fall. All soil and ecosystem sampling occurred during the growing season (June – August) of 2015 – 2018 with the exception of soil temperature, which was recorded year-round.

Decomposition Experiments

For all experiments described below, senesced but attached leaves were collected for litter bags from multiple plant individuals in the general vicinity of the experimental plots and dried at 60°C for 48 hours before litter bag creation. Litter bags were made of 1 g of litter placed into 5 x 10 cm bags created out of 1mm nylon mesh with litter species varying between the experiments. Litter bags were incubated *in situ* within each of the 36 plots, litter bags were placed at the soil surface of the but under added litter for 1x and 2x litter addition plots.

For objective 1; three replicates of litter bags containing *B. glandulosa* litter were installed into each plot in September 2015. A single litter bag was removed each subsequent fall, to determine 1-year (2016), 2-year (2017) and 3-year (2018) litter decomposition rates. Once litter bags were collected from the field, litter was removed from the bag and any foreign debris removed (roots, live plants, moss etc.). Litter was then dried at 60°C for a minimum of 48 hours and weighed. Litter was ground and analyzed for C:N using a dry combustion C and N analyzer (ElementarPyroCube®).

For objective 2; litter bags were created in September 2016 using litter from the dominant species of three functional groups: a deciduous shrub (*B. glandulosa*), a graminoid (*Carex consimilis*) and a prostrate shrub (*Salix reticulata*). Litter decomposition rates were measured for 1 year from 2016-2017. Once litter bags were collected from the field, litter bag processing was the same as objective 1.

Statistical Analysis

The mass loss measurement was reformulated as remaining mass (remaining mass = Initial mass – mass loss). %C and %N measurements were also re-structured as proportion remaining C and N for the litter in each litter bag. %C and %N measurements at each time step were converted into a proportion remaining C or N from initial values by converting %C or %N to mass of C or N within each litter bag.

Proportion remaining C or N =

(Final mass * (Final %C or %N * 0.01)) / (1g * (Initial %C or %N * 0.01))

Statistics were done on these re-formulated values (mass remaining and proportion C or N remaining). Data for the 1st research question was analyzed as a 3-way ANOVA with the main factors of shrub treatments, litter treatments, incubation time. Data for the 2nd research question was analyzed as a 4-way ANOVA with the main factors of shrub treatments, litter treatments, incubation time, the different litter types. Data that did not follow the ANOVA assumption of normality was either: transformed using a log transformation or was analyzed using a Kruskal Wallis test if data could not be transformed. Due to the nature of a Kruskal Wallis test, for these analyses only main treatment effects were examined and not their interactions. When there was a significant effect of litter treatment or incubation time, we used a post-hoc Tukey tests to test for differences between individual treatments. All stats were done using R software version 3.5.3 and JMP 12 (SAS Institute, Cary, NC, USA).

Results

Objective 1: Effects of treatments on decomposition rates of *B. glandulosa* litter

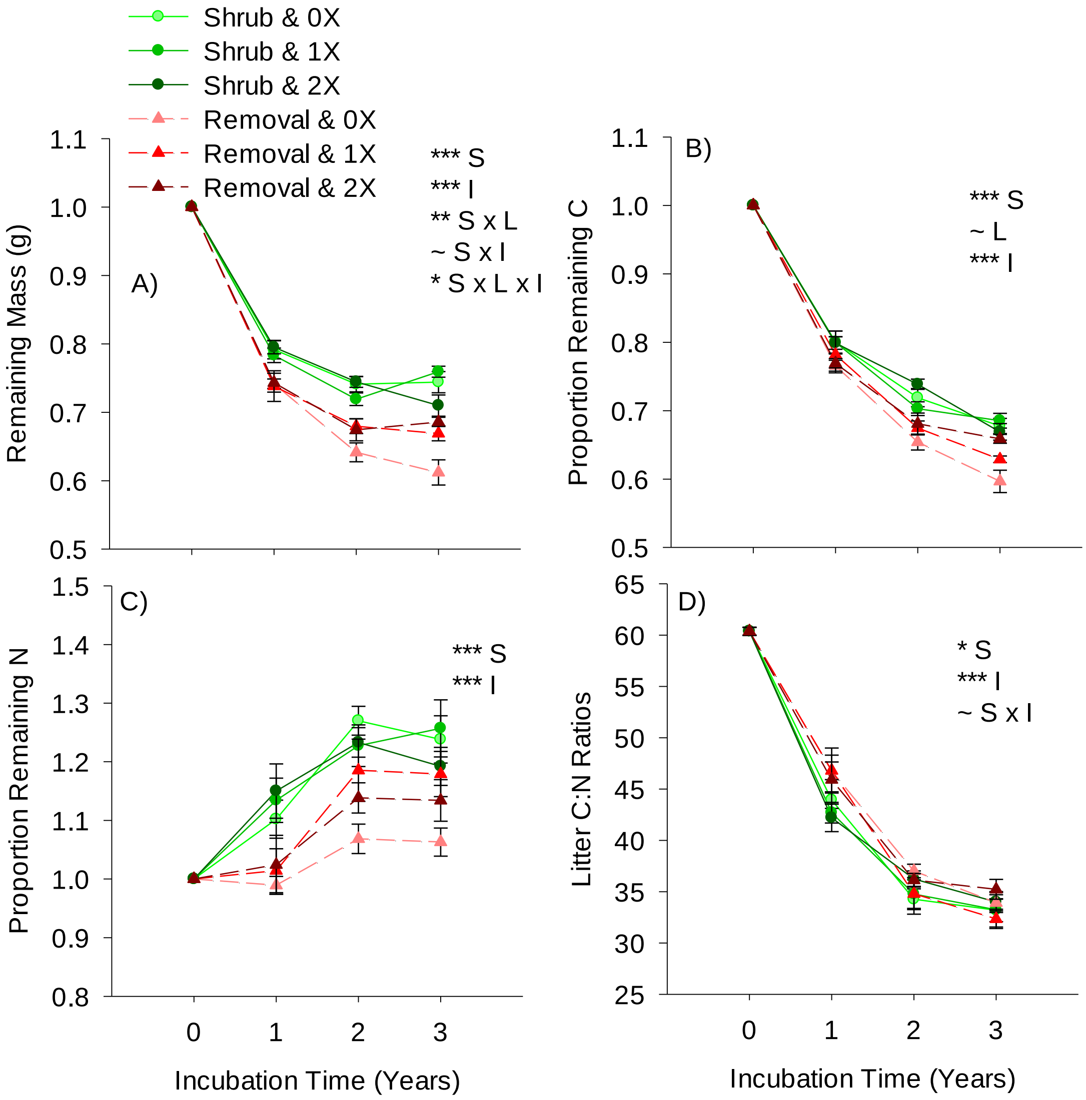

Figure 3.1. Mean (± SE) remaining litter mass (A), proportion remaining C (B), proportion remaining N (C) and litter C:N ratios (D) for *B. glandulosa* litter decomposition in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Statistical significance for I (Incubation Time), S (Shrub), L (litter), and their interactions is presented as: ~ 0.1 > p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.

Table 3.1. Summary of three-way ANOVAs on nutrients and mass loss for litter bags decomposing in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Bold values are significant at $p < 0.05$.

		S (Shrub)	L (Litter)	I (Incubation)	S x L	S x I	L x I	S x L x I
	df	1,90	2,90	2,90	2,90	2,90	4,90	4,90
Remaining	F	119.93	2.08	53.89	5.09	2.50	1.52	3.24
Mass	p	**<0.001**	0.131	**<0.001**	**0.008**	0.088	0.202	**0.016**
Prop C	F	48.74	2.66	168.69	2.18	1.52	0.94	1.55
	p	**<0.001**	0.0753	**<0.001**	0.119	0.224	0.444	0.1935
Prop N	F	42.74	2.24	19.51	2.28	0.07	0.46	0.87
	p	**<0.001**	0.112	**<0.001**	0.109	0.931	0.766	0.488
C:N	F	6.80	0.75	123.52	0.23	2.60	0.66	0.51
	p	**0.011**	0.478	**<0.001**	0.796	0.080	0.625	0.731

Remaining mass showed a shrub x litter x incubation time interaction. In general, shrub effects dominated over litter effects, and shrub plots had more remaining mass (slower decomposition) than shrub removal plots. Litter effects were few; increasing litter slowed decomposition (higher remaining mass) but only in removal plots and only in the 3rd year of the study (Figure 3.1, Table 3.1). Proportion remaining C, and proportion remaining N showed higher values in shrub present plots than shrub removal plots (Figure 3.1, Table 3.1). Litter C:N ratios had a marginal shrub x incubation time interaction where values were lower in shrub present plots than in shrub removal plots during the 1st year but for the subsequent years there was less distinction between the shrub treatments (Figure 3.1, Table 3.1). Proportional remaining C had a marginal litter effect where it was higher in the higher litter abundance plots (Figure 3.1). Incubation time significantly affected every measurement (Table 3.1). For remaining mass, proportion remaining C, and litter C:N ratios there was a decrease in mass over time, with the largest decrease in mass happening in the first year (Figure 3.1, Table 3.1). Proportion remaining N accumulated through the years (Figure 3.1).

Objective 2: Effects of treatments and litter type on decomposition rates

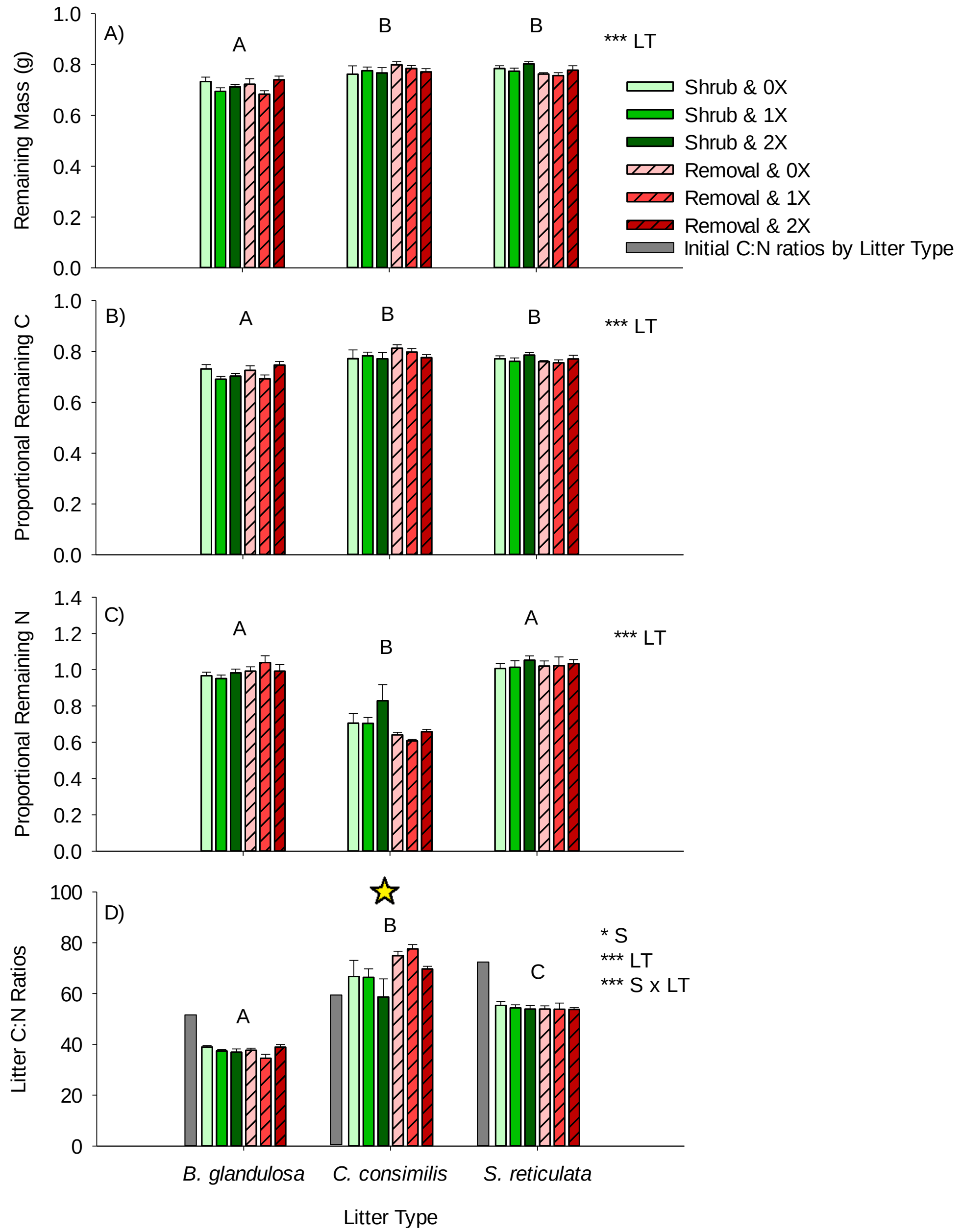

Figure 3.2. Mean (± SE) remaining litter mass (A), proportion remaining C (B), proportion remaining N (C) and litter C:N ratios (D) after 1 year of decomposition for *B. glandulosa, C. consimilis*, and *S. reticulata* litter decomposition in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Initial litter quality (C:N) is represented as grey bars (D). Statistical significance for I (Incubation Time), S (Shrub), L (litter), and their interactions is presented as: ~ 0.1 > p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001. Letters above the bars present statistically significant differences in litter type. Yellow star shows statistically significant differences between shrub treatments for a litter type.

Table 3.2. Summary of three-way ANOVAs on nutrients and mass loss for litter bags decomposing for a single year in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Bold values are significant at $p < 0.05$.

		S (Shrub)	L (Litter)	LT (Litter Type)	S x L	S x LT	L x LT	S x L x LT
Remaining Mass	df	1,90	2,90	2,90	2,90	2,90	4,90	4,90
	F	0.01	2.21	31.51	0.15	2.24	1.67	0.78
	P	0.914	0.116	**<0.001**	0.861	0.113	0.163	0.539
Prop C	F	1.01	1.56	31.44	0.09	1.55	1.67	1.03
	P	0.319	0.216	**<0.001**	0.918	0.218	0.165	0.397
C:N	F	4.66	1.42	223.54	0.54	8.05	1.96	0.18
	P	**0.034**	0.247	**<0.001**	0.585	**<0.001**	0.107	0.948
Prop N	df	1, N=108	2, N=108	2, N=108	—	—	—	—
	γ^2	0.05	1.95	59.20	—	—	—	—
	P	0.825	0.378	**<0.001**	—	—	—	—

Note: Degrees of freedom differ by measurement depending the type of statistical analysis.

B. glandulosa litter decomposed faster (lower remaining mass) and has lower proportion remaining C than the other two litter types (Figure 3.2, Table 3.2). *C. consimilis* showed the lowest proportion remaining N values out of the three litter types (Figure 3.2, Table 3.2). Final litter C:N ratios were different for all litter types the lowest being *B. glandulosa*, then *S. reticulata* and the highest being *C. consimilis* (Figure 3.2, Table 3.2). Litter C:N ratios had a shrub x litter type interaction where only *C. consimilis* showed lower values in shrub plots than removal plots (Figure 3.2, Table 3.2).

Discussion

Increasing shrub abundance is likely to influence decomposition both through effects on the decomposition environment and also through differences in decomposition rates between shrubs and other tundra species. In this study, the physical presence of shrubs reduced decomposition rate through effects on the decomposition environment, but there were no effects of shrub litter quantity on decomposition rate. However, in this study shrub litter was more labile (lower C:N ratios) and decomposed faster than litter from other common tundra species. When *Betula* litter decomposed under shrubs, N accumulation in *B. glandulosa* litter increased, consequently decreasing the C:N ratios of the litter, which could further amplify positive effects of shrubs on decomposition rates.

The slower decomposition rates (slower mass and C loss) in plots with shrubs are likely due to changes in the physical properties of the environment caused by shrub presence. In this study, the surface soil under shrubs had lower moisture (Figure S1) and was colder than in plots without shrubs (Figure 2.1) a pattern also found in other studies (Myers-Smith & Hik 2013). These changes in microclimate under shrub canopies could be responsible for the slower decomposition rate, as decomposition is sensitive to moisture and temperature (Cornelissen et al. 2007; Makkonen et al. 2012).

The quantity of litter had a weak effect on decomposition through effects on the decomposition environment; in this study only in shrub removal plots, and then only after 3 years of litter treatments did higher litter quantity decrease decomposition (decreased C loss). Because this effect was weak, and only happened in removal plots, it is likely that the insulation ability of increased litter quantity was overwhelmed by the insulation abilities seen of shrub presence. Although litter does have the capacity to insulate in other environments (Forest: Sayer 2006; Grasslands: Song et al. 2018), it was likely not the case in this study. Thus, this study finds that the major effects of shrub litter on the tundra will likely not be through its physical presence but rather its chemistry and form.

In this study *B. glandulosa* was more labile and decomposed faster than the other litter types, as has also been seen in other studies (McLaren et al. 2017). Faster decomposition may result from the lower initial C:N ratios seen in *B. glandulosa* litter than the other litter types (Figure 3.2). Faster decomposition may also be partially due to the shape of *B. glandulosa* litter as it has a high specific leaf area (SLA) which increases the surface area for microbial colonization. In support of this hypothesis, *B. nana*, a close relative to *B. glandulosa* with almost identical leaf structure, has higher SLA than other tundra litter types (Shaver et al. 2001) which has been suggested to lead to its faster decomposition rate (McLaren et al. 2017). Deciduous shrubs likely release higher amounts of litter each fall than other plants due to their size and productivity (Cornelissen et al. 2007; McLaren et al 2017). Further, many of the other dominant tundra species retain their leaves for multiple years, including evergreen shrubs and graminoids frequently retain their litter as standing dead for multiple years (Johnson & Tieszen 1976). In this study, *B. glandulosa* litter decomposed ca. 6% faster than the dominant species of the other plant functional groups, but studies have reported 3x (Crofts et al. 2018) to 5x (McLaren unpublished data) more litter under shrubs than open tundra. Therefore, regardless of the faster decomposition of shrub litter, the significantly higher inputs of fresh shrub litter each fall could accumulate and become a sink for C.

N accumulated in the *B. glandulosa* litter throughout the experiment, particularly in the shrub plots, suggesting immobilization of N from the environment by the microbes colonizing the litter (Manzoni et al. 2008). Higher N-immobilization in shrub plots was also seen in the soil microbes, where shrub plots had higher microbial N (Fig 2.3) and lower mineralization rates. This suggests faster microbial growth in shrub plots (accumulation of MBC, Fig 2.3), which may be a result of higher carbon inputs for increased root biomass and consequently higher exudate production (Nannipieri et al. 2008). Other studies have reported N-translocation into recalcitrant litter, or litter with a high C:N, from more labile litter types (Schimel & Hättenschwiler 2007; Handa et al. 2014; McLaren et al. 2017) and the same process could also transfer N from adjacent soil into decomposing litter.

B. glandulosa litter is more labile than the other dominant litter types and consequently decomposes faster. Depending on the rate that this carbon is incorporated into soil versus released as respiration, this may allow for the carbon that is stored in the litter to be released more quickly to the atmosphere. However, shrub presence decreases decomposition rate through its effects on the decomposition environment (lower soil temperature and moisture) by a similar magnitude. This decrease in decomposition caused by shrubs will have opposite effects on tundra carbon storage. Finally, shrubs have been reported to produce 3 – 5x more litter than surround tundra vegetation (Crofts et al. 2018; McLaren unpublished data). Because of these high litter inputs, over time shrub encroachment is causing litter to accumulate. Overall the effects shrub encroachment on carbon release through litter decomposition will depend on a combination of the increasing litter quantity, the slowing of decomposition through environmental effects, and the rate that carbon is released from the fallen litter versus the biomass that is retained over multiple years from other litter types.

4. SYNTHESIS: EFFECTS OF SHRUB ENCROACHMENT ON THE TUNDRA

Shrub presence affected tundra soil properties above and belowground. Shrub presence increased winter soil temperatures and decreased summer soil temperatures and moisture. In the winter shrubs are known to trap snow with their canopies and increase snow depth, consequently increasing soil temperatures (Sturm et al. 2001). In the summer shrubs are likely reducing soil temperatures through canopy shading, where they are preventing the suns energy from reaching the ground (Figure 2.1, Figure S1; Block et al. 2010; Myers-Smith & Hik 2013). The lower soil moisture under shrubs is likely through increased uptake by the large plant biomass outweighing the decreased evaporation resulting from canopy shading. Shrub effects on nutrient availability and microbial activity varied between years for most variables; Shrubs increased microbial biomass for most years of the study but only in 2016 was there a statistical difference. In the last year of the study shrubs increased enzyme activity and decreased N mineralization. Soil C:N ratios were higher under shrubs, likely resulting partially from shrub root exudates, because of their larger root biomass (Chapin et al. 1980; Wang et al. 2016) shrubs could potentially release more carbon in to the soil. Shrub presence also decreased decomposition rate through effects on the decomposition environment, where shrubs lowered soil temperature and moisture (Figure 2.1, Figure S1).

Contrary to expectations, and in contrast with the effects of the shrub presence itself, there were very few effects of shrub litter quantity on the physical environment, which translated into few effects on nutrient cycling and microbial functions. There were increases in microbial biomass with litter additions, which could be caused by the higher availability of organic matter to decompose. With increasing litter quantity soil C:N ratios also increased likely due to the increased inputs of litter material with a higher C:N ratio. The results of this study suggest that the primary impacts of shrub litter on the tundra ecosystem will be through changes in litter quality, and the quantity of the litter available to be decomposed, rather than effects of the litter on microclimate. Shrub litter decomposed faster than other tundra litter types due to differences in chemistry (Figure 3.2) and physical characteristics, such as higher SLA (McLaren et al. 2017) than the other dominant litter types.

Understanding the direction of the effects of tundra shrub encroachment on carbon storage or release from the tundra will depend on the balance of shrub and litter effects. Figure 4.1 shows the hypothesized effects of shrub encroachment on physical properties and microbial activity on the tundra, highlighting those found in this study, and consequently describes how this could affect carbon release from the tundra. As is evident by the mechanisms (arrows) directly connecting shrubs to decomposer activity, there are both positive (higher quality and quantity of litter, warmer winter soils) and negative (cooler summer temperatures and reduced soil moisture) effects of shrubs on decomposer activity. The net effect of those changes on decomposition rate, and ultimately CO_2 release to the atmosphere, will depend on balance of these effects. For example, the winter is long but has low microbial activity so the overall impact of temperature changes may result primarily from summer effects and be negative. There is also an increase in litter lability but a decrease in decomposition through shrub effects on the decomposition environment; these effects were of similar magnitude, but in opposing directions, and may cancel each other out. However, the increased quantity of litter being put into tundra ecosystems from deciduous shrubs may result in more microbial growth and biomass, as was seen in this study, which could then result in higher microbial respiration rates (although effects were only marginal in this study) or increased carbon storage in microbial biomass or necromass.

More research focusing on the magnitude of each of these effects of opposing directions for how shrubs affect tundra carbon storage and release to determine the net effect of shrub encroachment on the tundra carbon balance.

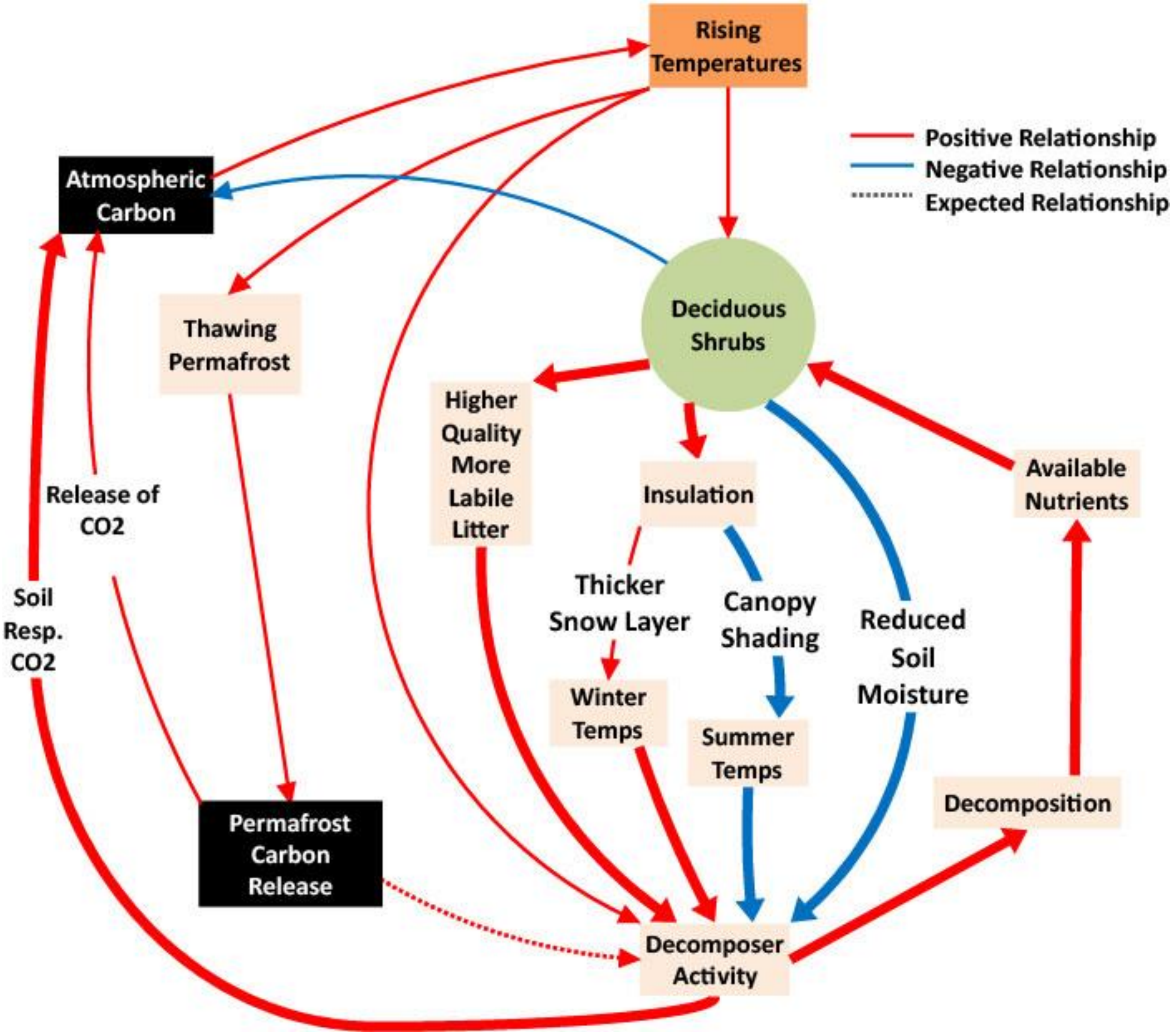

Figure 4.1. Diagram shrub encroachment effects that are supported (solid lines), expected (dashed lines), and seen in this study (Bolded Lines).

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APPENDIX

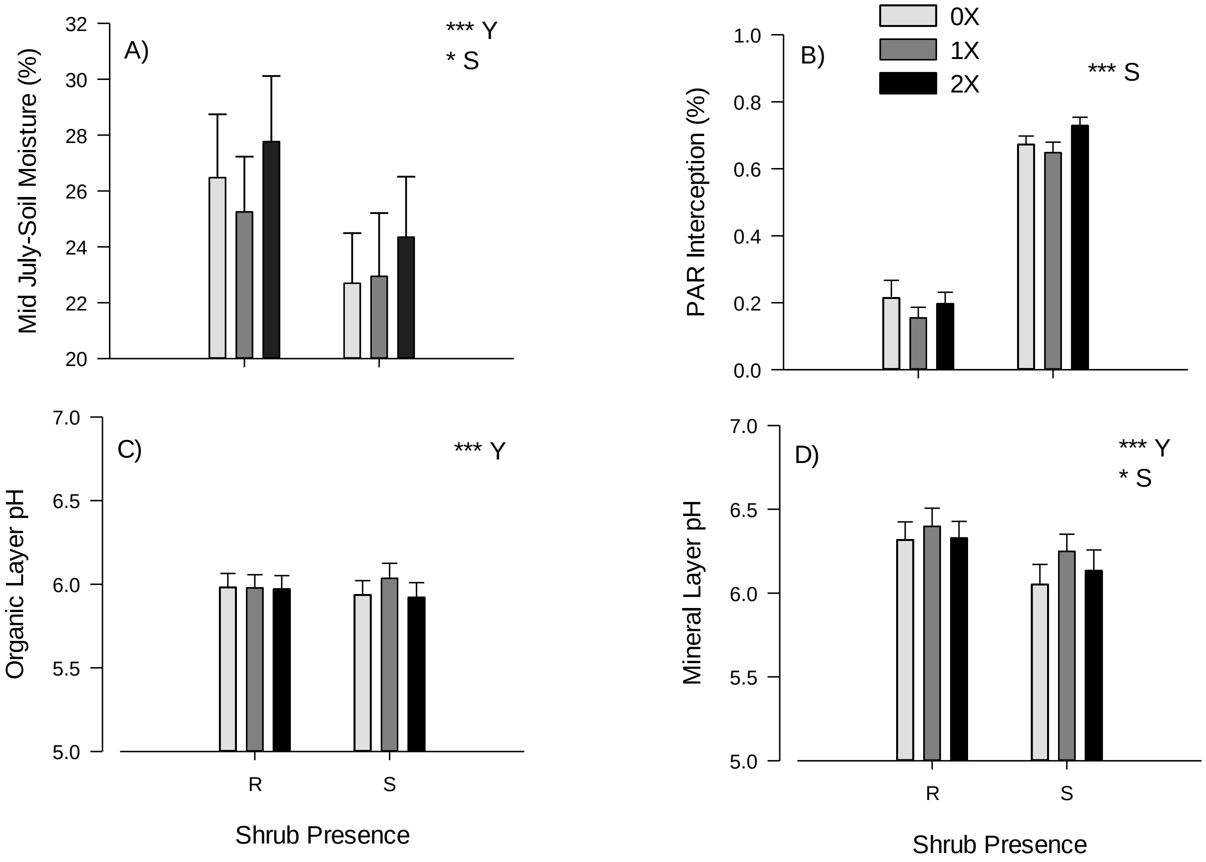

Figure S 1. Mean (± SE) for physical properties: soil moisture (A), PAR interception (B), organic layer pH (C) and mineral layer pH (D) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Means are across 4 years (2014-2018) with a single measurement in July of each summer. Statistical significance for Y (Year), S (Shrub), L (litter), and their interactions is presented as: ~ $0.1 > p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

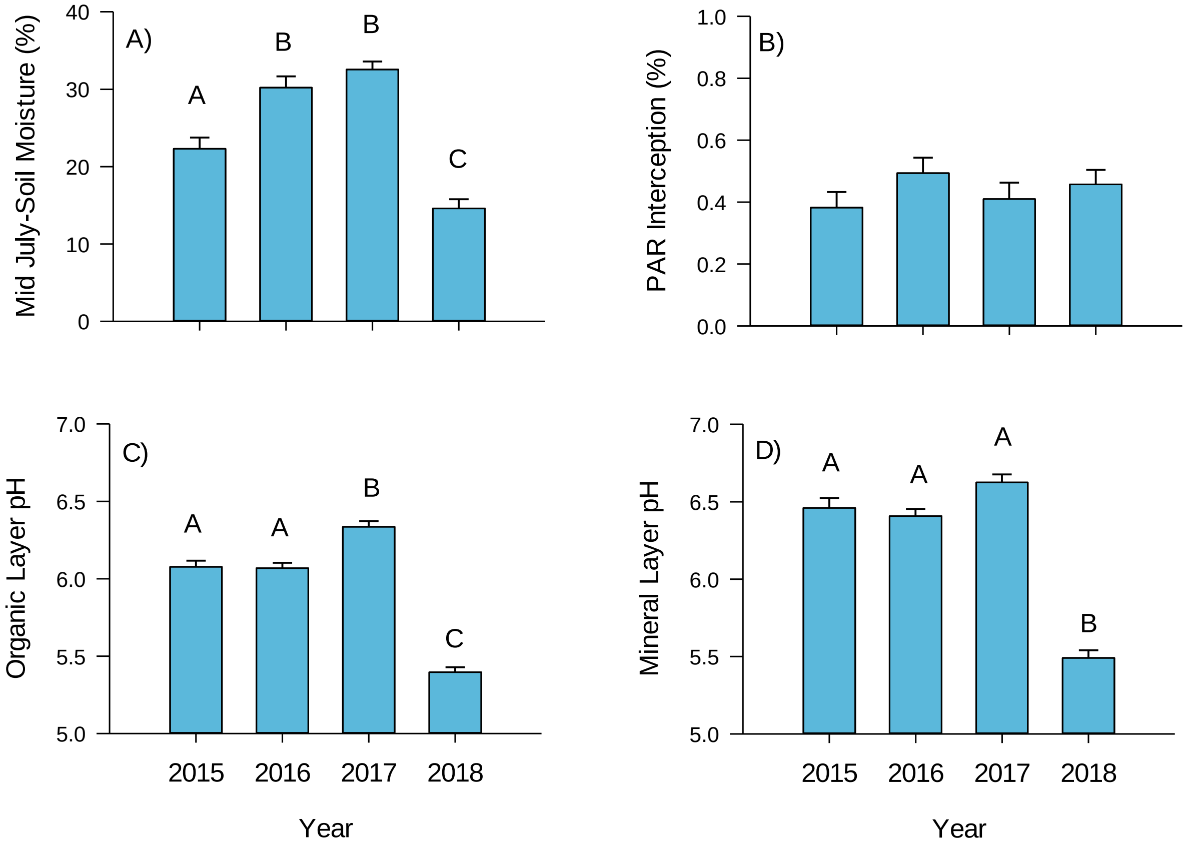

Figure S 2. Mean (± SE) for physical properties: soil moisture (A), PAR interception (B), organic layer pH (C) and mineral layer pH (D) of a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Letters above the bars present statistically significant differences in years.

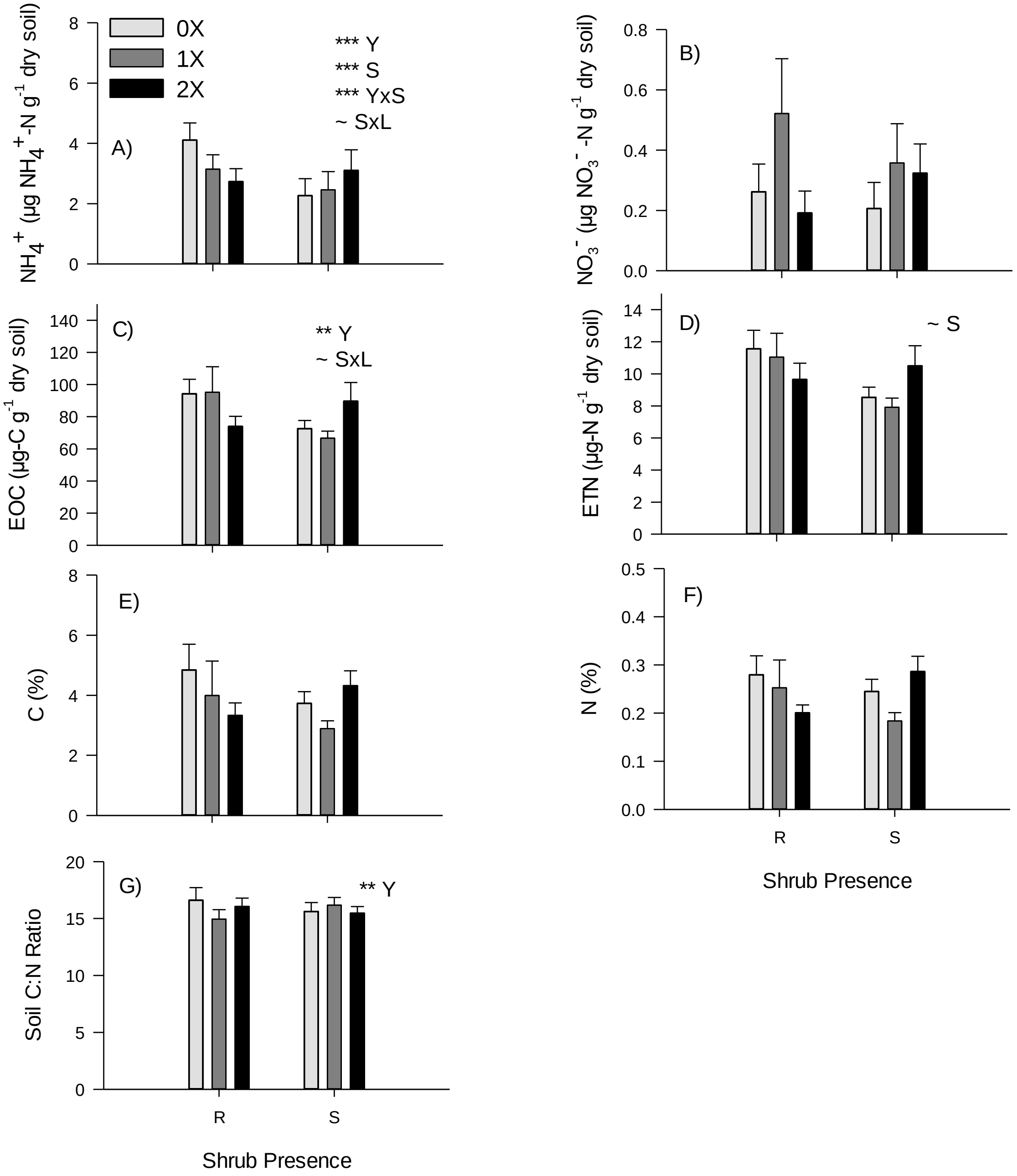

Figure S 3. Mean (± SE) of soil nutrient measures (NO_3^-, NH_4^+, EOC, ETN, %C, and % N and C:N ratios) in the mineral layer of the soil in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Means are across 4 years (2014-2018), for most variables, with a single measurement in July of each summer. Statistical significance for Y (Year), S (Shrub), L (litter), and their interactions is presented as: ~ $0.1 > p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

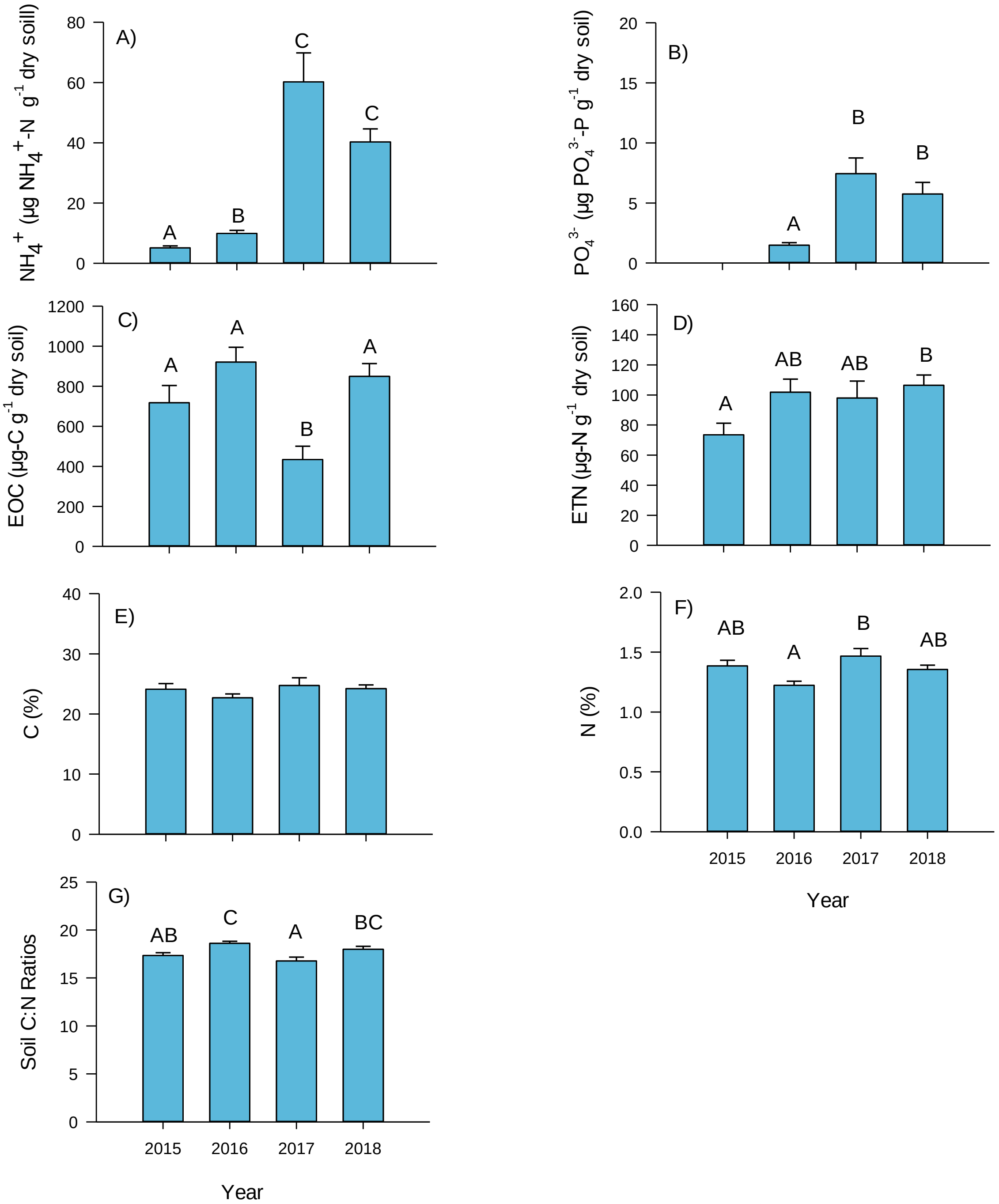

Figure S 4. Mean (± SE) of organic layer soil nutrients (NH_4^+, PO_4^{3-}, EOC, ETN, soil %C, and %N and C:N ratios) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Letters above the bars present statistically significant differences in years.

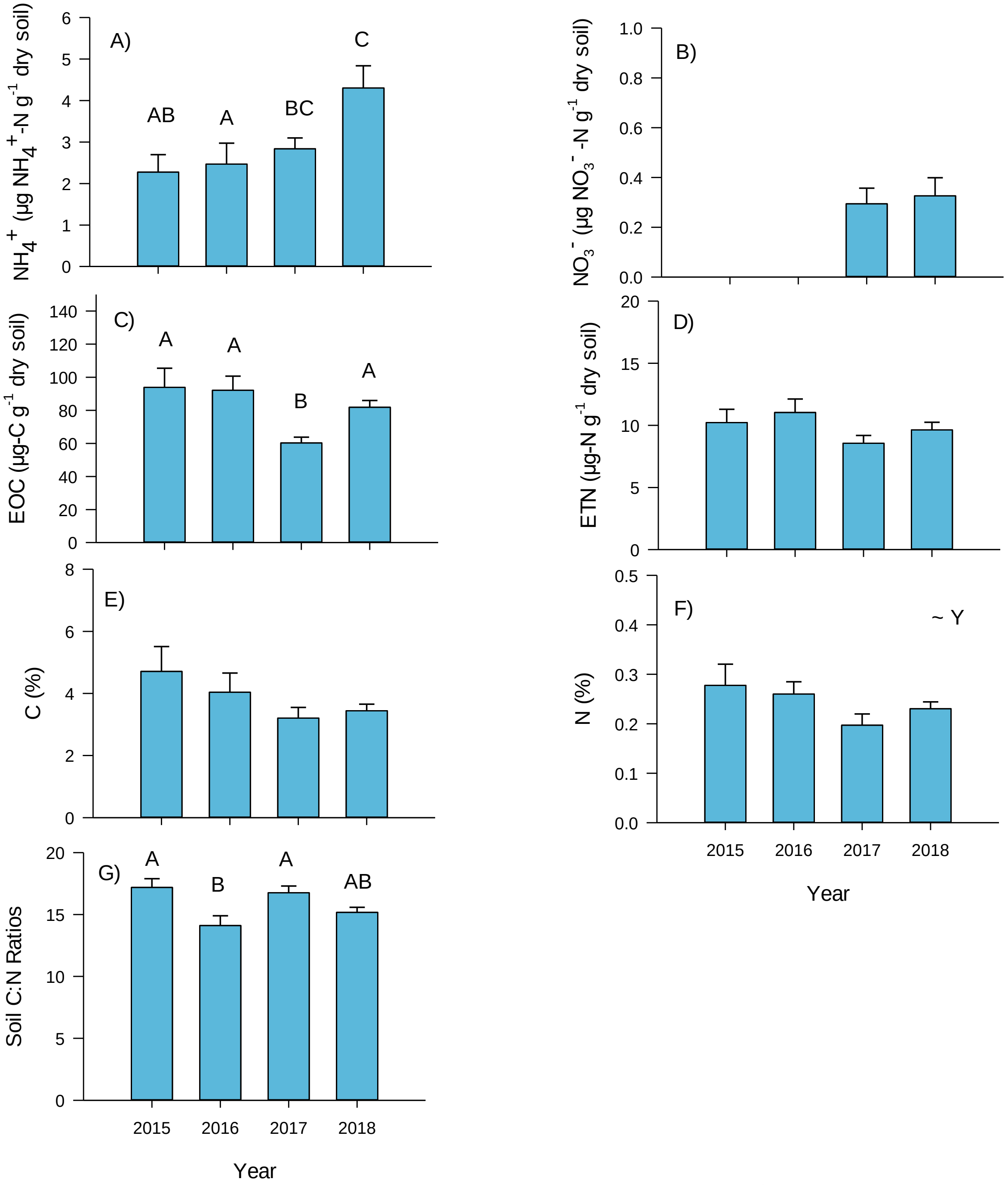

Figure S 5. Mean (± SE) for mineral layer nutrients (NO_3^-, NH_4^+, PO_4^{3-}, EOC, ETN, soil C%, and N% and C:N ratios) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake in Yukon Territory, Canada. Letters above the bars present statistically significant differences in years. Marginally significant year differences are noted as ~ Y.

Table S 1. Summary of three-way ANOVAs (or Kruskal-Wallis when data could not be normalized) for year, shrub, litter and their interactions in mineral layer soil nutrients in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Bold values are significant at $p < 0.05$.

Mineral Layer		Year (Y)	Shrub (S)	Litter (L)	Y x S	Y x L	S x L	Y x S x L
	df	3,120	1,120	2,120	3,120	6,120	2,120	6,120
NH_4^+	F	10.53	13.58	0.18	20.32	0.81	3.05	0.93
	p	**<0.001**	**<0.001**	0.834	**<0.001**	0.562	0.051	0.476
EOC	F	5.17	2.25	0.33	0.83	0.74	2.81	0.74
	p	**0.002**	0.137	0.718	0.479	0.622	0.064	0.619
EON	F	1.43	3.59	0.40	0.33	0.55	1.89	0.48
	p	0.236	0.061	0.671	0.803	0.772	0.156	0.823
Soil C%	F	1.33	0.57	1.47	0.59	0.93	0.54	0.58
	p	0.267	0.453	0.234	0.623	0.476	0.586	0.750
Soil N%	F	2.68	0.25	2.14	0.80	1.11	1.99	1.22
	p	0.050	0.621	0.122	0.498	0.359	0.141	0.299
CN Ratio	F	5.02	0.03	0.26	0.50	1.27	1.13	0.69
	p	**0.003**	0.858	0.769	0.685	0.275	0.326	0.660
NO_3^-	df	1, N=72	1, N=72	2, N=72	—	—	—	—
	γ^2	0.00	0.03	1.81	—	—	—	—
	p	0.954	0.855	0.405	—	—	—	—

Note: Degrees of freedom differ by measurement depending the type of statistical analysis.

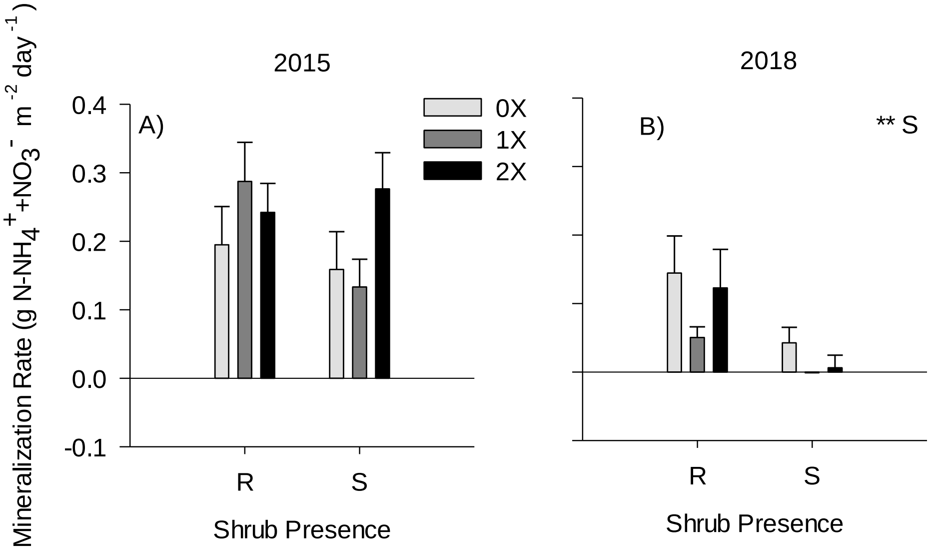

Figure S 6. . Mean (± SE) of organic layer N mineralization rates for summers 2015 and 2018 in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Statistical significance between S (Shrub), L (litter), and their interactions is presented as: ~ $0.1 > p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

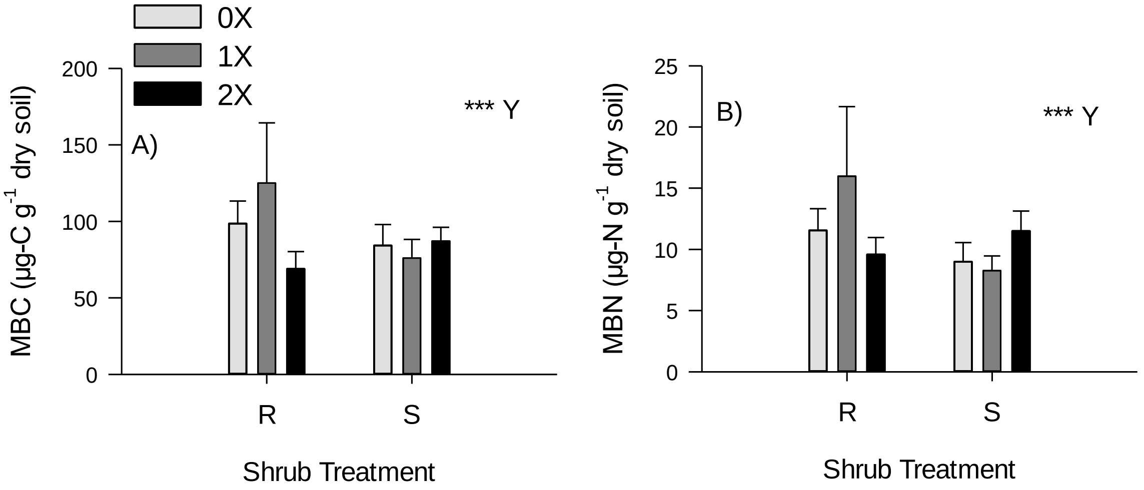

Figure S 7. Comparison of litter additions and shrub removals on mean (± SE) for mineral layer microbial biomass (MBC and MBN) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake in Yukon Territory, Canada. Means are across 4 years (2014-2018) with a single measurement in July of each summer. Statistical significance between Y (Year), S (Shrub), L (litter), and their interactions with each other is represented as: ~ 0.1 > p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.

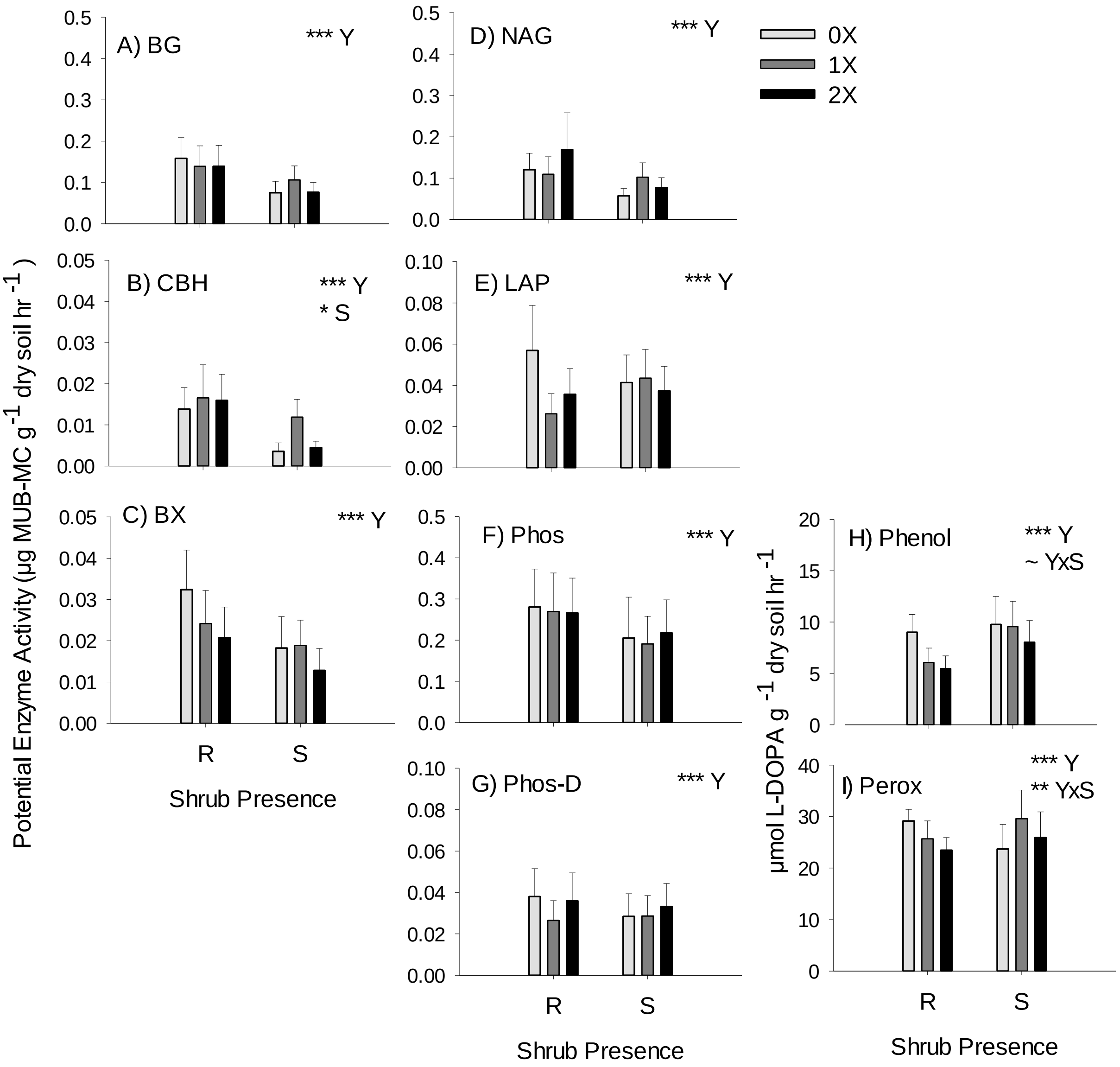

Figure S 8. Mean (± SE) potential activity of hydrolytic enzymes (BG, CBH, BX, NAG, LAP, Phos, and Phos-D) and oxidative enzymes (Phenol & Perox) in the mineral layer of a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Means are across 4 years (2014-2018), for most variables, with a single measurement in July of each summer. Statistical significance for Y (Year), S (Shrub), L (litter), and their interactions is presented as: ~ $0.1 > p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

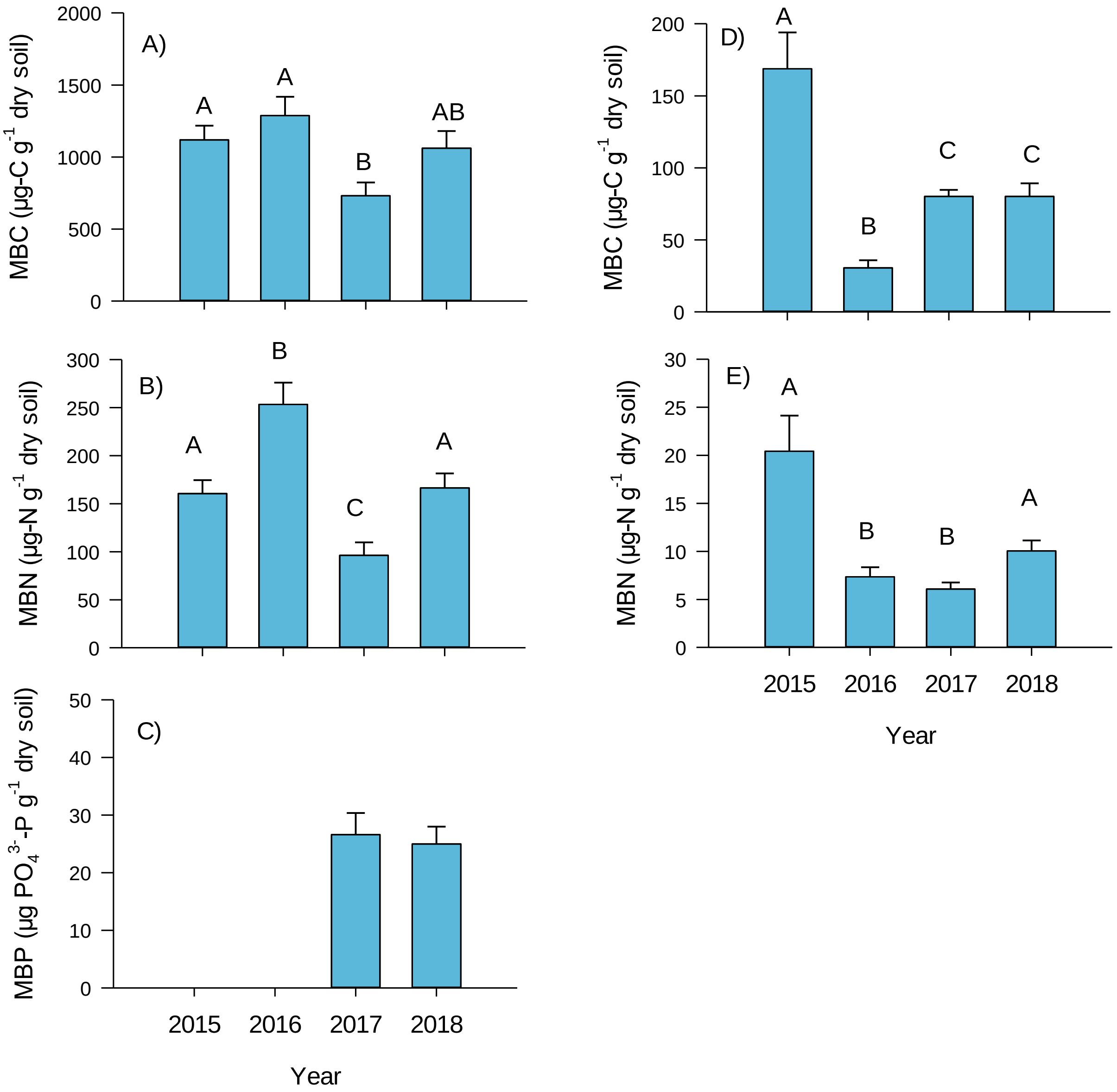

Figure S 9. Mean (± SE) for organic (A-C) and mineral (D-E) layer microbial biomass (MBC, MBN, MBP) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Letters above the bars present statistically significant differences in years.

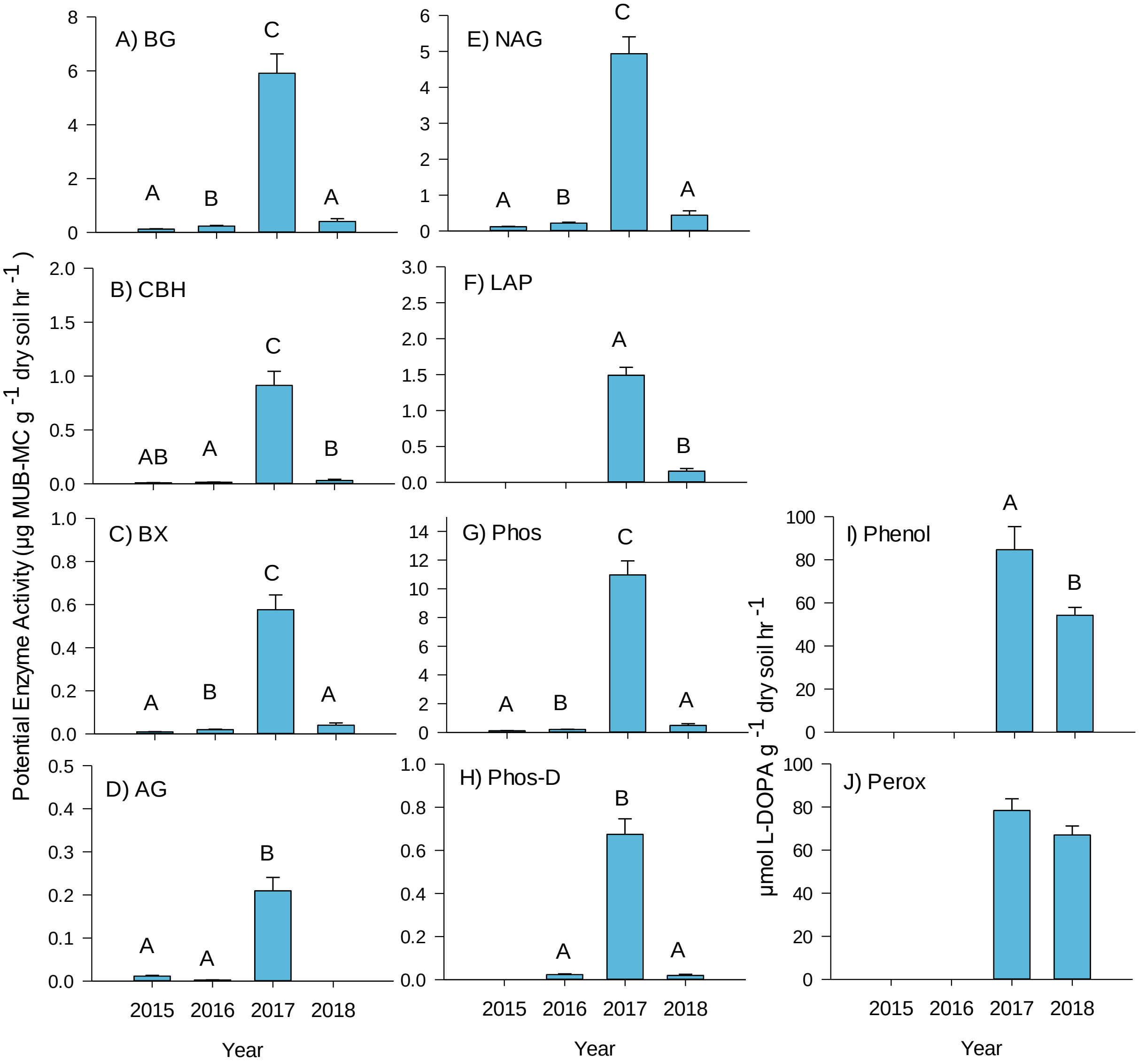

Figure S 10. Mean (± SE) potential activity of hydrolytic enzymes (BG, CBH, BX, AG, NAG, LAP, Phos, and Phos-D) and oxidative enzymes (Phenol & Perox) in the soil organic layer in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Letters above the bars present statistically significant differences in years.

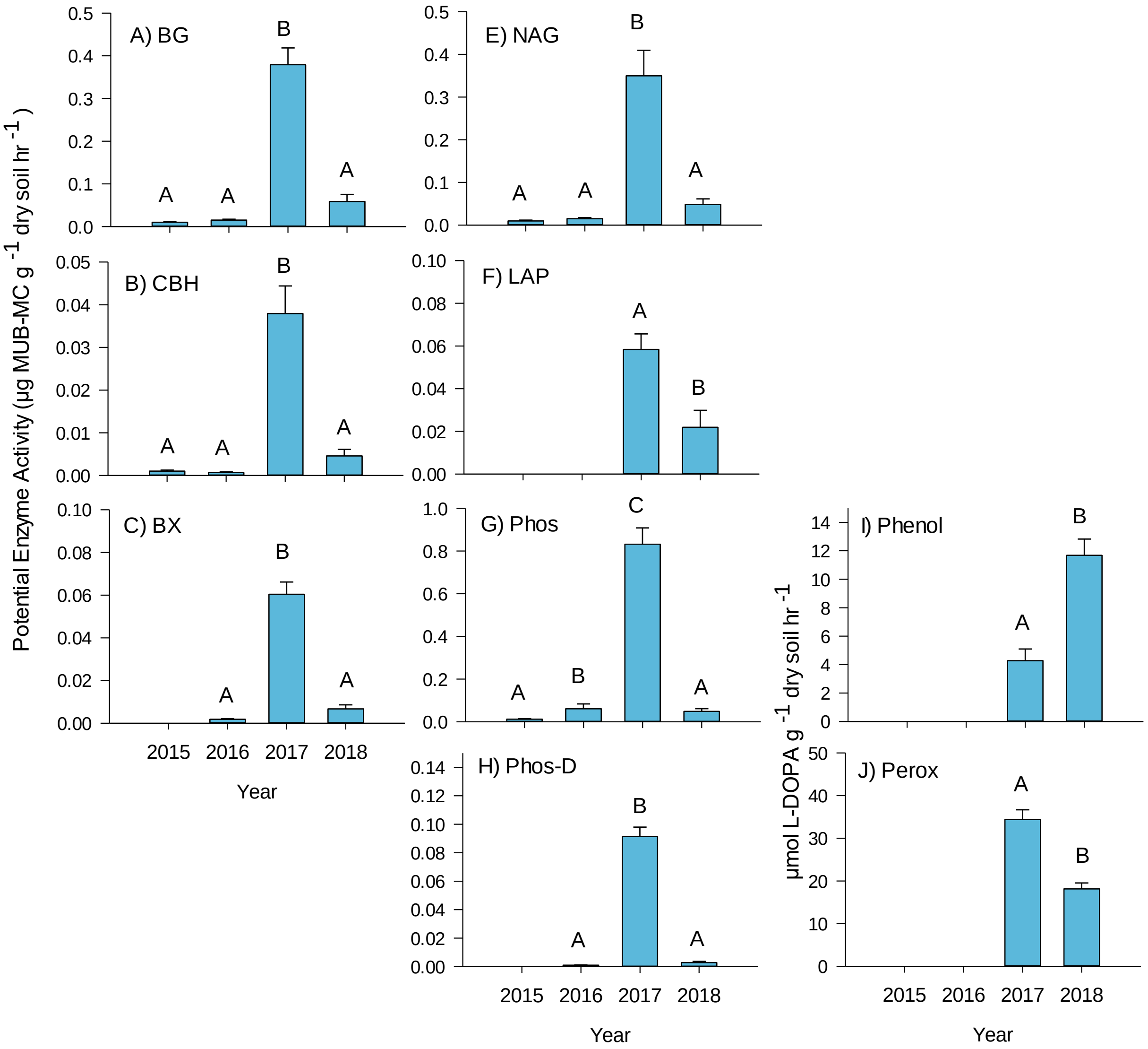

Figure S 11. Mean (± SE) potential activity of hydrolytic enzymes (BG, CBH, BX, NAG, LAP, Phos, and Phos-D) and oxidative enzymes (Phenol & Perox) in the soil mineral layer in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Letters above the bars present statistically significant differences in years.

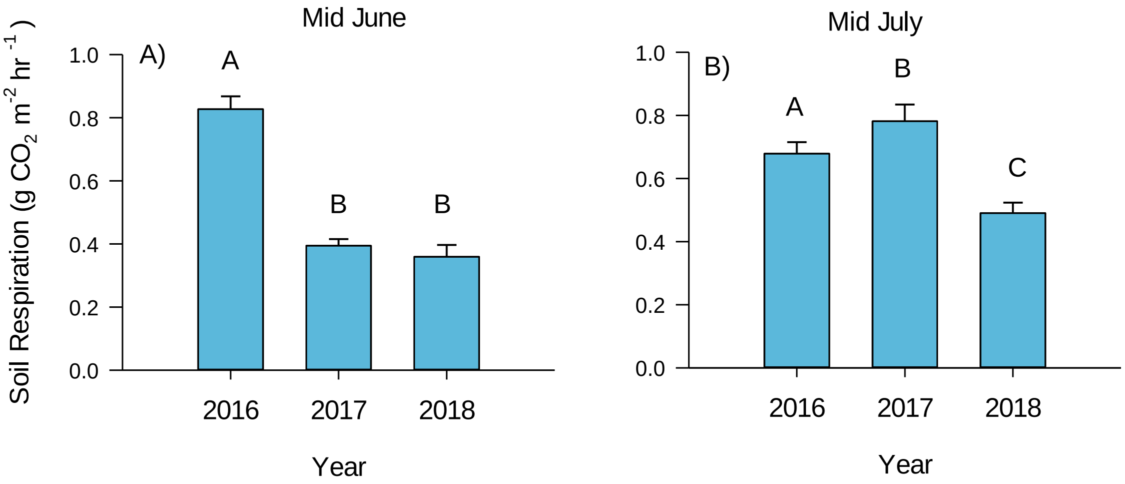

Figure S 12. Mean (± SE) for soil respiration (root + microbial respiration) on mid-June (A) and mid-July (B) in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Letters above the bars present statistically significant differences in years.

Table S 2 Summary of three-way ANOVAs (or Kruskal-Wallis when data could not be normalized) for year, shrub, litter and their interactions on mineral layer soil microbial processes and properties in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Bold values are significant at $p < 0.05$.

Mineral Layer		Year (Y)	Shrub (S)	Litter (L)	Y x S	Y x L	S x L	Y x S x L
	df	3,120	1,120	2,120	3,120	6,120	2,120	6,120
MBC	F	21.44	0.18	0.61	1.97	1.12	1.86	0.87
	p	**<0.001**	0.673	0.547	0.122	0.355	0.161	0.519
MBN	F	14.80	0.23	0.26	1.97	1.27	1.97	1.09
	p	**<0.001**	0.633	0.769	0.122	0.279	0.145	0.370
BG	F	61.25	1.04	0.06	0.53	0.11	0.57	0.37
	p	**<0.001**	0.310	0.942	0.664	0.996	0.570	0.895
NAG	F	54.41	0.51	0.04	0.26	0.19	0.67	0.35
	p	**<0.001**	0.476	0.957	0.857	0.979	0.515	0.907
Phos	F	79.53	1.01	0.25	0.16	0.37	0.33	0.12
	p	**<0.001**	0.317	0.778	0.923	0.895	0.719	0.993
	df	3, N=144	1, N=144	2, N=144	—	—	—	—
CBH	γ^2	55.78	6.14	0.47	—	—	—	—
	p	**<0.001**	**0.013**	0.789	—	—	—	—
	df	2, N=108	1, N=108	2, N=108	—	—	—	—
BX	γ^2	65.69	1.13	0.02	—	—	—	—
	p	**<0.001**	0.288	0.990	—	—	—	—
Phos-D	γ^2	73.05	0.02	0.12	—	—	—	—
	p	**<0.001**	0.883	0.944	—	—	—	—
	df	1, N=72	1, N=72	2, N=72	—	—	—	—
LAP	γ^2	16.72	0.03	0.44	—	—	—	—
	p	**<0.001**	0.866	0.802	—	—	—	—
	df	1,60	1,60	2,60	1,60	2,60	2,60	2,60
Phenol	F	33.27	0.01	0.27	3.50	1.21	0.72	0.90
	p	**<0.001**	0.931	0.767	0.066	0.307	0.490	0.412
Perox	F	46.33	1.37	0.43	10.61	0.45	1.50	0.28
	p	**<0.001**	0.247	0.650	**0.002**	0.639	0.231	0.760

Note: Degrees of freedom differ by measurement depending the type of statistical analysis.

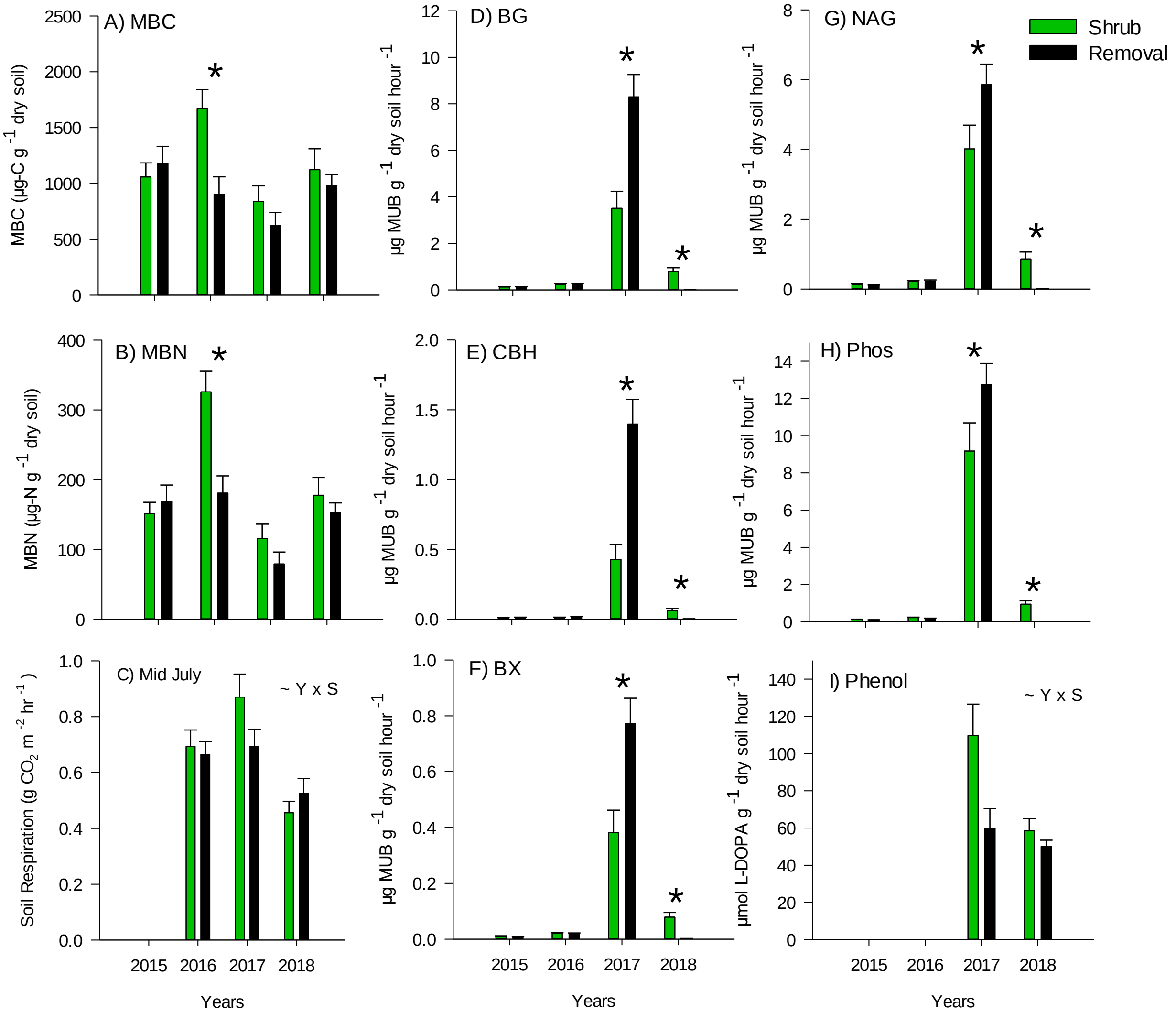

Figure S 13. Mean (± SE) of microbial processes in the organic soil layer measurements in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Asterisks show statistically significant differences in shrub treatments by year. Marginally significant shrub x year interactions are noted as ~ Y x S.

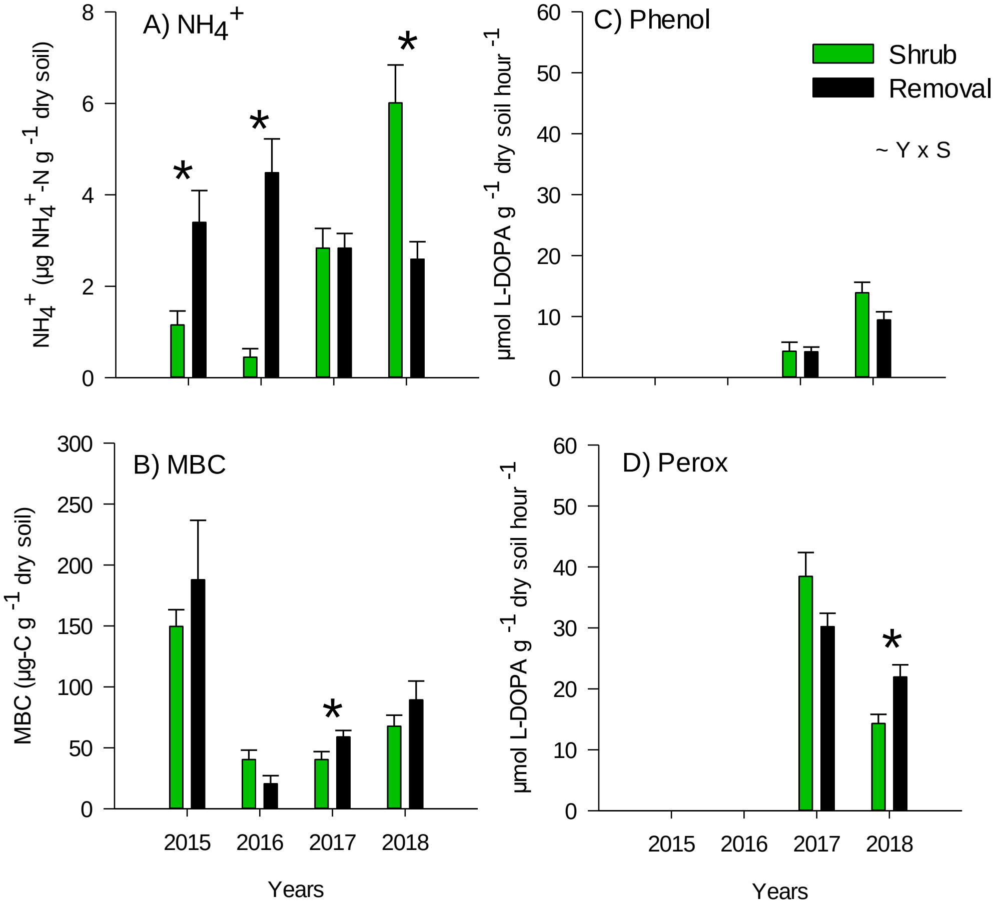

Figure S 14. Mean (± SE) of various mineral soil layer measurements in a fully factorial shrub and litter manipulation experiment in a sub-arctic alpine tundra area near Kluane Lake, Yukon Territory, Canada. Asterisks show statistically significant differences in shrub treatments by year. Marginally significant shrub x year interactions are noted as ~ Y x S.

VITA

Daniela Aguirre is an El Paso native who graduated from Socorro High school in 2012. Following high school, she enrolled at the El Paso Community College where she completed her Associate's Degree in Biology with honors. She then transferred to UTEP where she enrolled in the Environmental Science program with a concentration in Biology. During her Bachelor's program, she volunteered in several different labs researching ecological topics and helped develop multiple protocols that are still in use in those labs today. Through her devotion to research, she received the Outstanding Graduating Senior in Environmental Science award. She was also able to present her research at the Center for Undergraduate Research Initiative (COURI) symposium. She graduated with her Bachelors in Environmental Science in Spring 2017 with Magna Cum Laude honors. A semester later, in Fall 2017, Daniela began her Master's in Biology at UTEP. During her masters, she got certified in Geospatial Information Science and Technology (GIST) and was a teaching assistant at UTEP where she taught various topics in life sciences. She received the Outstanding Masters Student – Ecology and Evolutionary Biology honor, was a recipient to multiple grants and scholarships (the Frank B. Cotton Trust Scholarship, the Allien and Paul C. Davidson Scholarship, and the Dodson Research Grant), and graduated as the college of science graduate student marshal of students.