The effects of nitrogen availability on plant species in the boreal tundra ecotone

by

Gold Morrigan Cummins

A Thesis for submission to the Faculty of Graduate and Postdoctoral Studies of

The University of Manitoba

in partial fulfillment of the degree of

MASTER OF SCIENCE

Department of Biological Sciences

University of Manitoba

Winnipeg, MB

Abstract

In high latitude biomes, increasing temperatures due to climate change are predicted to increase nutrient availability. Given the boreal region is dominated by plant and fungal species that are adapted to surviving in low nutrient soil, an increase in nutrients like nitrogen may cause a variety of responses in mycorrhizae fungi and their host plants. I found that several plant species responded to three years of 10 – 100 kg ha^{-1} nitrogen addition by changing their leaf N isotope ratios, morphology and physiology. However, these changes showed no obvious correspondence with mycorrhizal status. Although ectomycorrhizal fungi slow down the mineralization rates of nitrogen, and help to immobilize nitrogen, both ectomycorrhizal host plants, *Picea glauca* (Moench) Voss, and *Dryas integrifolia* Vahl, increased in total chlorophyll $\partial^{15}N$ while lowering the C:N ratio within their tissue due to fertilizer. The ericoid host plants (*Ledum decumbens* (Aiton) Lodd. ex Steud*, Vaccinium uliginosum* L., and *Empetrum nigrum* L.) all responded differently from one another and did not respond to nitrogen unlike the ectomycorrhizal host plants. These results suggest that mycorrhizal types do not predict how boreal plant species will take up and utilize inorganic nitrogen.

In some regions, climate change has resulted in boreal forests range expanding northwards, especially with a northward shift of coniferous trees. Northward range expansion for conifers on the tundra may involve the establishment and expansion of tree islands: clusters of conifers that create microhabitats on their leeward side. While we predicted these microhabitats would benefit conifer establishment and survival, we did not find this. After planting *P. glauca* and *Pinus banksiana* Lamb, seed around tree islands near Churchill, Manitoba, I found higher germination further away from the tree islands and on the windward side.

Contributions of Authors

Manuscript Authors: Gold Morrigan Cummins, John Markham

This thesis describes two different vegetation experiments. One is a long-term fertilizer plot experiment that was created, designed and established by John Markham. The other is a short-term conifer treeline experiment that was designed and established by Gold Morrigan Cummins and John Markham. Gold Morrigan Cummins has written this manuscript with revisions from John Markham. Figure 1 – 4 were made using Biorender.com

Table of Contents

List of Tables

List of Figures

Introduction

The nitrogen cycle controls how nitrogen enters and in what forms it exists in an ecosystem. This cycle involves plants, microbes, fungi and occasionally animals, transferring nitrogen through many processes. In the soil, nitrogen exists as inorganic and organic nitrogen (Tamm, 1991). Organic nitrogen accumulates from dead plants, animals, and microbial matter and exists primarily as dead organic matter in the soil. With the exception of some simple amino acids like glutamine or asparagine, organic nitrogen is typically unavailable to plants. Instead, most plants rely on inorganic nitrogen that has been mineralized following decomposition of organic matter by soil saprotrophs (Zhu & Zhuang, 2013; Moreau et al., 2019). Depending on the amount and speed of decomposition in the environment, organic matter can exist in the soil as amino acids, oligopeptides, nucleotides, urea, proteins, or undecomposed litter. This leaves the vast majority of organic nitrogen inaccessible for plant roots to take up by themselves, and instead plants typically rely on inorganic nitrogen in the soil or through adaptations that allow them to gain nitrogen from other processes.

Inorganic nitrogen enters the soil through either the decomposition of organic nitrogen or microbial species converting atmospheric N_2 into ammonium (Moreau et al., 2019). Microbial communities can decay organic nitrogen sources into ammonia to generate energy and a source of nitrogen for themselves (Tamm, 1991; Mullen et al., 2015). A proportion of the ammonium created by microbes can be released back into the soil based on the carbon to nitrogen ratio of the mineralized organic matter, the amount of organic matter that was successfully decomposed, and the rate at which these microbial communities die (Laungani & Knops, 2012; Mullen et al., 2015; Tamm, 1991). Soil bacteria and archaea species can also transform ammonium into nitrate (Mullen et al., 2015, Tamm, 1991). Plants can uptake both ammonium and nitrate, with nitrate being the more common inorganic nitrogen source in high nutrient (low C:N) soils, whereas ammonium binds with the cation exchange complex of soil causing it to be immobilized in the soil and become less accessible to plants (Moreau et al., 2019). However, in nutrient poor soils, plants take up higher amounts of ammonium due to the low concentration of nitrate in the soil, and the low rate of nitrification in the soil. Nitrification and mineralization are controlled by soil temperature, microbial and fungal competition effects, soil moisture, and substrate C:N ratio (Liu et al., 2021; Moreau et al., 2019) Colder temperatures, organic matter containing a high C:N ratio, and a high presence of fungi that immobilize saprotrophs in the soil correspond to low nitrogen availability in the soil (Britto & Kronzucker, 2013). These factors cause high latitude habitats such as the boreal forest and tundra to have reduced mineralization and nitrification compared to other environments (Sierra et al., 2017; Tamm, 1991). As shown in Figure 1, each of these factors slows the transfer of nitrogen in the boreal, as saprotrophic and mycorrhizal fungi immobilize nitrogen within their hyphae and slow nitrogen mineralization in the soil by out competing and inhibiting saprotrophic microbes.

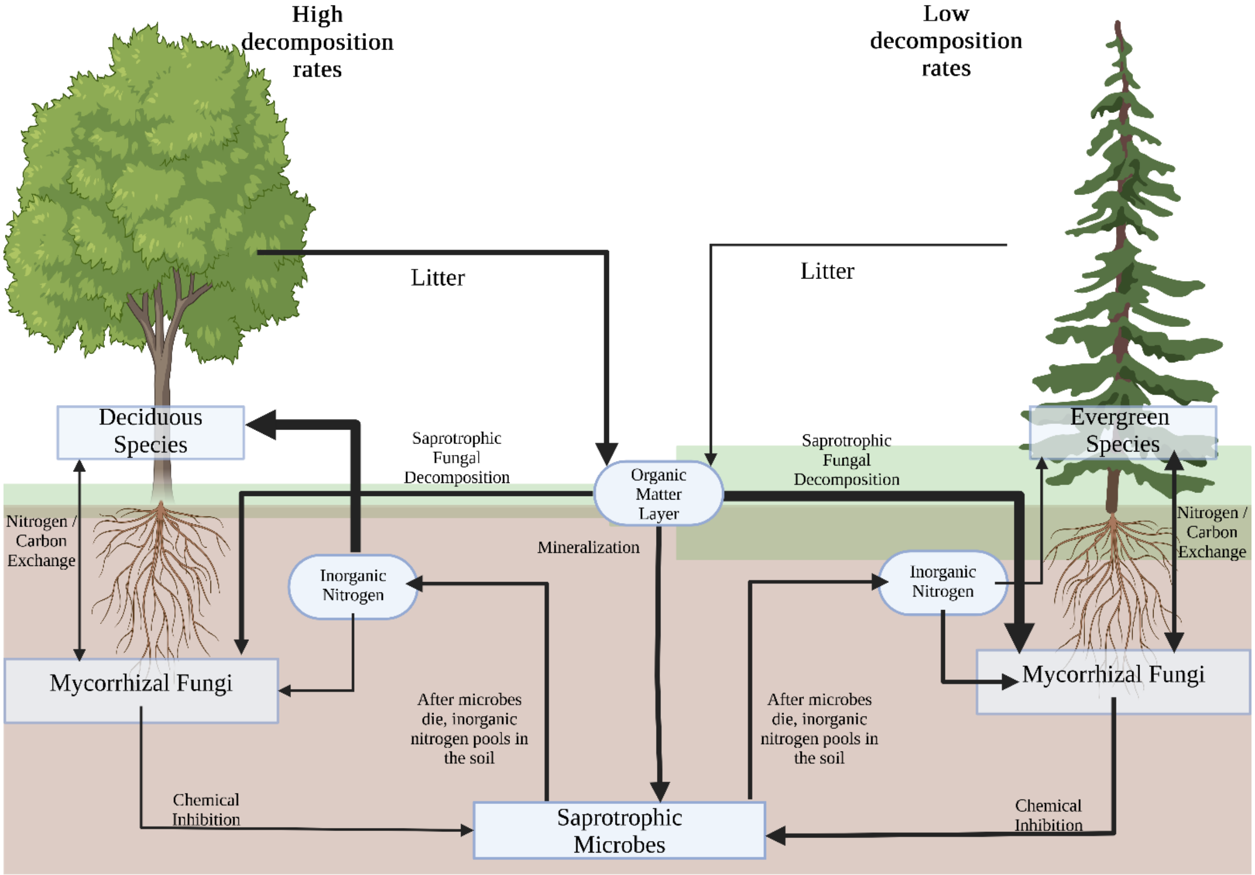

Figure 1. The nitrogen cycle in high and low productivity environments showing how mycorrhizal fungi affect mineralization rates and plant nitrogen uptake. Each arrow dictates the transfer of nutrients or, if named, the specific effects each group has on one another. The width of each arrow indicates the strength of these effects, where environments with low rates of decomposition commonly have low temperatures, a higher presence of saprotrophic and mycorrhizal fungi along with harder to decompose plant litter.

Climate is the major driver of the decomposition of organic substrates in the soil, with higher temperatures driving higher decomposition and higher nitrogen mineralization (Vanhala et al., 2008; Mullen et al., 2015). The low soil and air temperatures found in the boreal slows the decomposition of organic substrates. The quality of organic substrate also dictates the amount of carbon and nitrogen that saprotrophs can use (Laungani & Knops, 2012; Tamm, 1991). The quality of nutrients microbes live off of is controlled by many abiotic factors such as soil moisture content and air/soil temperatures along with biotic factors such as the content of lignin and the ratios of carbon to nitrogen and carbon to phosphorus found in the dead organic matter

(Flanagan, 1986). In nutrient-poor environments like the boreal forest, the dominant conifer species contain a high C:N ratio in their needles. The two most common conifers in boreal forests are *Picea glauca* and *Picea mariana* Britton, Sterns & Poggenb*.,* with *P. glauca* needles having a C:N ratio of 116.7 and *P. mariana* needles containing a C:N ratio between 39.6 – 58.5 (Fyles & McGill, 1987; Ueyama et al., 2018). In comparison, other abundant boreal plant species have leaf C:N ratios of 32.2 - 42 *(Ledum groenlandicum* Oeder*;* 33.8 (*Vaccinium uliginosum),* 45.0 - 56.6 (*Vaccinium vitis-idaea* L.*)*, and 17.4 – 30.0 (*Betula glandulosa* Michx.) (Ueyama et al., 2018).

In more temperate environments, plants have much lower C:N ratios in their litter than do boreal plants. For instance, the maples *Acer negundo* L*., Acer platanoides* L*.,* and *Acer pseudoplatanus* L. have C:N ratios between 23.90 – 45.05 in their leaves (Janusauskaite & Straigyte, 2011). Plant species that decompose faster have leaves with lower C:N ratios than those that decompose more slowly. The high C:N content in boreal plant litter indicates that saprotrophs may be inhibited in decomposing organic matter in the boreal due to the lack of nitrogen and by competition and inhibition effects between fungi and bacterial species (Orwin et al., 2011; Averill & Hawkes, 2016; Liu et al., 2021). Some fungal species can create secondary metabolites that inhibit the growth of bacterial communities (Yogabaanu et al., 2017; Liu et al., 2021). A common syndrome of fungi that can produce these metabolites is mycorrhizal fungi.

Mycorrhizal fungi are species of fungi that can associate with plants to gain photosynthetic carbon from the host plant in exchange for portions of the nitrogen and phosphorus that the fungus takes up from the soil (Van Der Heijden et al., 2015). The association between plants and mycorrhizae that allows both to gain nutrients is common in many different environments; over 200 000 plant species can host mycorrhizal fungi (Van Der Heijden et al., 2015; Smith & Read, 2008). Mycorrhizal fungi reside in contact with their host plants roots and grow out through the soil to access pools of nutrients in the soil. Each mycorrhizal syndrome differs in how the nitrogen pool they can access and the amount of nitrogen they are able to uptake and exchange with their host (Table 2 in Van Der Heijden et al., 2015). The five major syndromes are arbutoid, ectomycorrhizae, ericoid, arbuscular, and orchid mycorrhizae (Van Der Heijden et al., 2015; Crips & Eddington, 2005; Treu et al., 1995). Alongside these major types of mycorrhizal fungi, some plant species can have multiple types of mycorrhizal fungi within their roots, with these plant species described as “dual mycorrhizal” (Teste et al., 2020). To facilitate the exchange of nutrients between mycorrhizae and plants, the fungus grows structures around and, in some cases, inside the root. These structures differ between mycorrhizal types. Ectomycorrhizae form a mantle around the plant root that penetrates the outer layer of the roots while arbuscular and ericoid mycorrhizae form hyphae and structures in the interior of the plant’s roots (Razgulin, 2022). Mycorrhizal fungi provide differing amounts of nutrients from various nutrient pools, with the type and proportion of the nutrient that mycorrhizal fungi pass to their host plant being variable between mycorrhizal syndrome, plant species, and habitat. In the case of arbuscular mycorrhizae, a syndrome that associates with 74% of all plant species, are primarily involved in supplying their host with phosphorus (Van Der Heijden et al., 2015; Van Der Heijden et al., 2006). Arbuscular mycorrhizae can provide 0 – 90% of their host plant’s phosphorus acquisition, and up to 20% of their host plant’s nitrogen acquisition, where the nitrogen acquisition is dependent on the environment (Van Der Heijden et al., 2015; Corrêa et al., 2015; Leigh et al., 2009). Along with producing metabolites to inhibit bacterial growth, some mycorrhizal fungal species compete with saprotrophic species for organic matter in the soil (Sierra et al., 2016; Averill & Hawkes, 2016; Liu et al., 2021; Näsholm et al., 2013). These fungal species can create saprotrophic enzymes for the same sources of organic nitrogen as the microbes capable of mineralization, which causes ammonium to become immobilized in the mycorrhizal fungi and their host plant.

While arbuscular mycorrhizae have been studied extensively, the amount of nitrogen they pass to their host plant appears to be variable (Corrêa et al., 2015). Due to the amount of nitrogen in the soil depending on the environment, a variable amount of nitrogen is passed from arbuscular mycorrhizae to their host plant; a meta-analysis determined that the proportion of nitrogen passed to the host plants reduces under high and low amounts of nitrogen in the soil. (Corrêa et al., 2015). They argue that when the environment has high and low amounts of usable nitrogen in the soil, both the fungus and host plant will be limited in either carbon, nitrogen, or another nutrient necessary for growth, and will result in the mycorrhizal fungi immobilizing nitrogen in themselves and reducing the proportion of nitrogen passed onto the host plant (Figure 1 in Corrêa et al., 2015).

While arbuscular mycorrhizae are extremely common, they tend to associate with herbaceous or woody plant species in lower latitudes than the boreal. Ectomycorrhizae and ericoid mycorrhizae are the more prevalent mycorrhizal types in boreal, arctic and alpine environments due to the saprotrophic abilities both are capable of. Both mycorrhizal fungi can create enzymes to decompose organic substrates and transform them into inorganic nitrogen and amino acids to trade with their host plant for photosynthetic carbon (Van Der Heijden et al., 2015). This transfer of nutrients benefits their host plant with up to 80% of its nitrogen acquisition, depending on the environment (Read & Perez-Moreno, 2003). In environments where mineralization is limited and organic matter is abundant, ectomycorrhizae and ericoid mycorrhizae contribute a large portion of their host's total nitrogen acquisition (Read & Perez-

Moreno, 2003; Van Der Heijden et al., 2015). Saprotrophic mycorrhizal activity also slows microbial mineralization due to the removal of organic substrates from the soil and may decrease ^{15}N levels in their host plants tissue due to fractionation of the ^{14}N and ^{15}N isotopes (Hobbie et al., 2009; Högberg, 2011; Cui et al., 2020). Given that nitrogen fractionation occurs within the mycorrhizal hyphae, the majority of ^{15}N remains in the mycorrhizal fungi's hyphae while the host plant takes up the ^{14}N that is not immobilized by the plant's fungal mycorrhizal structures (Hobbie et al., 2009; Högberg, 2011; Cui et al., 2020). As ^{15}N becomes immobilized in mycorrhizal hyphae, this results in plants having negative $\partial^{15}N$ and the fungal structures in the host plants root cells having a positive $\partial^{15}N$. In other words, plant tissues are commonly high in ^{14}N and low in ^{15}N, while mycorrhizal fungi are high in ^{15}N and low in ^{14}N. The amount of ^{15}N in plant foliage, fungal bodies, and the soil differs between latitude, plant species and how much nitrogen the mycorrhizal fungi supply to their host plant (Hobbie et al., 2009). In general, higher levels of ^{15}N in the plant indicate that mycorrhizae are not immobilizing ^{15}N and that the host plant is not as reliant on mycorrhizal association to access necessary amounts of nitrogen Hobbie et al., (2009). Instead, in high nutrient environments, where nitrates are more common, host plants do not rely on their mycorrhizae as they can take up nutrients in the soil on their own, resulting in a higher amount of ^{15}N found in the host plant. Mycorrhizal fungi are dependent upon reciprocal exchange with host plants, where the fungal partner receives soluble organic carbon in exchange for providing the plant host phosphorus and nitrogen (Högberg, 2011; Högberg et al., 2010). The amount of carbon allocated to a host plants mycorrhiza is complicated and complex, controlled by many different factors, such as the mycorrhizal structures created by the fungus, plant species, the N:P ratio of the soil, and fungal species (Moreau et al., 2019; Grman & Robinson, 2013; Reddy et al., 2024). Although the actual transfer of carbon from host to mycorrhizal fungal hyphae is a complicated affair, in general, when nitrogen is added into the soil of a conifer forest carbon allocation from the host to the ectomycorrhizal fungal structures is reduced (Högberg et al., 2010; Högberg et al., 2011). In boreal environments, low carbon allocation reduces the extent to which ecto- and ericoid mycorrhizae compete with and inhibit microbes for soil organic matter (Moreau et al., 2019; Razgulin, 2022; Högberg et al., 2010).

The climate crisis is poised to change many boreal species' access to nutrients; how and if plant species will react to increasing temperatures is an active field of research Cameron & Lantz, 2016; Harsch et al., 2009). Over the past century, there has been an increase of 1.5˚ C in mean annual temperatures, with another increase of 4 – 6˚ C expected over the next century (Mucha et al., 2018). Due to the low temperatures and mineralization rates in the boreal environment, nitrogen and carbon can become inaccessible to plant, fungal and microbial species, causing the boreal to be a sink for nutrients (Jonasson & Shaver, 1999; Vallotton & Unc, 2024). Increasing annual temperatures will cause the boreal to have changes in water and nutrient availability as these nutrients stored in the boreal will become available (Price et al., 2013; Chapin et al., 2004). This increase in temperatures affects every aspect of the boreal, with changing precipitation, microbial action, plant/fungal responses, forest productivity and plants' access to nutrients expected to change with the temperature (Price et al., 2013; Mucha et al., 2018; Boonstra et al., 2008; Vanhala et al., 2008). Precipitation is highly variable across the boreal, but as temperatures and water availability increase, there will be higher amounts of precipitation and evaporation (Price et al., 2013). Higher precipitation and evaporation leads to higher amounts of snowfall in the winter, while the drier summer months tend to lead to droughts. The change in precipitation due to the climate crisis across Canada over the next 100 years was modeled by Hogg & Schwarz (1997) who predicted a more temperate and dry central

Canada, and a similar, if not, higher rate of annual precipitation in eastern and western Canada. However, these predictions are highly variable as annual precipitation does not show the precipitation over the individual seasons; some environments receive higher annual precipitation and still experience drought in the summer. Microbial activity is influenced by mycorrhizal fungi, other saprotrophs and the temperature in the boreal, with increased temperatures allowing for higher rates of decomposition (Price et al., 2013). Vanhala et al. (2008) demonstrated this trend with soil organic matter having a higher decomposition rate as temperatures increase in southern and northern spruce/pine forests. The increase in nitrogen availability and temperature has also been hypothesized to increase the productivity of boreal forests (Price et al., 2013; Mucha et al., 2018)

The boreal tundra ecotone contains a soil layer that rely on low temperatures and slow decomposition, permafrost (Tamm, 1991; Prescott et al., 2000). While several complex factors such as topography, relative humidity, and fuel conditions are required to interact with one another for a wildfire to ignite, it has been predicted that the climate crisis will cause wildfires to increase in frequency and severity (Flannigan et al., 2000;Li et al., 2023). One impact of these forest fires, and temperatures increasing globally, is the permafrost layer thawing. The permafrost layer is soil that has remained at or below 0°C for at least two years (Obu, 2021). While permafrost layer is constantly frozen, a portion of the soil, called the active layer, undergoes annual freezing and thawing (Hansen et al., 2023). The active layer is where plants, fungi and microbes can access nutrients and water; as more permafrost thaws, then the active layer will become deeper (Hansen et al., 2023). Wildfires and higher annual temperatures increase the soil temperatures in the boreal tundra ecotone causing the active layer to deepen as the permafrost layer thaws (Li et al., 2024). As the permafrost layer contains organic matter that plants, microbes, and fungi cannot access, permafrost thawing increases nutrient availability. Finger et al. (2016) showed that after permafrost thaw, leaves of boreal plant species saw an increase in total nitrogen. They concluded that as the active layer became deeper, saprotrophic microbes mineralized the organic matter into inorganic nitrogen that plants were able to take up.

While ectomycorrhizae and ericoid mycorrhizae are the two most dominant mycorrhizal types in the boreal tundra ecotone, an increase in nutrient availability may affect this dominance. Both ericoid and ectomycorrhizae thrive in nutrient-poor soil where they outcompete saprotrophic microbes for organic matter and slow mineralization rates through inhibiting these microbial communities. However, as nitrogen mineralization and decomposition rates increase in the boreal environment, mycorrhizal colonization, biomass and the amount of carbon given to them by their host plant may decrease. Högberg et al. (2010) showed this loss in carbon transfer to mycorrhizal fungi by examining the percentage of a carbon tracer found in the mycorrhizae passed to it by its host plant after fertilizing four boreal *Pinus sylvestris* L., forest plots. After fertilizing these plots with 100 kg ha^{-1} N for one year, the percentage of carbon passed to the mycorrhizae decreased by 48% compared to the control plots. Similarly, Urcelay et al. (2003) also found that an increase in nitrogen reduced colonization by mycorrhizal fungi in some boreal plant species roots. They added 100 kg ha^{-1} of nitrogen and 5 kg ha^{-1} of phosphorus every year to 102 plots in Alaska. After 4 years, they sampled the roots of *Betula nana* L.*,* a dual mycorrhizal host plant, a plant species that is capable of hosting both arbuscular and ectomycorrhizal fungal structures within its roots and *Ledum palustre* L.*,* an ericoid host plant (Teste et al., 2020). They found that after applying fertilizer, *B. nana* saw reduced ectomycorrhizal colonization in their roots, while the ericoid mycorrhizae within roots of *L. palustre* stayed the same. It appears that mycorrhizal fungi may react differently depending on the environment, and the plant species they associate with. While the colonization of ericoid mycorrhizae did not seem to change in the Urcelay et al. (2003) study, Nilsson et al. (2005) found a change in fungal biomass due to nutrient availability. Nilsson et al. (2005) examined the total fungal biomass and mycelial production in soil taken from four sites in northern Sweden that naturally differed in nutrient availability. They found that soil with the highest amount of nitrogen and phosphorus had significantly lower amounts of ericoid and ectomycorrhizal fungal biomass. They also found that as nutrient availability increased, arbuscular mycorrhizae began to produce higher amounts of extra-radicle mycelium. Overall, it appears that ecto- and ericoid mycorrhizal fungi are negatively impacted by increases in inorganic nutrient availability in boreal soils (Nilsson & Wallander, 2003; Wallenda & Kottke, 1998). As the soil in the boreal will begin to accumulate nitrogen and phosphorus due to increasing soil temperatures, then ectomycorrhizae and ericoid mycorrhizal fungi may see reduced abundance and growth.

Across the boreal ecotone, conifers have been observed changing their growth and germination rates due to climate change (Boonstra et al., 2008; Price et al., 2013). Many conifer species, like *Picea glauca,* have been seen taking advantage of the increased temperature and heightened nutrient availability to increase their growth and germination, and expanding northwards onto the tundra (Dial et al., 2022; Harsch et al., 2009; Munier et al., 2010). The annual radial growth from 1910 to 2000 for white spruce trees across four sites on the white spruce treeline was studied by Wilmking et al. (2004): increased temperatures since 1950 showing a correlation with growth in all sites. In the more northern sites with a latitude of 68 °N, higher temperatures showed an increase in annual ring growth. However, while the higher latitude sites had higher ring growth, the sites at 63 °N had decreased annual ring growth as temperatures increased (Wilmking et al., 2004). While this does not indicate that higher temperatures increase white spruce forest productivity across the entire boreal, it does show that white spruce favors certain latitudes as temperatures increase. An increase in temperatures may impact more than just the growth rates of *P. glauca,* with seedling establishment and survival of some *P. glauca* populations being positively correlated with increased temperatures (Mamet & Kershaw, 2012). White spruce encroachment in higher latitudes has been predicted for some time, with models showing treelines will expand northwards due to increased water availability, longer growing seasons, reduced wildfire frequency, and increased nutrient availability (Dial et al., 2022; Kambo & Danby, 2018; Hewitt et al., 2016). For instance, Hewitt et al. (2016) used ALFRESCO, a model that simulates vegetation success and fire occurrence across Alaksa on an annual basis, to simulate the effects of increasing temperature, mycorrhizal interactions, and wildfire frequency/severity over the next 100 years on white spruce. They ran four versions of this model, with different levels of seedling establishment and performance based on how available ectomycorrhizal fungi were above the tree line and in areas disturbed by wildfires. In each of these models, they found an increase in the percent of forest cover and the percent of spruce found in the boreal tundra, with an average of 8.8% of Alaskan tundra converted into boreal forest. This high percentage of tundra colonized by white spruce was primarily accomplished when conifer seedlings were able to associate with ectomycorrhizae. The other factors that helped conifers colonize tundra were increasing soil temperatures and wildfire frequency (Hewitt et al. 2016). This model's prediction for conversion of boreal tundra into boreal forest through conifer seedling establishment beyond the treeline with northward encroachment is consistent with a meta-analysis by Lett & Dorrepaal (2018). This meta-analysis examined biotic factors influencing plant species establishment beyond the boreal ecotone treeline. Although Lett & Dorrepaal did not find that nutrient availability influenced seedling germination in the three studies they examined, they were able to find an interaction between soil temperature and moisture availability. As soil temperatures increased, and where water was available, conifer germination increased in tandem. They also found an interaction between soil temperature and water availability, where each plant species had varied responses to the heightened temperature and soil moisture. While *Larix decidua* Mill, seeds saw an increase in germination due to higher soil moisture and temperatures, soil moisture had a negative effect on seedling emergence and survival of *Pinus cembra* L.*,* *Pinus uncinate* Steud*,* and *Picea abies* Mill, seeds (Loranger et al., 2016). However, even though the emergence and survival of these conifer seeds decreased due to high soil moisture, they found that high temperatures reduced or nullified this decrease. Due to this interaction between soil temperature and moisture, Loranger et al. (2016) concluded that heightened soil temperature and moisture may have helped *P. uncinata* and *P. abies* seeds germinate and survive beyond the treeline. While germination and survival rates are not the only factors that can influence seedling establishment, it does appear that heightened soil temperature and moisture help some conifer species germinate.

One way that white spruce colonists may be able to establish themselves on the boreal tundra past the treeline is through expanding “tree islands” - clusters of trees that can survive on the tundra due to the high density of neighboring trees providing shelter against harsh tundra winds that are capable of damaging or killing individual trees (Albertsen et al., 2014; Scott et al., 1993). These clusters of trees can provide better conditions for colonists through the way they interact with the wind and snowfall. To mitigate the effects of the high winds on the tundra that can damage white spruce needles, tree islands spread themselves into elongated oval shapes that contain tall spruce trees in the center (Albertsen et al., 2014; Scott et al., 1993). This shape reduces the number of spruce needles damaged during the winter and creates a protective layer for seedlings by increasing moisture and nutrient availability on the leeward side of the island. The tree islands found on the tundra near Churchill, a sub-arctic environment located near Hudson Bay at a latitude of 58°N, showcases how tree islands can create a protective area on the leeward side of the island. In this environment, tree islands are heavily affected by the cold winds blowing off Hudson Bay (Albertson et al., 2014). These winds blow down onto the northwest (windward) side, which causes an increase in snow buildup on the southeastern (leeward) side. The high snow depth on the leeward side insulates plants from the cold winds of the tundra and allows for an increased rate of growth after the snow melts and deposits high amounts of water and nutrients into the soil (Albertsen et al., 2014; Scott et al., 1993). Due to increased snow depth and protection against tundra winds, tree islands create microhabitats which benefit establishment of conifer seeds. Munier et al. (2010) compared the germination, seedling height, mortality and damage of *Picea mariana* seeds in the transition zone between alpine tundra and forest, open canopy boreal forest and the tree islands in Mealy Mountain, a mountain range in the southern portion of Labrador. In each of these sites they tested how vegetation disturbance, heightened soil temperatures, herbivore exclosure, along with the interaction between each three treatments, would affect *P. mariana* seeds. They found that across the three sites, *P. mariana* seeds had the lowest seedling mortality and highest germination when grown around the tree islands. Within the tree island and tundra transition zone, they also found that higher soil temperatures increased the number of germinated seeds. Furthermore, they found that overall damage to seedlings did not differ between sites, and the only increase to seedling damage was due to disturbing the vegetation before planting the *P. mariana* seeds. From this study, it appears that tree islands can create a microhabitat that benefits the establishment of conifer seeds. These factors suggest tree islands are a possible way for white spruce trees to colonize the boreal tundra.

Alongside the predicted northward expansions of conifer trees, tall shrubs are moving northwards onto the boreal tundra. These tall shrubs (*i.e., Salix,* willow; *Betula*, birch; and *Alnus*, Alder), have begun to colonize the tundra above 60 °N over the past several decades (Beck et al., 2011; Cameron & Lantz, 2016; Bonfils et al., 2012; Rew et al., 2020). Like the conifers, the range of tall shrubs is expanding northwards due to increased mean temperatures during the growing season, higher soil moisture, and increased nutrient availability. As tall shrubs enter the tundra, the active soil layer of the soil that plants, fungi, and microbes can access will increase in depth, owing to the tendency for tall shrubs to cause increased snow depth that thermally insulates the soil from sub-freezing temperatures (Cameron & Lantz, 2016; Bonfils et al., 2012). Similar to tree islands, tall-shrub mediated snow depth allows for increased nutrient availability and soil moisture, allowing saprotrophs access to previously inaccessible organic sources, and helping tall shrubs establish themselves onto the tundra. Thus, a feedback loop between snow depth and increased tall shrub establishment further drives tundra colonization by boreal forest species.

As tall shrubs and conifers expand onto tundra, the tundra will begin to become more productive and greener (Ropars & Boudreau, 2012). NDVI, normalized difference vegetation index, tracks how productive and green plants are, where an increase in chlorophyll should increase a plants NDVI (Beck et al., 2007). While commonly used to see phenological changes in plant species across a period, NDVI can also be used as an analog for primary plant productivity and plant biomass. NDVI quantifies the contrast between the near infrared and the red light that plants reflect into the atmosphere after sunlight hits their leaves. As chlorophyll content decreases, plants will reflect higher amounts of red light with near infrared light increasing in tandem as leaves increasing in area (Beck et al., 2007). Near infrared and red light can thus be used to determine when plants are becoming greener, gaining biomass, and increasing their photosynthetic rates. NDVI can also be used as a proxy for how productive plants are, with productivity defined as how quickly plants can gain additional biomass (Schulte-Uebbing & De Vries, 2018). For example, high productivity forests can create higher amounts of biomass than low productivity forests, with high productivity forests/plant species having higher NDVI values than low productivity forests/plants. Boreal forests commonly have low productivity due to low temperatures, slow nitrogen mineralization rates, and mycorrhizal fungi inhibiting saprotrophic microbes causing low rates of growth (Jarvis & Linder, 2000; Sierra et al., 2016; Averill & Hawkes, 2016; Liu et al., 2021; Näsholm et al., 2013). Due to this, it has been predicted that as the climate crisis increases annual temperatures in the boreal, plant species like *Picea glauca* will move northwards (Dial et al., 2022; Kambo & Danby, 2018; Hewitt et al., 2016).

While it has been predicted that plant species will advance onto the tundra and move northwards, it does not seem like this is happening everywhere (Harsch et al., 2009). The lack of successful reproduction for white spruce populations seems to slow or stop tree line advance (Kambo & Danby, 2018; Timoney & Mamet, 2020; Mamet & Kershaw, 2012). Timoney & Mamet (2020) assessed the white spruce treeline of Central and Northwestern Canada, from 57 °N to 68 °N, from 1960 – 2010 through historical cover photographs. They found that in high-latitude environments, forests with low to moderate tree cover had an average tree cover increase of 0.04% from 1960 to 2010. However, the areas closer to Churchill, near 58 °N, did not have any statistical increase in tree cover and instead, an estimated 0.12% decrease in average tree cover per year was found (Timoney & Mamet, 2020). While Timoney & Mamet (2020) did not hypothesize the factors that influence this decrease in tree cover, a possible reason is the lack of successful reproduction in the white spruce trees around Churchill. Benjamin et al. (2024) showed this lack of successful reproduction for Churchill's white spruce trees, with only 1% of the cones sampled across Churchill being reproductively viable. The number of viable white spruce cones in Churchill appears to change in the presence of increased available nutrients, as white spruce trees had higher cone and seed production in nutrient-rich red fox dens (Benjamin et al., 2024). The lack of reproductive success for white spruce appears to be a factor constraining the treeline advance in Churchill, causing the white spruce seedlings in Churchill to be variable in their ability to move northwards.

Hypothesis/Predictions

Given that there is conflict on whether, and to what degree, plant species in the subarctic are reacting to increased nutrients, I measured the responses of the dominant plant species in the boreal tundra ecotone on the west side of Hudson Bay after applying fertilizer to experimental plots. The predominant species in the forest are *Picea glauca, Empetrum nigrum, Vaccinium uliginosum*, and *Ledum decumbens*. In the tundra, *Arctostaphylos alpina* (L.) Spreng, *Shepherdia canadensis* Nutt., and *Dryas integrifolia* are dominant. Plant species in both the forest and tundra associate with ectomycorrhizal, ericoid, and arbuscular mycorrhizal fungi (Trofymow et al., 2020; Malloch & Malloch, 1982). *Picea glauca,* and *Dryas integrifolia* commonly associate with ectomycorrhizal species, and *Empetrum nigrum, Vaccinium uliginosum, Ledum decumbens*, associate with ericoid mycorrhizae. *Arctostaphylos alpina* and *Shepherdia canadensis* are unique, in that *A. alpina* has arbutoid mycorrhizae (Kühdorf et al., 2015; Kranabetter &

MacKenzie, 2010) while *S. canadensis* is a nitrogen fixing plant and therefore may not rely on its mycorrhizal partners for soil. A nitrogen fixer plant is one that associates with bacteria that can fix atmospheric nitrogen into a form that their host plant can use, allowing the host plant to access a nitrogen pool that many plant or fungal species cannot gain nitrogen from (Rousk et al., 2016). Similar to other actinorhizal nitrogen fixing plants, *S. canadensis* has been observed forming both arbuscules and a Hartig net around its roots (Teste et al., 2020). While uncommon, dual mycorrhizal plants have a greater degree of flexibility when multiple mycorrhizal syndromes are present in the soil (Teste et al., 2020). As nitrogen is limited in the boreal tundra ecotone, I predict that when nitrogen in the soil is increased, boreal plant species will rely less on mycorrhizal partners, and that this will alter their leaf physiology. Boreal plant species should increase their ∂^{15}N as they rely less on mycorrhizae for N, increasing chlorophyll due to increased access to N values while having a decreased C:N ratio in their leaves. As chlorophyll indicates how photosynthetically active and green a plant species is, as total chlorophyll increases in the leaves of boreal plants, plant productivity per unit land area should also increase. Furthermore, given that different mycorrhizal types allocate different amounts of nutrients from different pools, I predict that ericoid and ectomycorrhizae will allocate a smaller portion of nitrogen to their host plants as nutrients increase. This decrease in the proportion of nitrogen passed to the host plants can be interpreted through the ∂^{15}N values seen in plant leaves, with higher ∂^{15}N values showcasing that plants are up taking nitrogen from the soil rather than their mycorrhizae.

Alongside the physiological responses, I hypothesize that *Picea glauca* (White spruce) should see an increase in growth when nutrients are more easily accessible in both the boreal forest and tundra. White spruce trees have been seen increasing their growth due to an increase in soil nutrients in sub-arctic environments, with trees grown on red fox dens having higher growth than those elsewhere in the boreal sub-arctic (Lang et al., 2022). While white spruce trees, especially those in Churchill, are reproductively limited, planting white spruce seed in areas of high nutrient availability should allow for the growth rate to be determined. Alongside the white spruce trees, *Pinus banksiana* (Jack pine) will be planted to serve as a comparison given that jack pine is not native to Churchill. By planting jack pine, we will be able to determine if access to additional nutrients allows conifers other than white spruce to spread northwards. The northernmost range of *P. banksiana* stops at 55 ° N (Desponts & Payette, 1993; Payette et al., 2017), but its range may expand all the way to the northernmost limit of boreal forests (Figure 1 in Asselin et al., 2003). Given white spruce is currently establishing themselves on the tundra in areas of high snow depth and increased soil nutrient availability, I predict that both jack pine and white spruce growth and germination should increase on the leeward side of the tree islands along with increased growth for both species when they are planted in the higher fertilizer plots. This higher rate of growth and germination should correlate with increased temperatures on the leeward side during winter due to the thermal insulating effect of the increased snow depth on the leeward side.

Objectives

The objectives of this thesis were to find if:

1) Higher available nutrient levels change the physiology of species found in the sub arctic?
2) Different species react to nutrients more or less than other species? Does expected mycorrhizal status predict physiological response to nutrients?

3) *Pinus banksiana* can survive in Churchill, and does higher nutrients increase this survival?
4) the additional snow depth on the leeward side of tree islands increase the germination and growth of *Picea glauca* seedlings?

Methods

Fertilizer plots

In 2021, Dr. John Markham's lab created forty plots to examine the effects of fertilizer on plant species in the boreal tundra ecotone near Churchill, Manitoba. Plots were arranged in blocks containing each treatment, with 50 meters between plots. These blocks were replicated four times on the tundra, and six times in the forest. Each plot is a 200 m^2 circle, with each being given one of four fertilizer treatments: control, low, medium or high fertilizer (Table 1).

Table 1. The kg ha^{-1} of nitrogen and phosphorus of each fertilizer treatment. Nitrogen was added as urea and P was added as triple superphosphate. 16 plots were in Churchill's tundra, and 24 in the forest. Every four plots were organized into blocks, with four replicate blocks on the tundra and six blocks in the forest.

Treatment Level	Amount of Nitrogen (kg/ha)	Amount of Phosphorus (kg/ha)
Control	0	0
Low	10	2
Medium	50	10
High	100	20

Fertilizer plots conifer germination

Each fertilizer plot was seeded with *Picea glauca* and *Pinus banksiana* inoculated with one of three ectomycorrhizal fungi species, or not inoculated. Two *P. glauca* seed lots were chosen from a latitude similar to Churchill's to avoid any differences in growth, with *P. glauca* seedlot A and B coming from 58.258 °N and 58.653 °N respectively. As for *P. banksiana*, seedlot C came from 56.116 °N with seedlot D coming from 57.333 °N. Each set of seeds came from the National Tree Seed Center, with in-lab germination found to be 100% after, in the case of white spruce, seeds had been cold stratified for at least two weeks. *P. banksiana* is not found in the boreal sub arctic, as it requires a warmer climate to grow effectively and reproduce (Asselin et al., 2003). I planted *P. banksiana* to act as a comparison to the native white spruce seed and to determine if additional soil nutrients or tree islands would create a microhabitat that would allow them to germinate and grow past their current range. The original plan was to harvest a subset of the seedlings at the end of 2024’s growing season, but due to poor growth of the conifer seedlings I was only able to record germination rate.

To test the effect of fertilizer on mycorrhizae formation and their effect on plant performance, I inoculated each conifer seeding site with one of three ectomycorrhizal species, with one seeding site kept uninoculated to serve as a control (Figure 2).

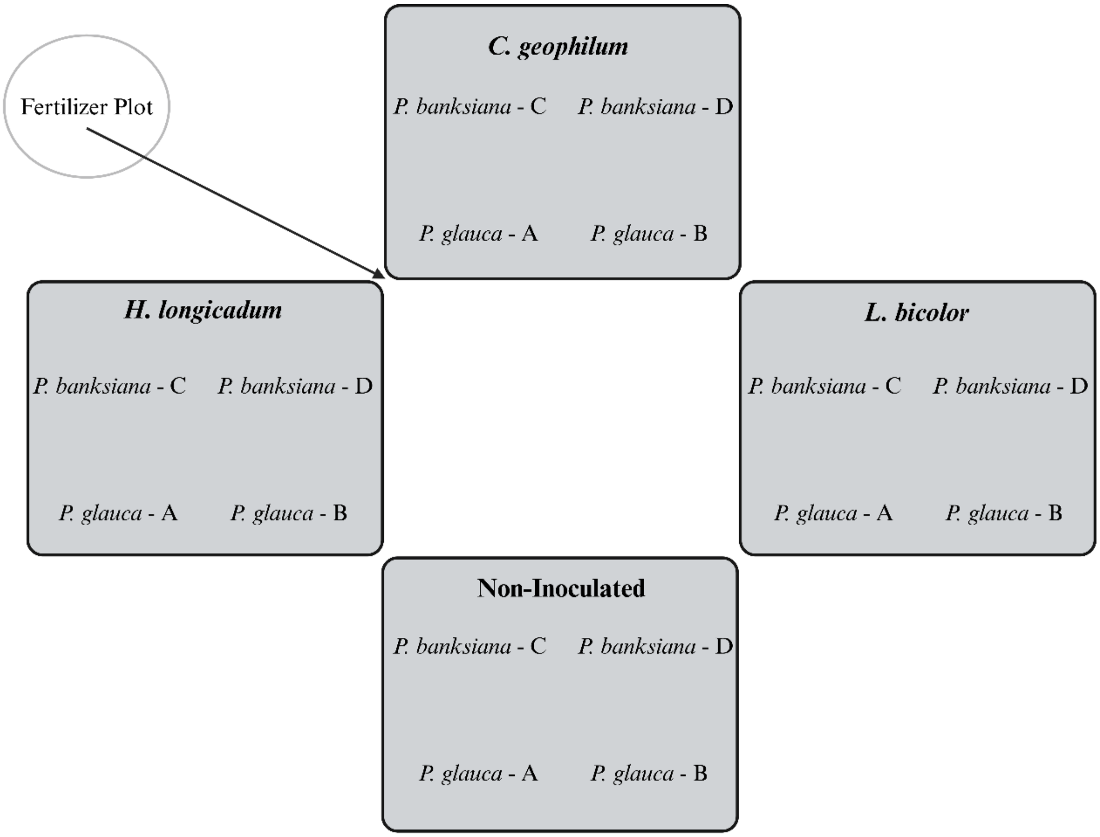

Figure 2. The layout of each seeding site for the conifer seeds planted inside each fertilizer plot. Mycorrhizal species is detailed for each seeding site, with the layout for each conifer species and seedlot displayed. Each seeding site contains 20 conifer seeds.

I planted 20 *Pinus banksiana* and 20 *Picea glauca* seeds from each seedlot into separate seeding sites inside each fertilizer plot, which were each supplemented with a mycorrhizal fungal treatment. At 0 and 8 weeks post-sowing, I inoculated each seeding site with one of three ectomycorrhizal fungal species: *Cenococcum geophilum* Fr., *Hebeloma longicadum* (Pers.) P.

Kumm*.,* and *Laccaria bicolor* (Maire) P.D. Orton., I chose these three fungal species as they are commonly found in white spruce-dominated forests, and all three ectomycorrhizal species used in the experiment provided by Dr. Leonard Hutchison from Lakehead University (Trofymow et al., 2020). Given that I planted these seeds in the field I was worried that if we adopted the traditional method of placing nutrient rich media agar inoculated with mycorrhizae, I may attract animals that could disturb our site. To solve this issue, instead I injected a mycelial slurry into each seeding site. This slurry was created by growing mycorrhizal cultures before moving them into liquid cultures (Repáč et al., 2011; Shimomura et al., 2012). Each of the three ectomycorrhizal species was grown on Modified Melin Norkrans media, with 0.2% Tween 80 added following Shimomura et al. (2012) and Vaario et al. (2002) to increase the rate at which mycorrhizae can associate with host plants and survive in the soil. These liquid cultures were suspended into a phosphate buffer and homogenized using a blender, with 10ml of each liquid culture injected 2 cm under the soil of each seeding site (AAT Bioquest, 2025). The conifer seeds planted into the non-inoculated seeding sites received 10ml of phosphate buffer, so each seed received the same amount of nutrients.

Tree Islands

I planted *Picea glauca* and *Pinus banksiana* seeds around tree islands on the tundra to determine how tree islands impact conifer germination and growth. To do so, I planted conifer seeds at eight tree island at two, five, and ten meters from the edge of the island on both the leeward and windward sides. At each of these distances I made six seeding sites on both the leeward and windward side for all eight tree islands. Overall, I created 36 seeding sites around each tree island. The windward side of each tree island was evident by abrasion on the tree stem. To reduce the human influence on each tree island, I chose islands that were at least 50 meters from roads and human structures. As well, each tree island chosen had emergent stems and were not clustered near other tree islands to ensure that snow retention would be consistent across each island. While the soil moisture level of each island was not measured, I chose tree islands that were 50 meters from wetlands and lakes. At 2 meters, 5 meters and 10 meters from the edge of the islands I planted three sets of 20 seeds of each species: one for *Cenococcum geophilum,* another for *Hebeloma longicadum,* and one more for a non-inoculated treatment. I planted 20 seeds of either *P. glauca* or *P. banksiana* in each of these seeding sites, with a total of 2,880 *P. glauca* and 2,880 *P. banksiana* seeds sown (Figure 3).

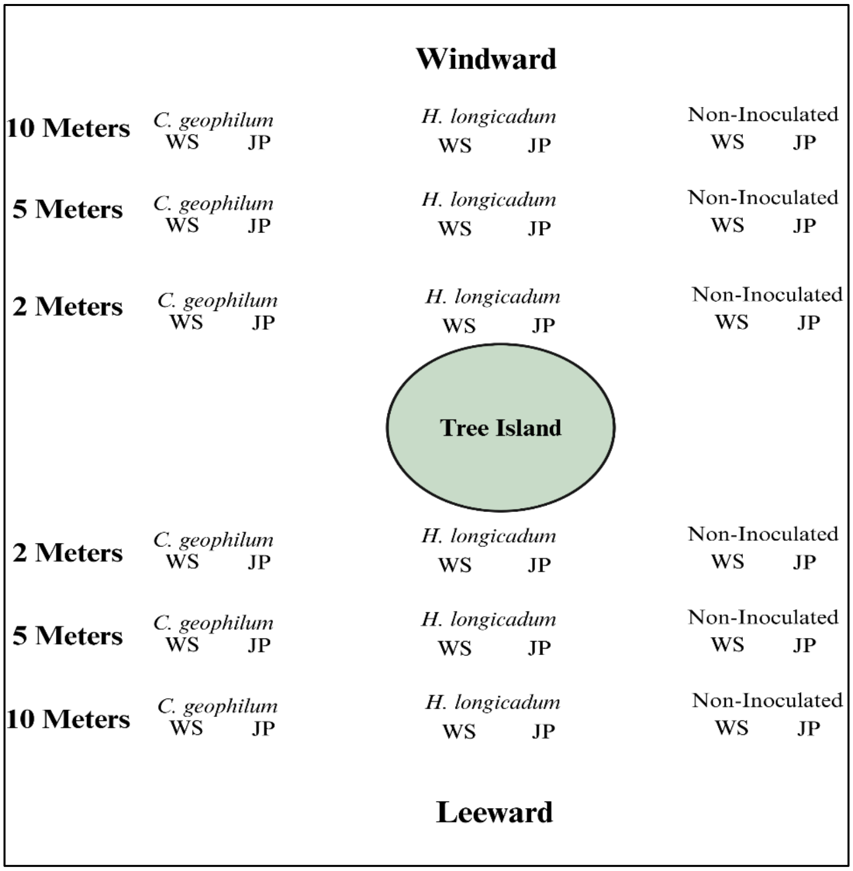

Figure 3. The layout for each seeding site around each tree island. At 2, 5 and 10 meters on both sides, there were 6 seeding sites. Each seeding site was inoculated with a different ectomycorrhizal species. WS corresponds to *Picea glauca* seeding sites and JP corresponds to *Pinus banksiana* seeding sites.

Seedlot B for *P. glauca* and seedlot D for *P. banksiana* were used for each of the seeding sites. These seeding sites were inoculated using the same 10 ml mycelial slurry as the fertilizer plots, with each seeding site inoculated 0 and 8 weeks post sowing. At the end of the 2024 growing season temperature probes were placed at each distance on both sides of the tree islands. This was done to determine the soil temperature around the tree islands during the winter. After 8 weeks, I measured the germination rate of each seeding site.

Species response to fertilizer

To determine if differing nutrient conditions will change the physiological responses of boreal plant species, the most abundant species in each fertilizer plot had their leaves sampled. To determine which species were most abundant, we recorded the cover of each species in each plot using a line intercept approach in 2023. Lines were run from the center of each plot in each cardinal direction, and in every one-meter section we recorded the presence of plant species that covered more than 10 cm in that segment (i.e., had more than 10 % cover). From this, we selected the three most abundant plant species in each fertilizer plot for sampling (Table 2).

Table 2. Number of plots each plant species occurred as a dominant species, and the mycorrhizal type each species associates with

	Forest					Tundra						
	Control	Low	Medium	High	Average	Control	Low	Medium	High	Average	Total Abundance	Mycorrhizal Status
Arctostaphylous alpina	2	1	2	2	1.75 ± 0.25	4	4	4	2	3.5 ± 0.5	21	Arbutoid
Betula glandulosa	1	2	3	1	1.75 ± 0.48	0	0	0	0	0	7	Arbuscular/Ecto
Empetrum nigrum	5	6	5	5	5.25 ± 0.25	1	0	0	0	0.25 ± 0.25	22	Ericoid
Ledum decumbens	4	4	4	5	4.25 ± 0.25	0	0	0	0	0	17	Ericoid
Shepherdia canadensis	3	3	4	3	3.25 ± 0.25	4	3	3	3	3.25 ± 0.25	26	Arbuscular/Ecto *N-Fixer
Vaccinium uliginosum	6	5	4	5	5.00 ± 0.41	2	1	0	1	1 ± 0.41	24	Ericoid
Picea glauca	6	6	6	6	6 ± 0	0	0	0	0	0	24	Ectomycorrhizae
Chamerion angustifolium	0	2	1	3	1.5 ± 0.65	0	0	0	4	1 ± 1	10	Arbuscular
Dryas integrifolia	0	0	0	1	0.25 ± 0.25	4	4	3	4	3.75 ± 0.25	16	Ectomycorrhizae
Salix reticulata	6	4	6	4	5.00 ± 0.58	1	1	0	1	0.75 ± 0.25	23	Arbuscular/Ecto
Draba alpina	0	0	0	0	0	2	3	2	2	2.25 ± 0.25	9	None
Lesquerella artica	0	0	0	0	0	0	2	3	3	2 ± 0.71	8	None

For each species we collected multiple samples of their newest leaves to compare the stable N isotope, C:N ratio, chlorophyll, and the specific leaf area. As every plant species was not in every fertilizer plot, there was a range of samples sizes for each species and treatment combination, between 7-26 samples for a species across the fertilizer plots. The fresh leaf samples were photographed, and I used. ImageJ to determine the leaf area from these records. Specific leaf area (SLA) was determined by dividing the leaf area found in ImageJ by the dry mass. SLA was not measured for *Picea glauca, Empetrum nigrum*, *Draba alpina* L., and *Lesquerella arctica* (Worms. ex Hornem.) S.Watson, since they do not produce flat leaves. Each leaf sample was then dried and weighed before being ground into a fine powder using a ball mill. Carbon and nitrogen isotope analyses were then performed by the University of Windsor Stable Isotope lab, to determine ∂^{15}N, and the C:N. Soil samples from each control fertilizer plot were taken and the urea used for our fertilizer treatments were also analyzed for stable nitrogen isotopes. The average ∂^{15}N of the urea used in our fertilizer treatments was -1.69. The average ∂^{15}N of the control plots soil was 0.87, with a minimum ∂^{15}N of -0.1, and a maximum level of 1.24.

The mycorrhizal status of each plant species was determined (Table 2) through reference to the following studies: Treu et al. (1995), Newsham et al. (2009), Baum et al. (2009), Dhillion (1994), Bledsoe et al. (1990), Chapin (1995) and Teste et al., (2020). Given that nitrogen fixing plants like *S. canadensis* can utilize a nitrogen pool that no other plant species sampled in the fertilizer plots has access to, I categorized *S. canadensis* by itself as a nitrogen fixer.

Unfertilized species physiology

Alongside the samples we collected from the dominant plant species in each fertilizer plot, I was given the ∂^{15}N data for various plant species found in unfertilized areas near the experimental plots that were collected previously by the Markham and Roth lab. The plant species sampled were: *Potentilla palustris* (L.) Scop.*, Betula glandulosa, Salix reticulata* L.*, Salix spp.*(various tall willow species in the upland habitat)*, Arctostaphylos alpina, Dryas integrifolia, Picea glauca, Empetrum nigrum, Ledum decumbens, Vaccinium uliginosum, Vaccinium vitis-idaea, Shepherdia canadensis, Elymus arenarius* L.„ *Equisetum variegatum* Schleich. ex F.Weber & D.Mohr.*, Salix lanata* L.*, Pyrola grandiflora* Radius*, Carex rupestris* All.*, Draba alpina, Eleocharis palustris* (L.) Roem. & Schult.*, Pinguicula vulgaris* L.*,* and *Scirpus caespitosus* Willd. Ex Kunth. *Bartsia alpina* L.was sampled, however, as it was hemiparasitic, and highly variable, having ∂^{15}N values between -7 and 0.9, it was removed. I added the ∂^{15}N data to the samples I collected from our control fertilizer treatment plots to get a more comprehensive data set for plant species a mycorrhizal types in the area.

Chlorophyll

Each dominant plant species in the fertilizer plots, and the tagged *Picea glauca* trees, had their chlorophyll content measured. Chlorophyll was measured by extracting pigments from 25 mg of powdered leaves in 10 ml of 100% ethanol and placing the solution in the dark for 24 hours. The solution was then centrifuged at 5,000 rpm for 5-10 minutes, with the supernatant's wavelength at 665nm and 650nm recorded using an Ultrospec 2100 Pro spectrophotometer. Both wavelengths were then used in Equation 1 to determine the total chlorophyll content in the leaf sample (Wintermon and De Mots, 1965).

$$\text{Chlorophyll Total} = (4 * 665_{\text{nm}}) + (25.5 * 650_{\text{nm}}) \qquad \text{(Equation 1)}$$

NDVI

Multispectral images of the fertilizer plots were created to determine the mean normalized difference vegetation index (NDVI) values of the whole plot and the individual species where they formed dominant patches within the plots. We used a Mavic 3M drone to photograph each fertilizer block at four wavelengths: green (560nm), red (650nm), red edge (730nm), and near-infrared (860nm). We flew the Mavic at a height of 30 meters above each fertilizer block, capturing multispectral images for each plot at a resolution of 1.5cm/pixel with an overlap of 70%. Each wavelength photo was stitched together into an orthomosiac using Pix4D and then imported into QGIS. NDVI values were found using the contrast between near infrared and red light that the vegetation in each fertilizer plot reflected (Beck et al., 2007) (Equation 2).

$$\text{NDVI} = (\text{Near Infrared} - \text{Red})/(\text{Near Infrared} + \text{Red}) \qquad \text{(Equation 2)}$$

The overall mean NDVI of each plot was found, along with the NDVI value of any plant species visually identified in the orthomosiac. For each fertilizer plot, I removed any bare ground in the tundra, and any lichen patches (*Cladonia* species) in the forest. This was done by filtering out any NDVI values below 0.2. While we sampled 12 total plant species in the forest and tundra fertilizer plots, due to difficulty in positively identifying plant species in each fertilizer plot, only five of the twelve plants had their NDVI values analyzed. These species were: *Picea glauca, Ledum decumbens, Dryas integrifolia, Vaccinium uliginosum,* and *Shepherdia canadensis.* Within plots, the individual NDVI of up to 18 patches of each species were measured where possible. Out of all the plant sampled throughout the fertilizer plots, these species were either extremely abundant and easy to spot on the orthomosiac, such as *D. integrifolia* or *P. glauca,* or, as in the case of the rest of the plant species, they had an easily noticeable shape or texture that allowed me to correctly identify them. Due to this, the sample size for some species, such as *P.*

glauca, given they were abundant and easy to identify in the orthomosaics*,* was far higher in the NDVI than in the isotope data set.

Limitations/Changes to proposed methods

The growth and germination rate of both conifer species was very low, with no buds found on any seedling by mid-August, when mature trees had formed buds. Given the lack of growth, we assumed that each conifer seedling only used the nutrients found in their seed instead of the soil and did not have enough time to associate with mycorrhizal fungi, so we did not harvest any seedlings for destructive analyses. We initially planned on determining mycorrhizal colonization and species inside each seedling's roots, along with the isotopic, chlorophyll and NDVI values of their needles. Instead, we recorded only the germination rate of each seeding site in the fertilizer plots and tree islands after 8 weeks post planting, with the germination analyzed to determine any differences. While germination may be affected by many factors, the low temperatures in Churchill during the 2024 growing season may have slowed germination and growth. When planted in June 2024, the average temperature was 7.4 °C, a decline from 2023, which may have impacted germination for our conifers. As shown in Figure 4., although Churchill's growing season steadily increased in average temperature, in 2024, June's average temperature was colder than the previous two years.

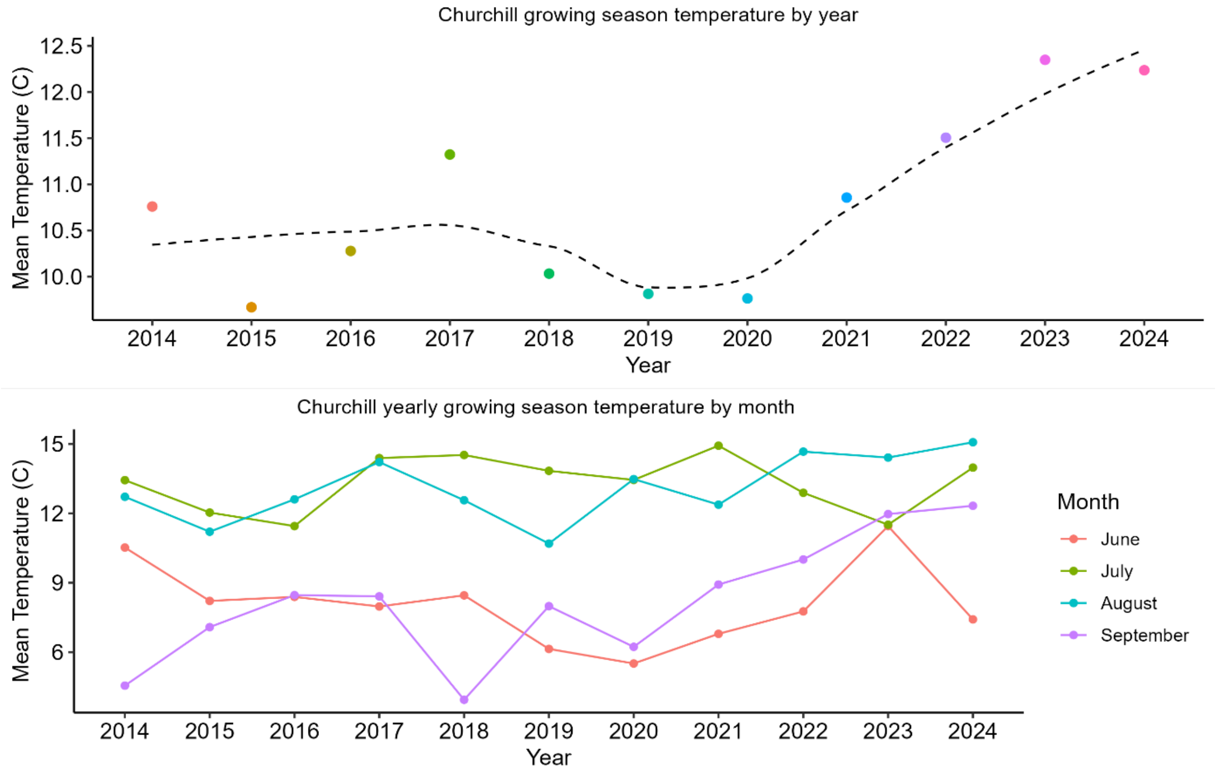

Figure 4. The average temperature during the Churchill growing season across the past eleven years. June 2024 temperatures were low, which may have slowed initial growth and germination for the conifer seeds.

This lack of growth was unexpected given that in order to survive the harsh winters present in the subarctic, conifer seedlings must form buds on their shoots (Wallin, 2018; Bigras & Colombo, 2001). Buds are vital to the success of conifers, where they help to safeguard the conifer from the winter and allow for the plant to create seed. The formation of buds on conifers correlates with seedlings beginning to form tolerance against freezing temperatures and indicates that they are exiting their initial growth stages. Once temperatures become low enough, a conifers bud will enter endodormacy, where they will only exit into bud burst once certain air temperatues are achieved (Sapkota et al., 2023; Sutinen et al., 2009). As the seedlings planted in both the fertilizer plots and tree islands did not form buds after eight weeks of growth, the survival rate of germinated seedlings is currently unknown. As shown in Figure 5., the conifer seedlings planted in the fertilizer plots and around the tree island did not form buds, nor produce true leaves.

Figure 5. The conifer seedlings in the fertilizer plots after 8 weeks of growth. The lack of true leaves and buds has made the survival of the *Picea glauca* and *Pinus banksiana* seedlings unclear.

While this is a significant issue, we will continue to monitor the growth of the conifer seedlings, with the next field expedition to Churchill planned in the summer of 2025. We will determine survival and growth rate of the conifer seedlings during the 2025 field season, and if possible, sample a subset of seedlings in each seeding site. If enough seedlings survive over the winter for an adequate number of samples, we will be able to determine whether additional soil nutrients are changing the seedlings isotopic and chlorophyll levels in their needles, along with the mycorrhizal density inside the roots. I will continue to support this experiment, but direct ownership and authorship for this experiment will be given over to Dr. John Markham and his lab.

Data Analysis/Models

Conifer germination in the fertilizer plots

Given the high amounts of ungerminated conifer seed in the fertilizer plots and around the tree islands, I used zero inflated poisson distributed general linear mixed models to analyze the conifer germination. To develop each GLMM, I used the glmmTMB package in R. Many different models were tested for the conifer germination, with non-zero inflated models utilizing various distributions such as gaussian, negative binomial and t-family. After various testing, fertilizer treatment (as a nominal variable), the conifer species, the mycorrhizal inoculant and seedlot were included as fixed independent variables. If I found any of these fixed effects to be non-significant, I dropped them and then included plot as a random effect to reduce variation in environmental conditions between plots. I also separated the conifer germination models, such that one model addressed germination in the forest plots and the other in the tundra plots. I did this to investigate the difference in germination between the two habitats and see if there was any difference in germination for either conifer species in the forest or tundra. I used the DHARMA package to test the model fit of each GLMM. Both models were analyzed using Anova type II. I will go into further detail for each model in the result section, but the general formula I made for analyzing conifer germination in the fertilizer plots is shown in Equation 3, where everything but plot number is a fixed effect.

Conifer Germination = Conifer Species + Fertilizer Treatment + Mycorrhizal Inoculant + Conifer Seedlot + (1|Plot Number)*

*(1|) indicates that factor is a random effect

(Equation 3)

For both models, I initially included an interaction term between fertilizer treatment and conifer species, but I dropped this as it gave me the same results as the model shown in Equation 3.

Conifer germination around the tree islands

I analyzed the germination around the tree islands similarly to the conifer germination in the fertilizer plots. I made a zero inflated generalized poisson distributed general linear mixed model, where I included which side of the tree islands that the seeding sites were located, and how far away the seeding sites were from the edge of the tree islands, the mycorrhizal inoculant and the conifer species as fixed effects. As I did not apply fertilizer around the tree islands, I did not include any interaction terms into the model. Similarly, in the fertilizer plot germination model I include tree island as a random effect to reduce variation between the tree islands if there were multiple sample replicates around the same tree island.

$$\text{Tree Island Germination} = \text{Tree Island Side} + \text{Seeding Site Distance} + \text{Conifer Species} + \text{Mycorrhizal Inoculant} + (1|\text{Island Number})$$

(Equation 4)

Unfertilized plant species ∂15N

I created a linear mixed model to determine if a plant species ∂^{15}N, when unfertilized, would be affected by mycorrhizal type, using data from the fertilizer plots, and from the surrounding area. I included the mycorrhizal type each host plant associated with as a fixed effect, and plant species as a random effect (Equation 5).

$$\partial^{15}\text{N (Unfertilized Plant Tissue)} = \text{Mycorrhizal Type} + (1|\text{Species})$$

(Equation 5)

Gaussian distribution was used in the linear mixed model analyzing $\partial^{15}N$ in the unfertilized plant species tissue. I then used an Anova type II test to determine model effects. I used the lme4 package in R to make each linear mixed model.

Effect of fertilizer on plant physiology

To analyze the physiology of the plant species sampled in the fertilizer plots, I created models with physiological variables (C:N, $\partial^{15}N$, and total chlorophyll), NDVI or SLA as a dependent variable, with plant species, the kg ha^{-1} of nitrogen added and the interactions between species and N added as independent variables. I did not run separate models for the forest and tundra habitats for the physiology variables. This was due to only having two species, *Arctostaphylos alpina* and *Shepherdia canadensis,* in the forest and tundra plots so I did not include habitat as an effect. I included habitat as a factor in the NDVI model, as I wished to investigate the difference in NDVI in the forest and tundra. Each of these models used a gaussian distribution and were analyzed with an Anova type III test. I will go in further depth for each of these models in the result section, but Equation 6 is the general formula I used for the physiology linear models.

$$\text{Dependent Variable} = \text{kg ha}^{-1}\text{ nitrogen added} + \text{Plant Species} + \text{kg ha}^{-1}\text{ N:Species Interaction} + (1|\text{Fertilizer Plot}) \qquad \text{(Equation 6)}$$

If any fixed effect was found to be non-significant, I dropped that factor and include plot as a random effect. This was true for each linear model besides the NDVI by species model. For the model investigating each plant species NDVI as fertilizer level increased, as there were multiple samples in the same fertilizer plot, I included plot as a random effect from the start.

To investigate how NDVI changed between the forest and tundra, I created another linear mixed model that included habitat as a factor. I included an interaction between the amount of N applied to the plot and habitat and included fertilizer block as a random effect as there were multiple plots in a block (Equation 7).

$$\text{Mean Fertilizer Plot NDVI} = \text{kg ha}^{-1}\text{ nitrogen added} + \text{Habitat} + \text{kg ha}^{-1}\text{ N:Habitat Interaction} + (1|\text{Fertilizer Block})$$

(Equation 7)

I made another linear model to investigate the relationship between cone production and chlorophyll content or tagged *Picea glauca* trees within the fertilizer plots that had their cones measured each year. For this model, I included the total chlorophyll content of each tagged trees needle as an independent fixed variable (Equation 8).

$$\text{2022 Mast Cone Number} = \text{Total Chlorophyll Content (A+B)}$$

(Equation 8)

This linear mixed model used a gaussian distribution and was analyzed using a type II Anova test. While the chlorophyll data of the tagged *Picea glauca* trees came from 2024, the number of cones each tagged *P. glauca* tree produced came from 2022. This was due to a lack of cone production in 2024, therefore I used the year when cones were last abundant (*i.e.,* 2022).

Due to low sample size, I included plant species sampled from outside the fertilizer plots, with these additional samples taken from the unfertilized plant species model. From the additional samples, *Vaccinium uliginosum, Arctostaphylos alpina,* and *Salix reticulata* only had 1-4 additional control samples added. However, for *Shepherdia canadensis* I added 28 additional samples in the forest and 14 additional control samples into the tundra. I tested the difference between the physiological variables between *S. canadensis* taken from the unfertilized areas and those sampled the control fertilizer plots in both the forest and tundra. From these linear models, I found no difference in the C:N, but ∂^{15}N levels were significantly different. I decided to include the data in the ∂^{15}N linear models as I wished to keep the sample size consistent across the models.

Mycorrhizal syndromes physiology

To look at how a plant's mycorrhizal status affected its response to fertilizer, models with mycorrhization type, the amount of fertilizer added, and their interaction as fixed effects and species as random effects were run. I tested species as a nested effect, where I found no difference between including species as a random and as a nested effect (Equation 9).

$$\text{Dependent Variable} = \text{kg ha}^{-1}\text{ nitrogen added} + \text{Mycorrhizal Syndrome} + \text{kg ha}^{-1}\text{ N:Mycorrhizal Interaction} + (1|\text{Species}) \qquad \text{(Equation 9)}$$

Post Hoc Tests

For each of the dominant plant species mycorrhizal models, I used emmeans in RStudio to produce pairwise comparisons for each species' dependent variable by their mycorrhizal type, to determine which mycorrhizal type was significantly different from one another. I also used emmeans to compare the germination of *Picea glauca* and *Pinus banksiana* in each of the seeding sites in the fertilizer plots and around the tree islands.

Results

Fertilizer plots conifer germination

Overall, germination in the fertilized plots was low with 9.5 ± 0.5% (mean ± SE) of the seeds germinating in each seeding site. Fertilizer level only significantly impacted conifer germination in the tundra, while conifer species significantly impacted germination in both the forest and tundra. The species of ectomycorrhizal fungi inoculated into each seeding site did not significantly impact conifer germination in both the forest and tundra. I found that the conifer seedlots impacted germination in the forest, but not in tundra (Table 3).

Table 3. Model effects for the fertilizer plot conifer germination factors. Both models were zero inflated. Inside each fertilizer plot, I planted 320 conifer seeds: 80 conifer seeds were inoculated with *C. geophilum, H. longcadium, L. bicolour* and non-inoculated. Across all 10 fertilizer blocks, a total of 800 conifer seeds were planted into each inoculant site.

Forest Fertilizer Plots	Chi-Square	Degrees of Freedom	P-Value
Feritlizer Treatments	0.311	3	0.957
Conifer Species	11.377	1	<0.001
Mycorrhizal Inoculant	2.215	3	0.528
Conifer Seedlot	5.414	1	0.019

Tundra Fertilizer Plots	Chi-Square	Degrees of Freedom	P-Value
Feritlizer Treatments	23.932	3	<0.001
Conifer Species	113.409	1	<0.001
Mycorrhizal Inoculant	0.628	3	0.889
Conifer Seedlot	3.039	1	0.081

Within the forest plots *Pinus banksiana* seedlot C had a germination rate of 5.75 ± 1.15% of seeds per seeding site while seedlot D had 7.80 ± 1.45% seeds germinated. *Picea glauca* seedlot A had 3.20 ± 0.80% germinations, while seedlot B had 5.10 ± 0.85%. *Pinus banksiana* seedlot D had higher germination than both *P. glauca* seedlots in the forest.

In the tundra, both species in the medium fertilizer treatment plots had the lowest level of seed germination. *Pinus banksiana* had significantly lower germination in the medium fertilizer treatment plots when compared to the low and high fertilizer plots. *Picea glauca* had significantly lower germination in the medium fertilizer plots compared to the control fertilizer plots (Figure 6).

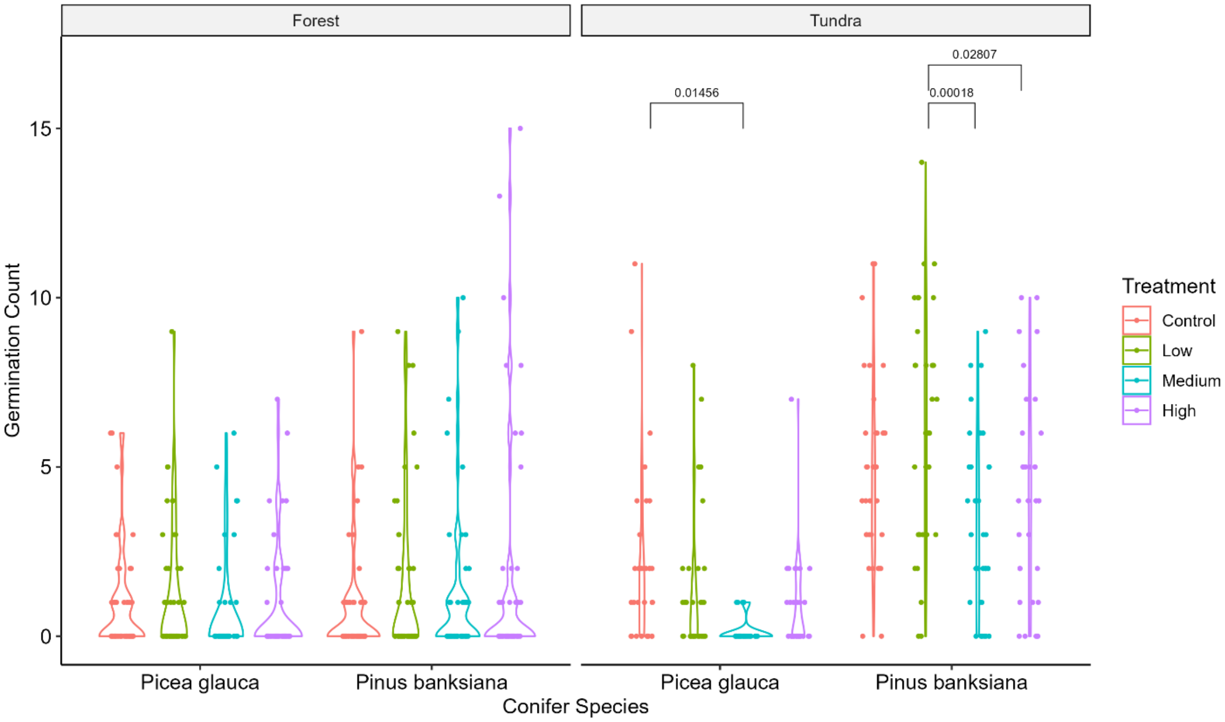

Figure 6. The germination of both conifer species in the forest and tundra fertilizer plots. The width of the line indicates how common an observation is, i.e. if 0 is the most common observation then it will be wider. In plots with a small number of germinated conifer seeds, then that line will be widest at 0.

Within the forest plots, *Picea glauca* had an average germination rate of 4.75 ± 1.1% in the control plots, 5.8 ± 1.4% in the low fertilizer plots, 3.2 ± 1.1% in the medium fertilizer plots, and 4.4 ± 1.2% in the high fertilizer plots. *Pinus banksiana* in the forest fertilizer plots had an average germination rate of 4.4 ± 1.3% in the control plots, 7.3 ± 1.7% in the low fertilizer plots, 6.5 ± 1.7% in the medium fertilizer plots, and 8.9 ± 2.6% in the high fertilizer plots. Within the tundra, *P. glauca* had an average germination rate of 11.0 ± 2.3% in the control plots, 7.8 ± 1.9%

in the low fertilizer plots, 0.6 ± 0.3% in the medium fertilizer plots and 4.2 ± 1.2% in the high fertilizer plots. *Pinus banksiana* in the tundra had an average germination rate of 24 ± 2.5% in the control plots, 30.5 ± 3.2% in the low fertilizer plots, 16.6 ± 2.4% in the medium fertilizer plots, and 20.8 ± 2.7% in the high fertilizer plots.

I found that in the tundra and the forest, *P. banksiana* germination was significantly higher than *P. glauca*. On average, in the tundra plots 53.9% of *P. glauca* seeding sites had no seeds germinate within them and 11.7% of *P. banksiana* seeding sites had no seeds germinate within them. Within the forest plots, 64.6% of *P. glauca* seeding sites had no seeds germinate within them and 58.3% of *P. banksiana* had no seeds germinate within them (Figure 7).

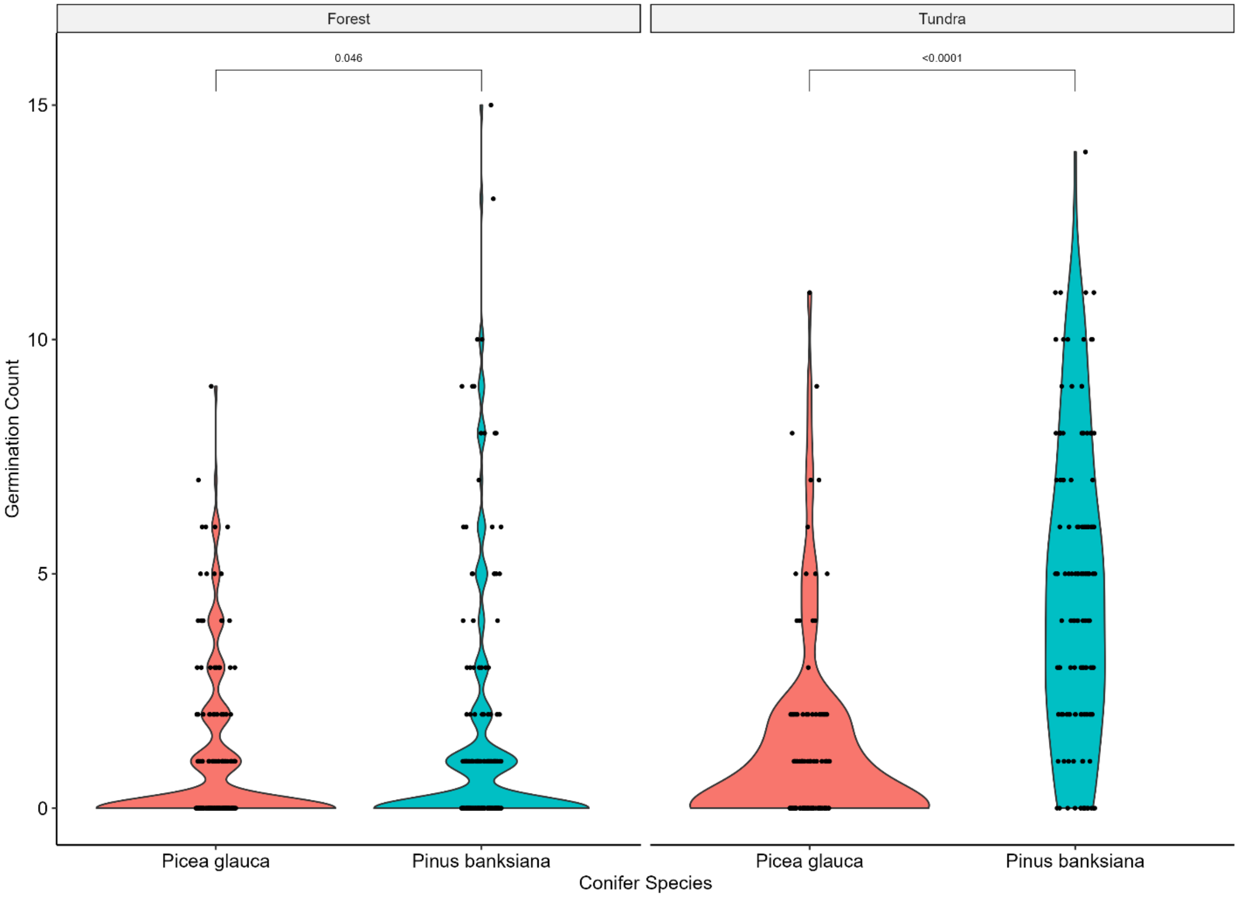

Figure 7. The germination of *Pinus banksiana* and *Picea glauca* in the forest and tundra. *P. banksiana* had on average, 74.32% higher germination than *P. glauca* in the tundra.

Tree island seed germination

I found that the tree island side (leeward or windward), how far away the seeding sites were from the edge of the tree island and the conifer species, impacted conifer germination. I also found the mycorrhizal inoculation treatment did not affect the germination of the conifers planted around the tree islands (Table 4).

Table 4. Model effects for the tree island conifer germination factors. Every tree island had 720 conifer seeds planted around it: 120 conifer seeds inoculated with *C. geophilum, H. longcadium* or left non-inoculated on both the leeward and windward sides. Across all eight tree islands, 960 conifer seeds were sown into one of the three types of inoculated seeding sites on both sides.

Tree Island Conifer Germination Factors	F-Value	Degrees of Freedom	P-Value
Tree Island Side	24.165	1	<0.001
Seeding Site Distance	47.794	2	<0.001
Conifer Species	5.708	1	0.017
Mycorrhizal Inoculant	4.878	2	0.087

When including mycorrhizal inoculant, tree island side, conifer species and seeding site distance there were no significant differences in germination between *Picea glauca* and *Pinus banksiana.* However, once mycorrhizal inoculant and tree island number were included as random effects the pairwise comparison showed a significant difference in the germination of *P. glauca* and *P. banksiana*. Across the tree islands, on average, 57.6% of *P. banksiana* seeding sites did not have any seed germinate and 68.8% of *P. glauca* seeding sites did not have any seed germinate (Figure 8).

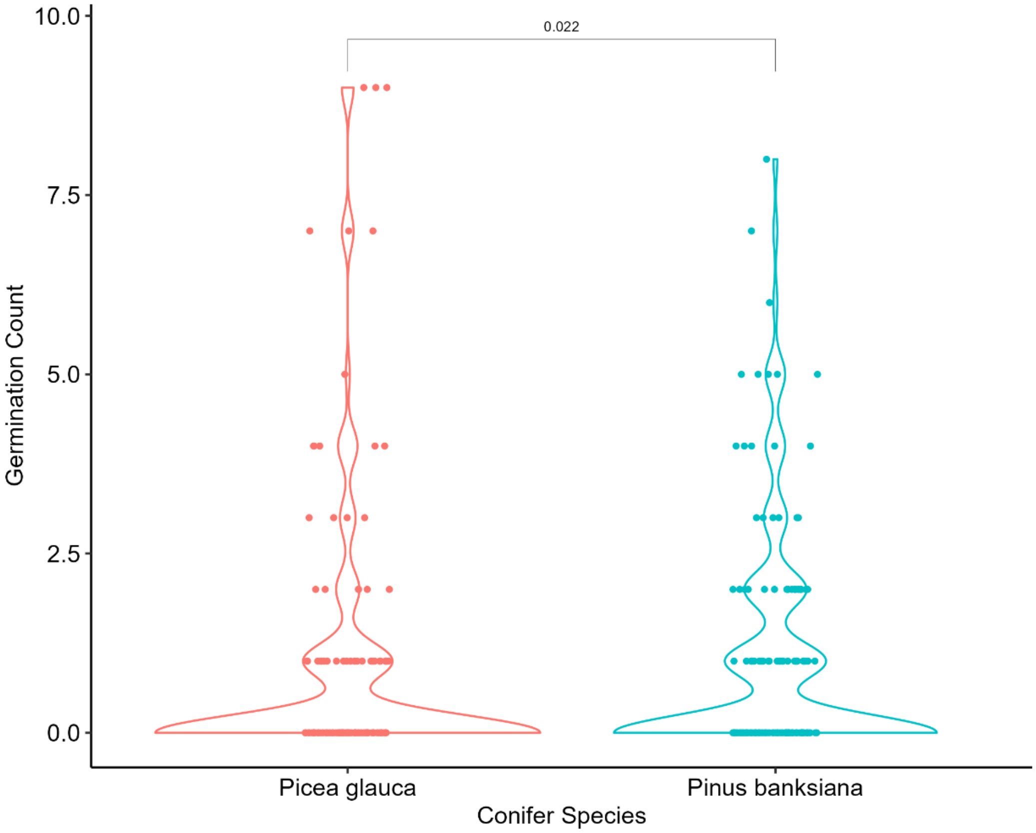

Figure 8. The germination of *Pinus banksiana* and *Picea glauca* across each of the tree islands. *Pinus banksiana* had on average, a 15.5% higher germination than *P. glauca.*

I found that the germination on the leeward side was significantly lower than the germination on the windward side of the tree islands. Furthermore, the windward seeding sites at 10 meters saw higher germination than that of the seeding sites at 2 and 5 meters (Table 5).

Table 5. Pairwise comparison effects of the tree island sides and distances

Tree Island Conifer Pairwise Comparison	Side	T-Ratio	P-Value
2 Meters - 5 Meters	Leeward	1.57	1
2 Meters - 10 Meters	Leeward	1.76	0.237
5 Meters - 10 Meters	Leeward	-0.196	0.354
2 Meters - 5 Meters	Windward	6.47	0.081
2 Meters - 10 Meters	Windward	4.25	<0.001
5 Meters - 10 Meters	Windward	-2.22	<0.001
Leeward - Windward		-3.52	<0.001

On the windward side, each seeding site 2 meters away had an average germination rate of 1.65 ± 0.50%, the seeding sites 5 meters away from the island had a germination rate of 5.20 ± 1.05%, and the seeding sites 10 meters away had an average rate of 11.95 ± 2.1%. The seeding sites 2 meters from the tree island on the leeward side had an average germination rate of 1.75 ± 0.6%, while the seeding sites 5 meters away had a germination rate of 2.1 ± 0.6%. The seeding sites 10 meters away on the leeward side had an average germination rate of 4.6 ± 1.05%. Across all distances and both conifer species, the windward side had 123% higher germination success than the leeward side (Figure 9).

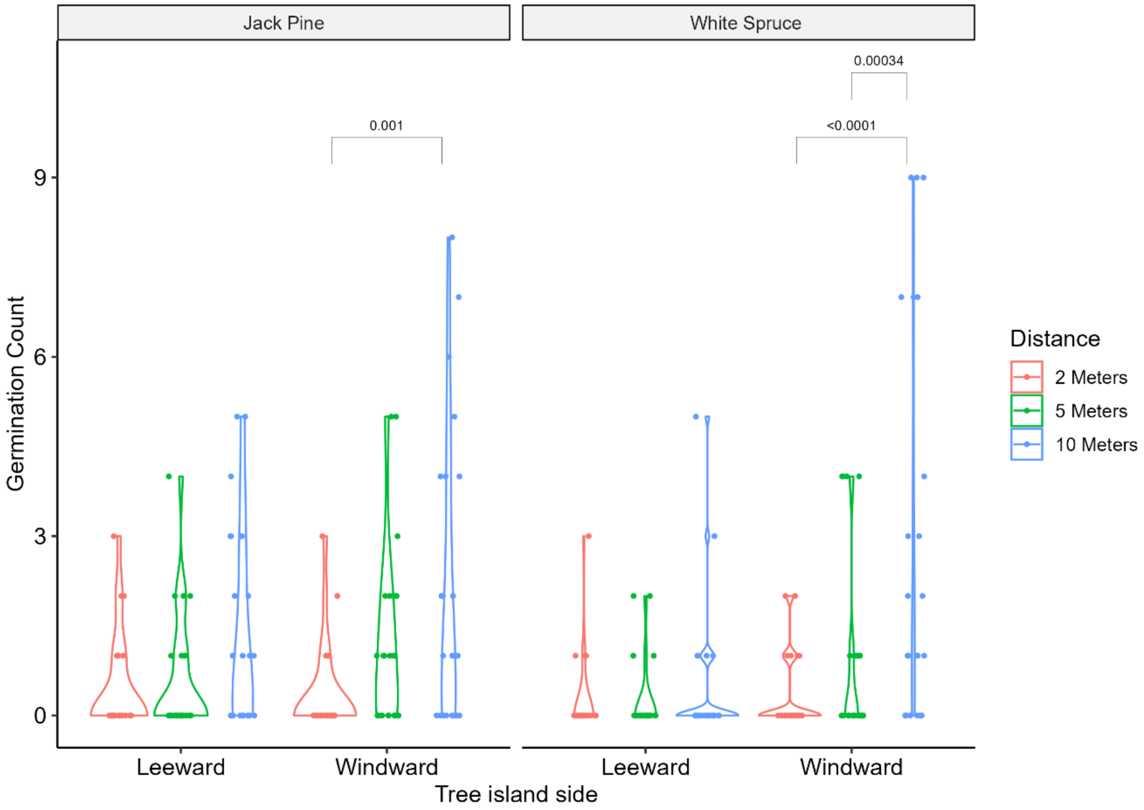

Figure 9. Germination of *Picea glauca* and *Pinus banksiana* at three distances away from the tree island, and on both sides of the tree island. On the windward side, *P. banksiana* had 500% higher average germination at 10 meters compared to germination at 2 meters. *Picea glauca* had a similar trend, where it had 970% higher average germination 10 meters away on the windward side than 2 meters away.

Unfertilized plant isotopic values

Using the formula described in Equation 8, I determined how mycorrhizal types impacted the ∂^{15}N levels within their host plants leaves. I found that mycorrhizal types ($\chi 2 = 46.169$, df = 6, p = < 0.001) significantly affected the ∂^{15}N leaf levels. Unfertilized boreal plant species generally had negative ∂^{15}N values in their leaves (Figure 10).

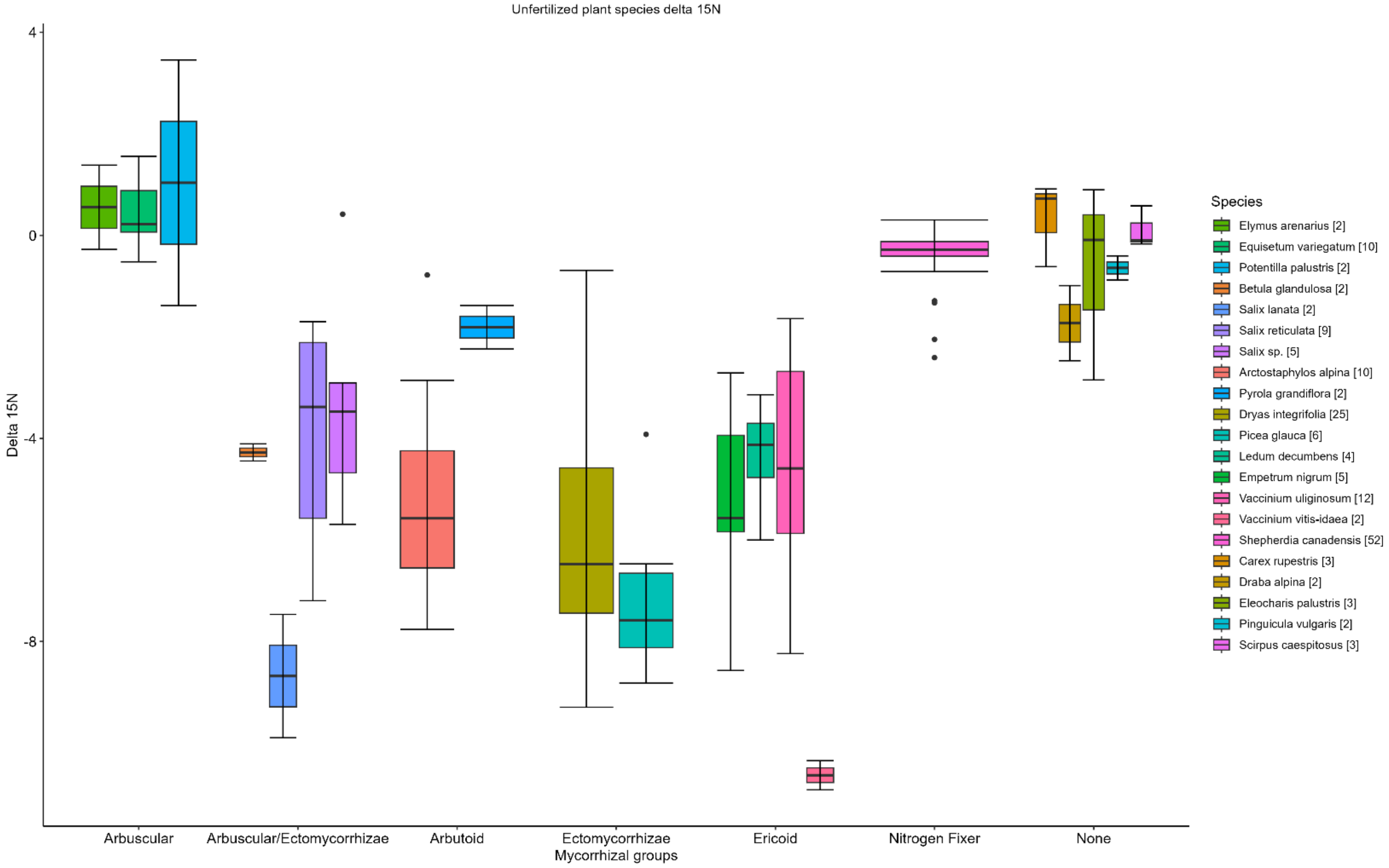

Figure 10. Delta ^{15}N values for unfertilized plant species in Churchill's boreal forest and tundra. Each species is labeled left to right, where the number to the right of the species in the legend corresponding to that species sample size.

The only mycorrhizal host plants that had, on average, ∂^{15}N values within their leaves close to 0 were those associated with arbuscular or dual mycorrhizae. While there were three other dual mycorrhizal host plants, *Shepherdia canadensis* was the only dual mycorrhizal (arbuscuclar/ectomcyorrhizal) host plant that also contained nitrogen fixing bacteria within it's roots. The only non-mycorrhizal plants with an average ∂^{15}N within their leaves that was above 0 were *Scirpus caespitosus* and *Carex rupestris*. All other plant species had an average ∂^{15}N within their leaves below 0.

I used pairwise comparison test to compare mycorrhizal types by the ∂^{15}N within their host plants leaves. Within this comparison, I included species as a random effect. From this pairwise comparison, I found differences in ∂^{15}N in plant species leaves, when grouped by their mycorrhizae (Figure 11).

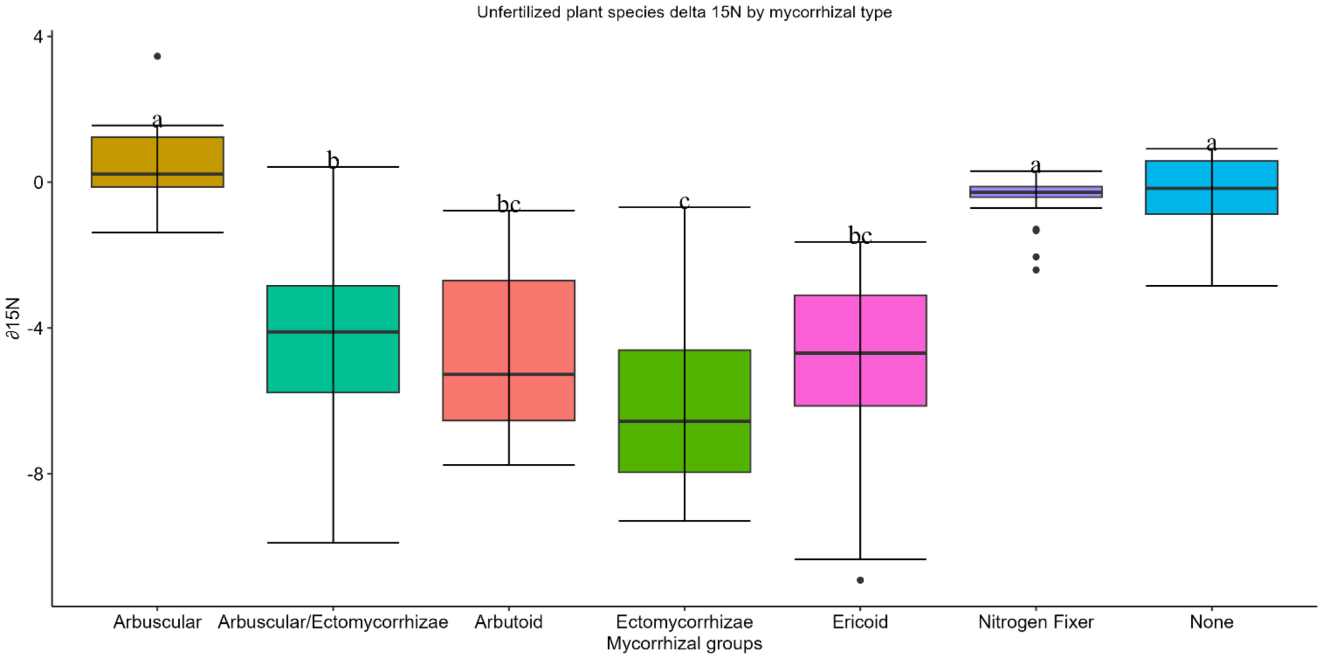

Figure 11. The ∂^{15}N levels for each plant species when grouped by their mycorrhizae. The host plants that associated with ericoid, ecto and arbutoid mycorrhizae had the lowest average $\partial^{15}N$. Arbuscular, non-mycorrhizal and *Shepherdia canadensis* had the highest average $\partial^{15}N$ levels within their leaves.

Arbuscular mycorrhizal host plants had an average ∂^{15}N of 0.51 ± 0.29 (mean ± SE), nitrogen fixing plants (*Shepherdia canadensis)* had an average ∂^{15}N of -0.34 ± 0.07 and non-mycorrhizal plants had an average ∂^{15}N of -0.42 ± 0.33. Dual (arbuscular/ectomycorrhizal) host plants had an average ∂^{15}N of -4.3 ± 0.56. Arbutoid host plants had an average ∂^{15}N of -4.7 ± 0.69, and ericoid host plants had an average $\partial^{15}N$ of -5.2 ± 0.51. Ectomycorrhizal host plants had an average $\partial^{15}N$ of -6.3 ± 0.37.

Fertilizer plot dominant species $\partial^{15}N$

Within the fertilizer plots, I found that $\partial^{15}N$ levels were significantly affected by the level of fertilizer, plant species, and the interaction between these two factors (Table 6).

Table 6. The model effects for the dominant species $\partial^{15}N$ model

Dominant Species $\partial^{15}N$ Factors	F-Value	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	11.499	1	<0.001
Plant Species	25.227	9	<0.001
Nitrogen added - Species Interaction	2.617	9	0.007

I performed individual regressions for each plant species comparing the species $\partial^{15}N$ versus the kg ha^{-1} of nitrogen applied to the plot. I found that *Arctostaphylos alpina, Dryas integrifolia, Ledum decumbens, Salix reticulata, Shepherdia canadensis* and *Picea glauca* significantly increased their $\partial^{15}N$ as the level of fertilizer increased. *Vaccinium uliginosum, Empetrum nigrum, Draba alpina* and *Lesquerella artica* did not change the $\partial^{15}N$ within their leaves due to fertilizer level (Figure 12).

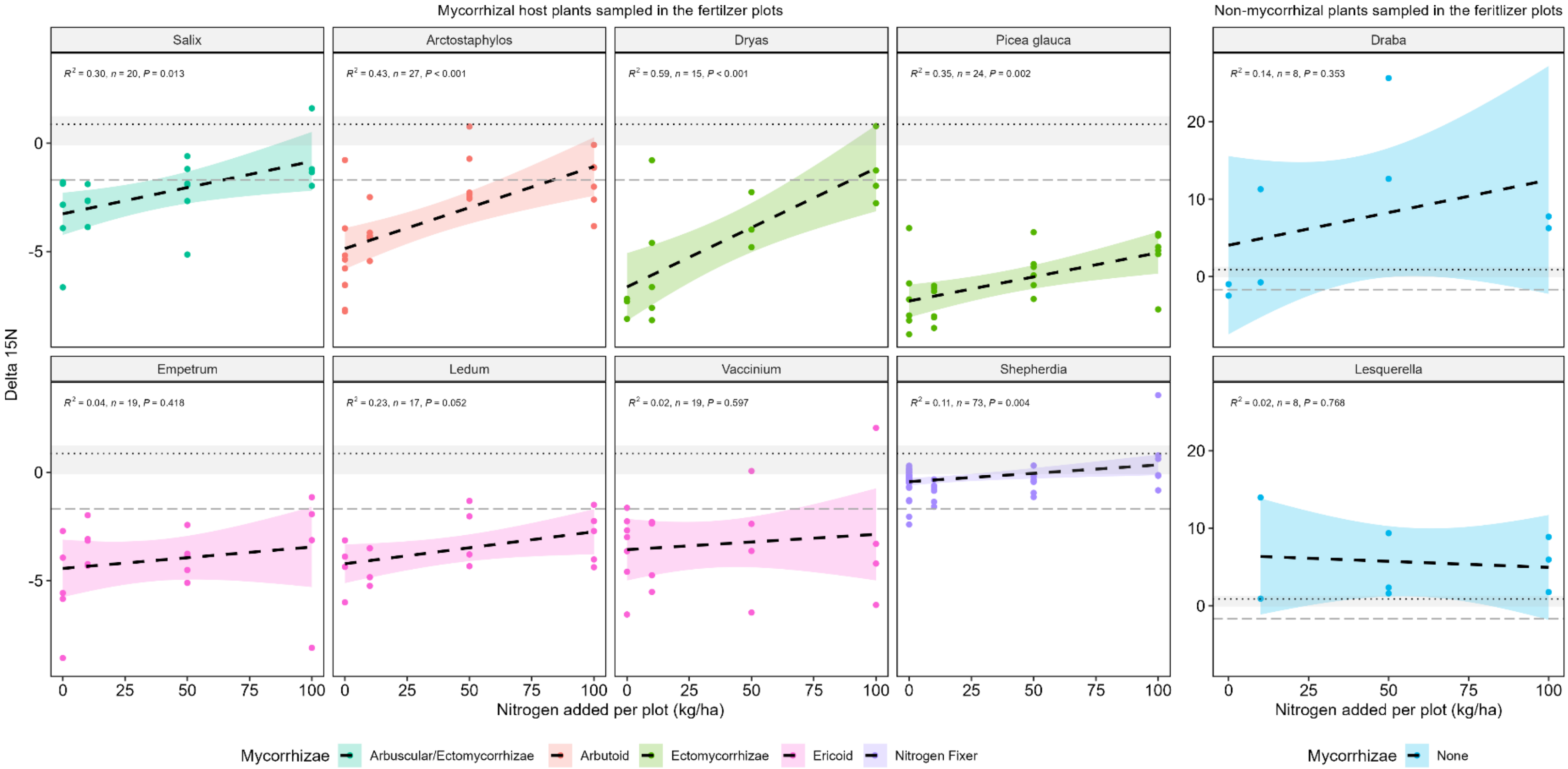

Figure 12. The *∂*^{15}*N* levels within each plant species leaves sampled from the feritlizer plots. The average *∂*^{15}*N* of the urea fertilizer at -1.69 is the light grey line, with the dark grey line being the average *∂*^{15}*N* in the control plots, at 0.87. The grey block represents the min and max *∂*^{15}*N* in the control plots. The non-mycorrhizal plants, *Shepherdia canadensis* and *Salix reticulata* were the only species in the feritlizer plots that had *∂*^{15}*N* levels in their leaves that went past the average *∂*^{15}*N* of the urea.

Dryas integrifolia had the strongest response to fertilizer. The ∂^{15}N within *D. integrifolia's* leaves increased 145.5% faster than *P. glauca*. The ∂^{15}N in *Picea glauca* needles increased 57% faster than *Empetrum nigrum* and *Ledum decumbens* due to fertilization. The ∂^{15}N inside *P. glauca's* needles increased 450% faster than *Vaccinium uliginosum*. Between *V. uliginosum, E. nigrum* and *L. decumbens*, the ∂^{15}N in *V. uliginosum* leaves changed 71.4% slower than *E. nigrum* and *L. decumbens.* The ∂^{15}N within *S. reticulata's* leaves increased by 78.2% between the control and high fertilizer plots. Only *Shepherdia canadensis* is a nitrogen fixing plant, and although this species can host dual mycorrhizae, these plants responded to fertilizer very differently. The average ∂^{15}N within *S. canadensis's* leaves was -0.32 ± 0.08 (mean ± SE), while the average ∂^{15}N within *S. reticulata's* leaves was -2.37 ± 0.38. The ∂^{15}N within *S. reticulata's* leaves also increased 68% faster than the ∂^{15}N within *S. canadensis's* leaves due to fertilization.

I created a model to determine if the mycorrhizal fungi a plant associates with would affect how leaf ∂^{15}N responds to fertilizer application, where I found that mycorrhizal syndrome was the only significant factor impacting ∂^{15}N levels (Equation 7, Table 7).

Table 7. The model effects for the mycorrhizal ∂^{15}N model

Mycorrhizal ∂^{15}N Factors	Chi-Square	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	3.041	1	0.081
Mycorrhizal Type	81.217	5	<0.001
Nitrogen added - Mycorrhizal Interaction	8.714	5	0.121
Random Effect - Species			

When grouped by mycorrhizal syndrome (aside from nitrogen fixing plants), arbuscular/ectomycorrhizae, arbutoid, ectomycorrhizae and nitrogen fixing plants significantly increased leaf ∂^{15}N due to increasing levels of nitrogen but ericoid mycorrhizal plants did not (Figure 13).

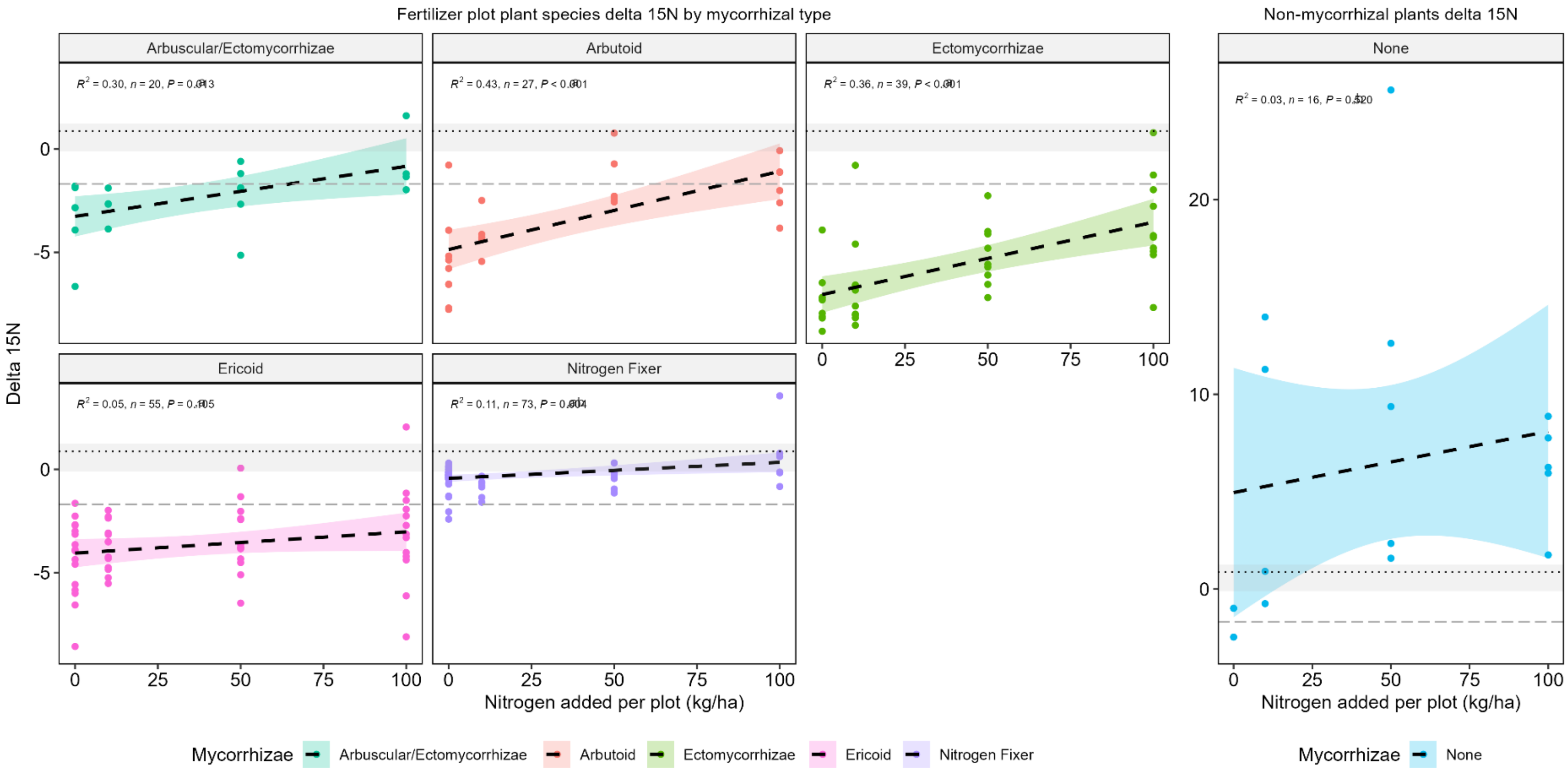

Figure 13. The ∂^{15}N of each pant species sampled in the fertilizer plots when grouped by their mycorrhizae. When grouped like this, the slope of each species is averaged across their mycorrhizal syndrome. This means that species like *Ledum decumbens,* that singificantly increased their *∂^{15}N* due to fertilization would not be singificant when grouped with other ericoid host plants. The letter after the *P* values for each mycorrhizal type corresponds to each syndromes compact letter display. This means that non-mycorrhizal plants have higher aerage ∂^{15}N in their leaves when compared to all other types besides nitrogen fixers.

I then conducted a pairwise comparison between the average ∂^{15}N in each plant species leaves when grouped by their myocrrhizae. I found that non-mycorrhizal host plants had significantly higher ∂^{15}N than ericoid, ectomycorrhizal, arbuscular/ectomcyorrhizal, and arbutoid host plants.

C:N Ratio

The amount of fertilizer added to the plots, plant species and their interaction significantly impacted the C:N ratio in the plant species leaves (Table 8).

Table 8. Model effects for the dominant species C:N ratio

Dominant Species C:N Factors	F-Value	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	27.858	1	<0.001
Plant Species	125.190	9	<0.001
Nitrogen added - Species Interaction	8.795	9	<0.001

Arctostaphylos alpina, Dryas integrifolia, Empetrum nigrum, and *Picea glauca* had the C:N ratios in their leaves lowered with increasing fertilizer. The only species that significantly lowered their C:N ratio and did not increase their ∂^{15}N values was *Empetrum nigrum*. This was also the only ericoid mycorrhizal host plant to have their C:N ratio respond to the fertilizer treatment, decreasing their average C:N ratio by half from control to high fertilizer treatments (Figure 14).

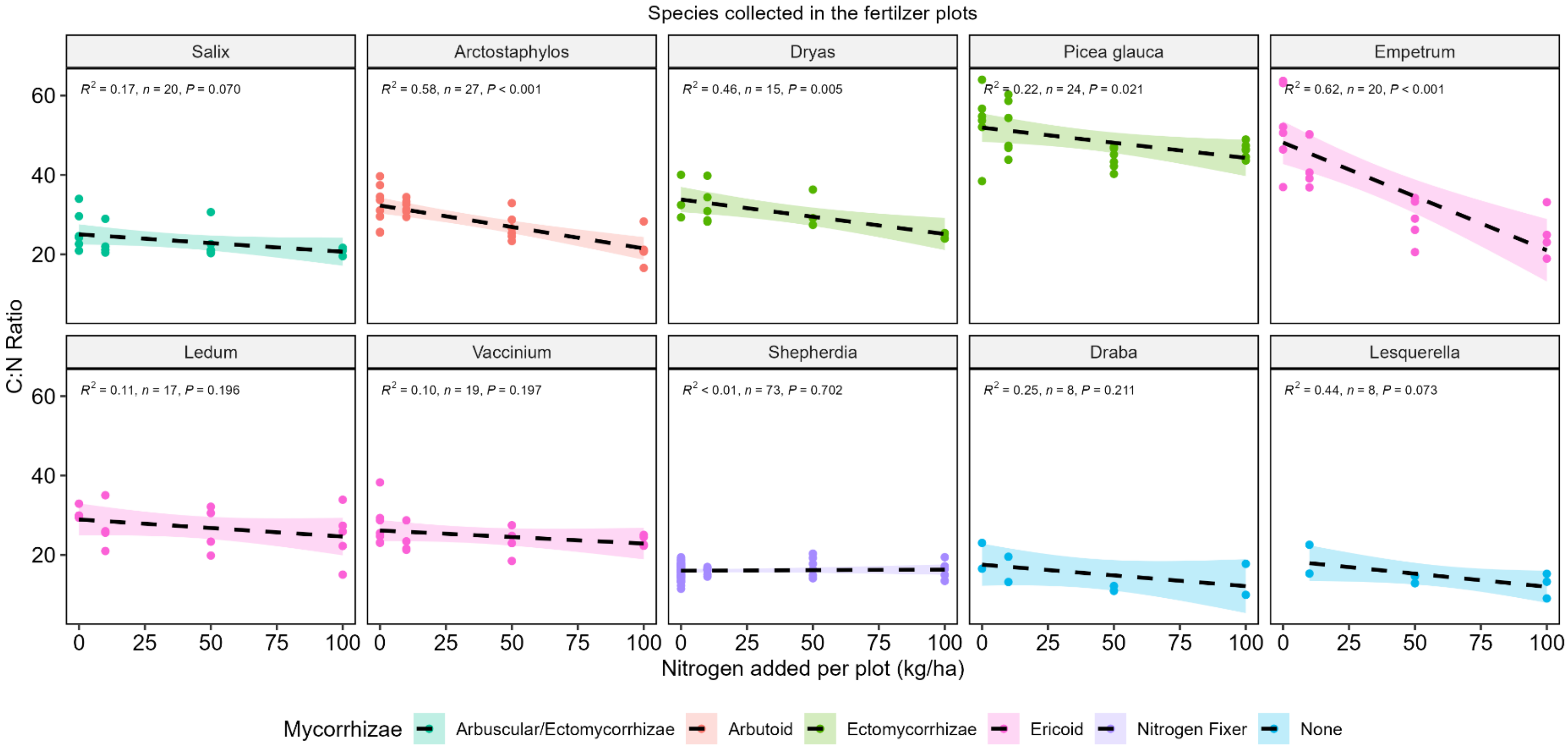

Figure 14. The C:N ratio within each species that was sampled from the fertilizer plots leaves. While *Salix reticulata* appeared to increase in $\partial^{15}N$ due to fertilization, it did not change in C:N. However, *Empetrum nigrum*, a species that did not see an increase in $\partial^{15}N$, lowered the C:N within its needles when grown in high feritlizer plots.

The C:N ratio within *Empetrum nigrum*'s needles changed the most due to fertilization, where *E. nigrum's* slopes changed 83% faster than *Ledum decumbens* and 91% faster than *Vaccinium uliginosum*. *Dryas integrifolia* and *Picea glauca* slopes were similar, where the C:N within *D. integrifolia's* leaves changed 8% faster than *Picea glauca*. *Arctostaphylos alpina's* slope changed 21% faster than *D. integrifolia* while *A. alpina's* slope changed 152% slower than *E. nigrum*.

I grouped each plant species by their mycorrhizae to find out if mycorrhizal syndrome would predict plant C:N ratio response to nutrients (Equation 7). I found that fertilizer level did significantly impact the C:N ratio of plant species, but it was dependent on mycorrhizal group (Table 9).

Table 9. Model effects for the C:N mycorrhizal model

Mycorrhizal C:N Factors	Chi-Square	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	2.3091	1	0.129
Mycorrhizal Type	12.644	5	0.027
Nitrogen added - Mycorrhizal Interaction	25.943	5	<0.001
Random Effect - Species			

When grouped by their mycorrhizae, arbutoid mycorrhizal, ericoid mycorrhizal, and non-mycorrhizal plants significantly lowered their C:N ratio. Ectomycorrhizal host plants and nitrogen fixing plants (*Shepherdia canadensis)* did not change the C:N within their leaves due to fertilization (Figure 15).

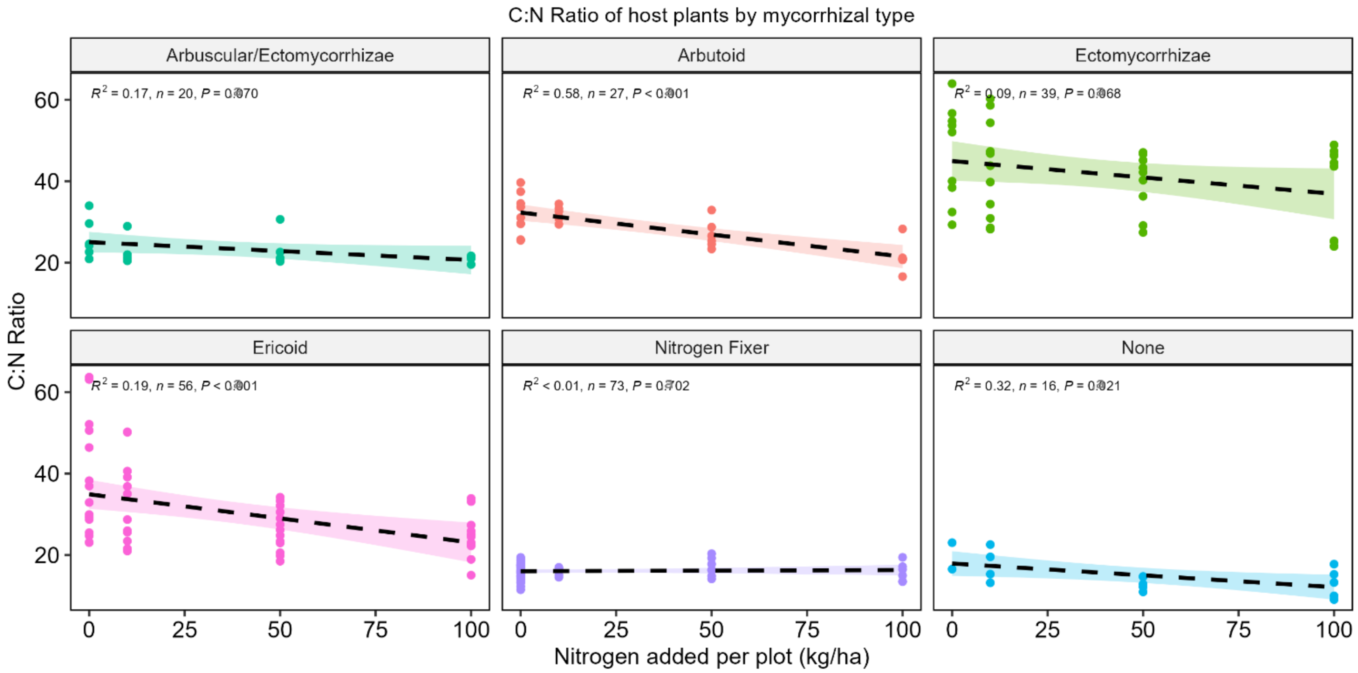

Figure 15. The C:N ratio within each plant species leaves when grouped by their mycorrhizae. When grouped together, ectomycorrhizal host plants have a non-significant change in C:N, while ericoid host plants see a significant change in C:N. This is due to how much *Empetrum nigrum* lowered the C:N ratio within its needles, and the variability in C:N between *Dryas integrifolia* and *Picea glauca.*

I then conducted pairwise comparisons to compare the C:N ratio among the mycorrhizal types. From this comparison, I found that there was no significant difference in the C:N ratio among the mycorrhizal types.

Chlorophyll

The amount of fertilizer added into each plot did significantly impact leaf chlorophyll, but it was dependent on plant species (Table 10).

Table 10. Model effects for chlorophyll in dominant species sampled in the fertilizer plots

Dominant Species Chlorophyll Factors	F-Value	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	2.198	1	0.140
Plant Species	14.219	9	<0.001
Nitrogen added - Species Interaction	2.771	9	0.005

When the effect of fertilizer on leaf chlorophyll was examined for each species separately, I found a significant effect on four of the ten species sampled. The total chlorophyll in *Dryas integrifolia, Picea glauca* and *Lesquerella artica* significantly increased due to fertilization. The total chlorophyll in *Vaccinium uliginosum's* needles significantly lowered as fertilizer level increased. The rest of the plant species chlorophyll levels did not significantly change as fertilizer levels increased (Figure 16).

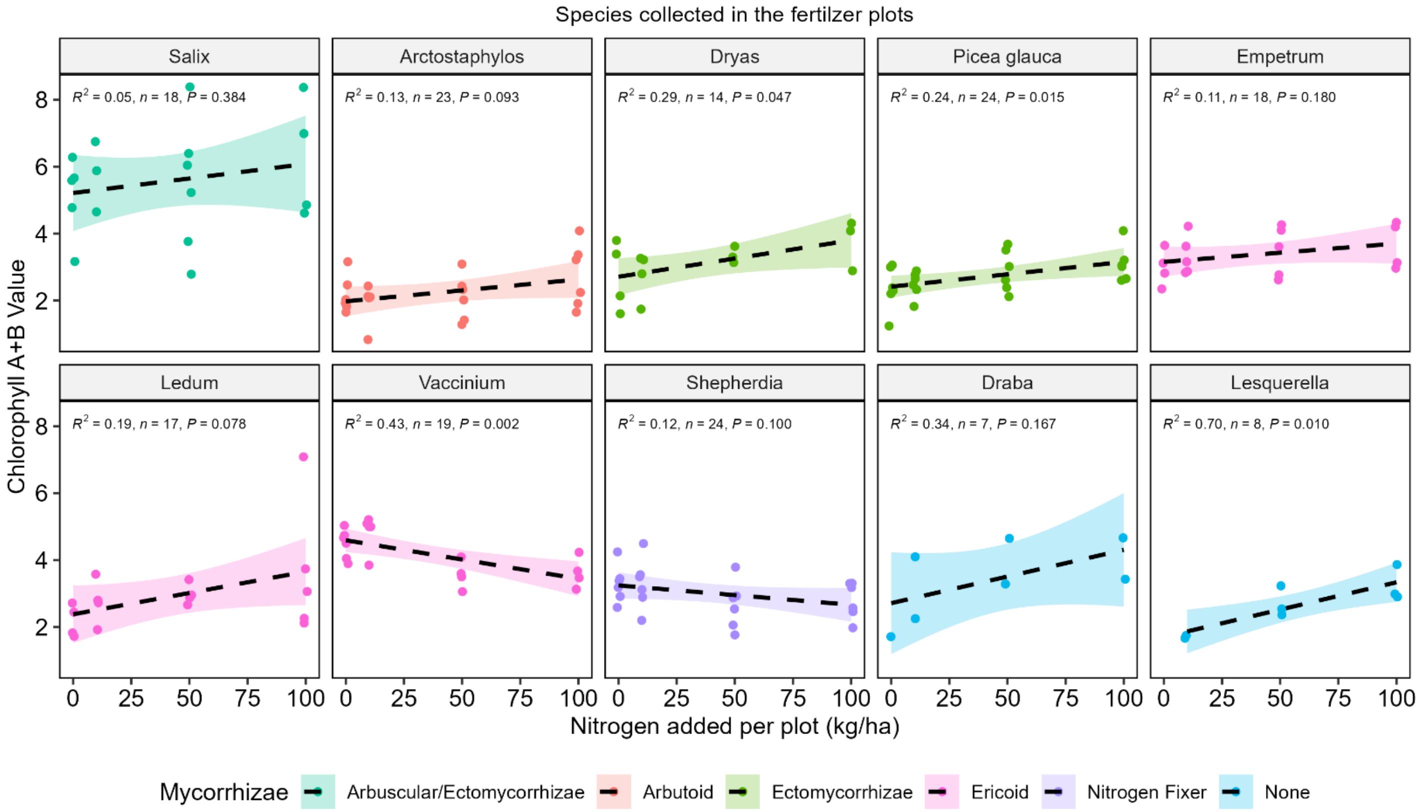

Figure 16. The total chlorophyll in each fertilizer plot leaves. *Picea glauca, Dryas integrifolia,* and *Lesquerella artica* significantly increased the chlorophyll within their leaves due to fertilization. *Vaccinium uliginosum* was the only species to have a significant decrease in chlorophyll due to increasing fertilizer level.

Lesquerella artica had the largest chlorophyll response to fertilization, where its slope increased 33% faster than *Dryas integrifolia* and 55% faster than *Picea glauca. Dryas integrifolia's* slope increased 32% faster than *P. glauca*. *Vaccinium uliginosum* was the only species that had a negative slope, where the average total chlorophyll within its needles decreased by 19% between the control and high fertilizer plots.

When samples species were grouped by their mycorrhizal status the effect of fertilizer on leaf chlorophyll was found to be dependent on mycorrhizal syndrome (Table 11).

Table 11. Model effects for the mycorrhizal chlorophyll model

Mycorrhizal Total Chlorophyll Factors	Chi-Square	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	2.586	1	0.108
Mycorrhizal Type	20.130	5	0.001
Nitrogen added - Mycorrhizal Interaction	11.478	5	0.043
Random Effect - Species			

Ectomycorrhizal host plants and non-mycorrhizal plants significantly increased the total chlorophyll within their leaves due to the addition of nitrogen in the soil. Ectomycorrhizal host plants, overall, increased the total chlorophyll within their leaves by 34.7%. Non-mycorrhizal plants increased the chlorophyll within their leaves by 108.7% between the control and high fertilizer plots (Figure 17). None of the other mycorrhizal types had their leaf chlorophyll affect by the fertilizer treatment.

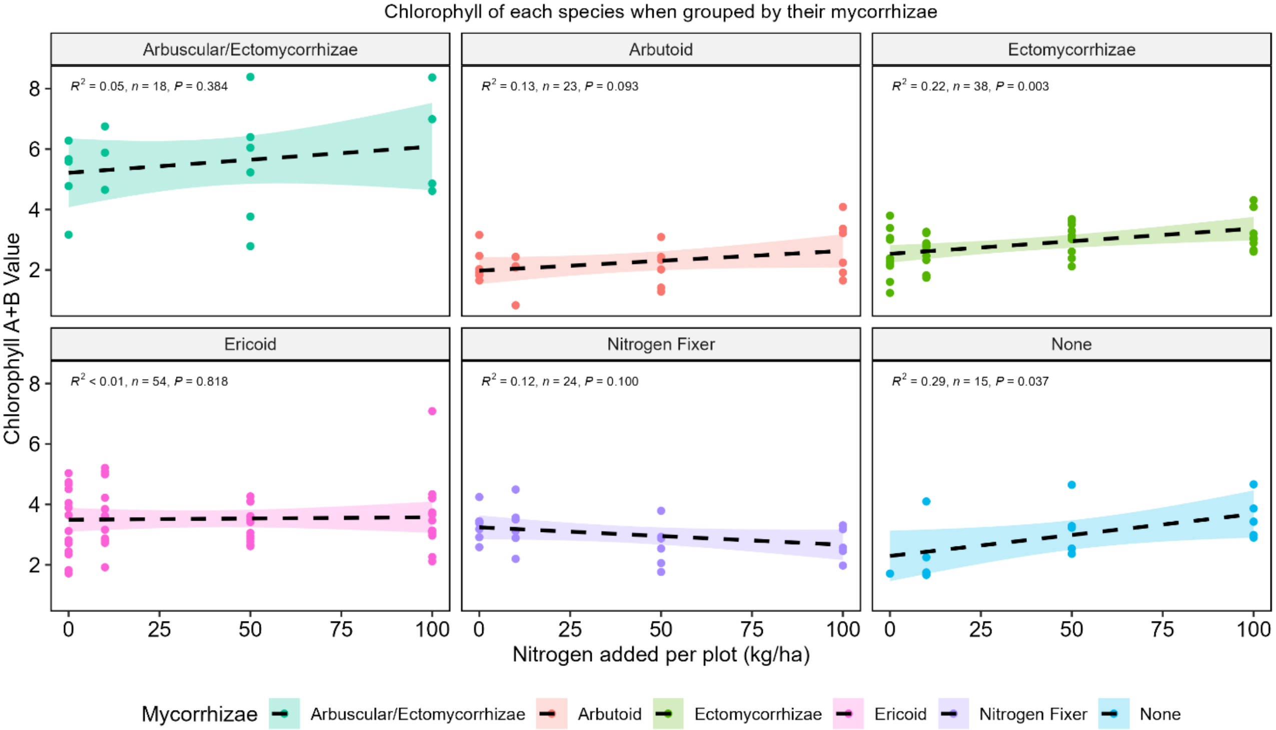

Figure 17. The total chlorophyll within each plant species leaves when grouped by their mycorrhizae. Given the grouping effect, non-mycorrhizal plants saw a significant increase in total chlorophyll due to fertilization when only *Lesquerella artica* had a significant effect.

After performing a pairwise comparison, I found that when each plant species was grouped by their mycorrhizae, there was no difference in among the mycorrhizal type in the leaf chlorophyll response to fertilizer.

To determine if an increase of chlorophyll would cause higher cone production, I compared the number of cones that each tagged *Picea glauca* tree had in 2022 (the last year with a successful cone crop) with their total chlorophyll content in 2024. I found that each tagged trees' total chlorophyll content was significantly correlated with the production of cones in 2022 (Figure 18).

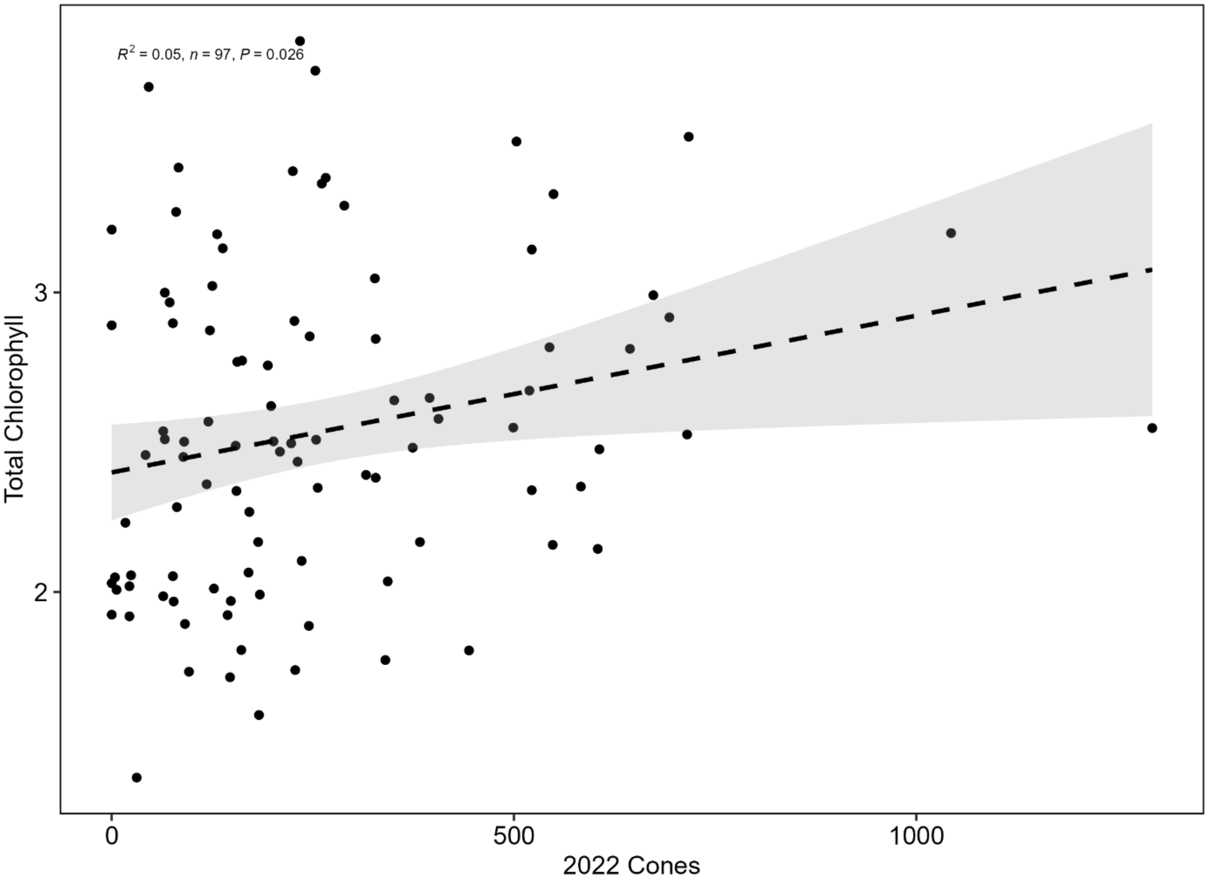

Figure 18. The total chlorophyll and cone numbers of each tagged *Picea glauca* tree in the fertilizer plots. While heavily varied, overall if *P. glauca* needles had high chlorophyll then that tree would produce higher amounts of cones.

Specific Leaf Area

Through the linear model described in Equation 6, I found that only plant species significantly affected the specific leaf area of plants species sampled in the fertilizer plots (Table 12).

Table 12. The model effects for each factor in the SLA model

Dominant Species SLA Factors	F-Value	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	0.001	1	0.977
Plant Species	13.633	5	<0.001
Nitrogen added - Species Interaction	1.634	5	0.158

I then compared each plant species specific leaf area as nitrogen in the plots increased. Through these individual models, I found that no plant species changed their SLA due to the fertilization treatments (Figure 19).

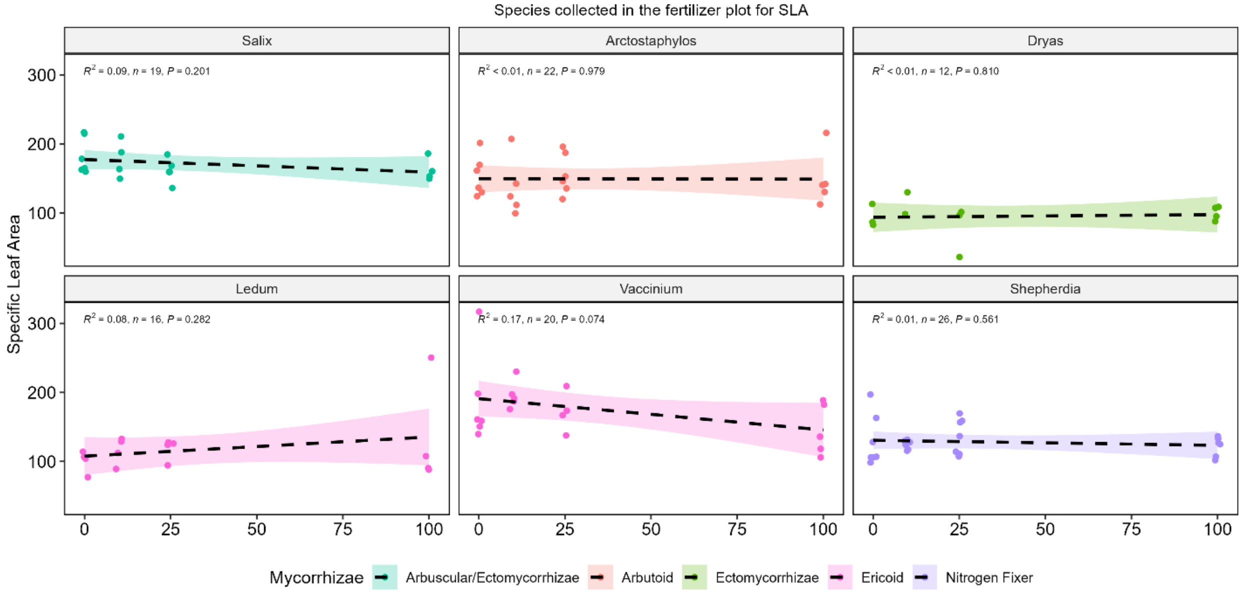

Figure 19. The specific leaf area for some of the plant species sampled in the fertilizer plots. There was not a significant change in the SLA of each species due to fertilization.

NDVI

Using the linear mixed model described in Equation 7, I was able to find that fertilizer level, habitat and the interaction between these two factors significantly impacted the mean NDVI of each fertilizer plot (Table 13).

Table 13. Model effects of NDVI linear mixed model comparing habitat

Fertilizer Plot NDVI Factors	Chi-Square	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	12.692	1	0.004
Habitat	91.802	1	<0.001
Nitrogen added - Habitat Interaction	6.593	1	0.010
Random Effect - Fertilizer Block			

I found that the forest and tundra fertilizer plots saw a significant increase in average NDVI values due to increasing fertilizer. Within the forest, the mean NDVI of the forest plots increased from 0.72 to 0.77 when 100 kg ha^{-1} of nitrogen was introduced into the soil. As for the tundra, the control plots had an average NDVI of 0.49, and the high fertilizer plots had an average NDVI of 0.58 (Figure 20).

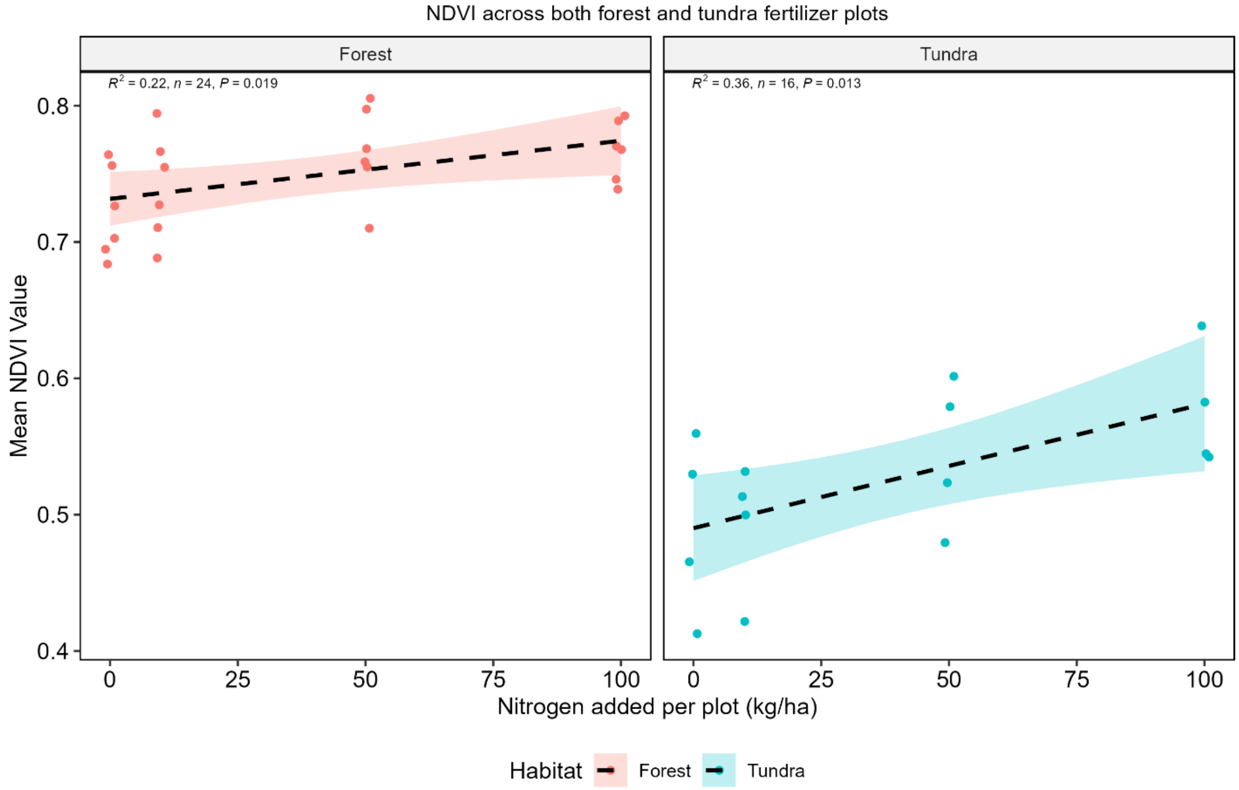

Figure 20. The overall NDVI values for each plot in the forest and tundra. Both habitat's plots were significantly increased by the fertilizer treatments, with the forest having a 7% increase in NDVI while the tundra increased in overall NDVI values by 17%.

I also found that while the forest plots had higher average NDVI ($T = 9.237$, df = 8, $p = <0.001$) the tundra plots increased their average NDVI 114% faster than the forest plots when supplied with the same amount of fertilizer.

Using the model described in Equation 6, I determined that the amount of fertilizer added into the soil, and plant species significantly impacted the NDVI of 5 of the dominant plant species sampled in the fertilizer plots (Table 14).

Table 14. Model effects for the plant species NDVI in the fertilizer plots

Dominant Species NDVI Factors	F-Value	Degrees of Freedom	P-Value
Amount of nitrogen added (kg/ha)	24.952	1	<0.001
Plant Species	462.861	4	<0.001
Nitrogen added - Species Interaction	5.017	4	0.286
Random Effect - Plot			

I found that in the forest and the tundra every plant species significantly increased their NDVI due to the fertilization treatments (Figure 21).

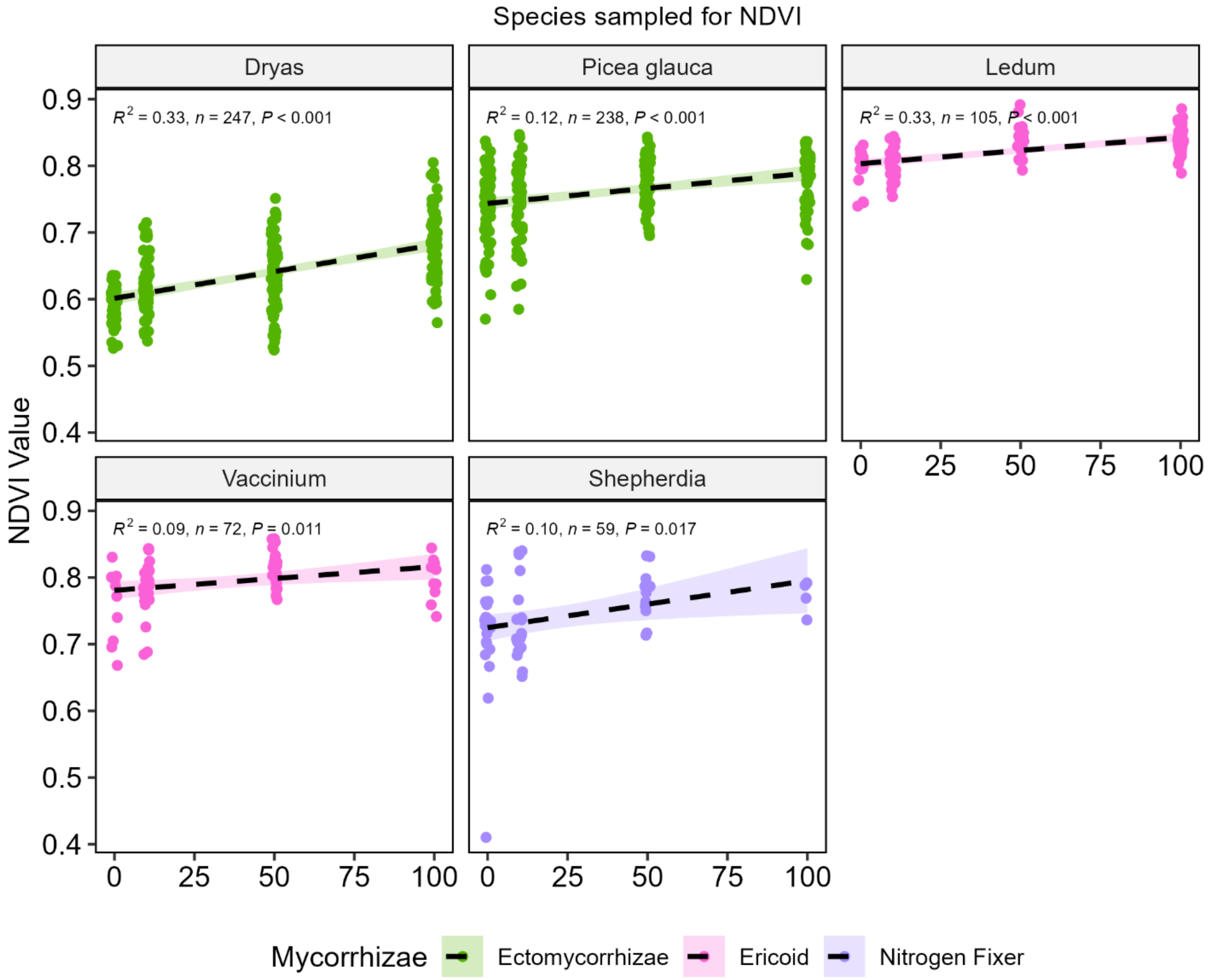

Figure 21. The NDVI values found for each plant species identified in fertilizer plot orthomosaics. As each species was found to increase their NDVI values in presence of additional nitrogen, it appears that boreal plants are overall becoming greener in the presence of additional nutrients.

Discussion

Fertilizer plot conifer germination

I found that neither the mycorrhizal inoculation nor the amount of fertilizer in each plot impacted the seedling recruitment of *Picea glauca* or *Pinus banksiana*. This lack of influence of mycorrhizal inoculation is most likely due to our seedlings failing to grow to the point where mycorrhizae could infect their roots. However, mycorrhizae should begin to affect the conifer seedlings growth rate once they exit their early stage of development. The soil in our sites is likely heavily populated by mycorrhizal fungi, since their host plants are present. Heavily disturbed northern boreal sites may have lower abundance of mycorrhizal fungi. For instance, Trofymow et al. (2016) found that soil from oil sands reclamation sites had half the ectomycorrhizal species richness of a nearby undisturbed forest. Given this high level of fungal richness, and that I inoculated almost every seeding site with an ectomycorrhizal species, it can be assumed that there was adequate mycorrhizal presence in the soil (Tedersoo et al., 2012; Shimomura et al., 2012). While I found that the medium fertilizer treatment in the tundra significantly decreased the germination of *P. banksiana* and *P. glauca*, I do not believe that an increase in nitrogen decreased conifer germination. The germination of alpine and arctic species is not affected by nitrogen fertilization (Milbau et al., 2017). *Betula nana, Solidago virgaurea* L. and *Vaccinium myrtillus* L.*,* all of which are plant species present in the subarctic, were grown by Milbau et al. (2017) in soil with additional 14 kg ha^{-1} N per year. While fertilization improved seedling establishment in *B. nana*, it had no effect on *S. virgaurea* establishment and reduced seedling establishment in *V. myrtillus*.

In our fertilizer plots, *Pinus banksiana,* a conifer not native to Churchill, was capable of surviving and germinating in Churchill's boreal tundra ecotone. *P. banksiana* is found throughout the boreal forest of North America and can grow and survive in areas that are frequented by wildfires. As wildfires remove the established species in an area, *P. banksiana* recovers faster than other species, which allows it to establish itself in the aftermath (Asselin et al., 2003; Carroll & Bliss, 1982). Furthermore *P. banksiana* has been seen to germinate even in environments with a temperature between -0.5°C and 15°C, with the range of *P. banksiana* being limited by fire frequency rather than temperature (Asselin et al., 2003; Haavisto & Winston, 1974; Wang & Lechowicz, 1998). As *P. banksiana* is capable of germinating in a wide range of environments, the high amount of *P. banksiana* germinating in our fertilizer plots may not tell the full story of their survival in Churchill. While the germination rate of *P. banksiana* during the initial growing season was higher than *Picea glauca*, their growth and survival rates after one of Churchill's winters may showcase how effective *P. banksiana* is at establishing itself in Churchill's boreal forest and tundra. It could be that the high germination rate of *P. banksiana* may occur, with an equally high number of *P. banksiana* seedlings dying off in the winter season. An increase in temperature has been seen to cause increased germination in boreal species, but seedling mortality increases as well after initial germination (Milabu et al., 2017; Angell & Kielland, 2009). This increased mortality rate may not be due to the temperature increase, but instead competition between seedlings. *Pinus banksiana's* high germination rate could cause a high mortality rate as seedlings begin to compete for light and nutrients. As the *P. banksiana* seedlings in the fertilizer plots only began to leave their initial growth stages, the behavior of *P. banksiana* or *P. glauca* cannot be assumed currently, but Dr. Markham's lab will continue to monitor and document the growth and survival of both conifer species. As a result of my current studies, we now know that *P. banksiana* is capable of germinating in Churchill's tundra ecotone, and that it does germinate in higher numbers than the native *P. glauca* during the initial growing season.

Tree island conifer germination

I predicted that the leeward side would evidence increased germination and survival of *P. banksiana* and *P. glauca* conifers close to the tree island. Contrary to my predictions, however, the windward side at farther distances instead saw the highest rate of germination for both conifer species. As in the fertilizer plots, the mycorrhizal species inoculated into each seeding site had no effect on the germination rate of either conifer species. Unlike the tundra fertilizer plots however, *Pinus banksiana* did not germinate in higher amounts than *Picea glauca.* Given that the windward side of the islands should be buffeted by harsher winds and have comparatively lower nutrient and water access to the leeward side, this high rate of germination was unexpected. Albertsen et al., (2014) showed the microclimate created by tree islands can influence the germination of conifer seeds. Out of the 12 tree islands Albertsen et al., (2014) studied, eight tree islands had seedlings around them, implying they were expanding onto the tundra. They found that *P. glauca* trees on the windward side of the island steadily increased their height while seedlings/saplings and other boreal species germinated on the islands' leeward side. Furthermore, they found that moss, *Vaccinium uliginosum*, *Andromeda polifolia* L., *Salix lanata* L., and *Vaccinium vitis-idaea* cover increased on the leeward side, compared to the open tundra. Fitzpatrick et al., (2020) also found snowfall was greater on the islands' leeward side of tree islands; snowfall precipitation was associated with increased *P. glauca* seedling density on the leeward side. Holtmeier & Broll (1992) further emphasized that tree islands' leeward size has increased soil moisture compared to their windward side, with this difference being particularly evident during dry weather conditions. Mamet & Kershaw, (2013) and Mamet et al., (2015) also concluded that tree islands accumulate snow fall due to their shape, causing increased leeward soil moisture and nutrient availability. Given that each of these studies concluded that being closer to the tree islands' leeward sides creates microhabitat that enhance seedling establishment onto the tundra, I predicted that *P. glauca* and *P. banksiana* seeds would germinate in higher numbers on the leeward side. As conifer germination was significantly lower on the leeward side it appears that there are other factors that cause both *P. glauca* and *P. banksiana* to germinate in higher amounts on the windward side of the tree islands.

While Fitzpatrick et al., (2020) did not ultimately find any significant difference in the *Picea glauca* expansion between sides, they found that snow fall accumulates closer to the leeward side. How far the microhabitat created on the leeward side extends past the tree island has not been well documented. While snow accumulation does not affect the temperature of the seeding sites around our seeding sites during the growing season, the snow fall from the previous winter should have impacted soil moisture around tree islands, with Holtmeier & Broll (1992) finding increased soil moisture on the leeward side even during July and August. Each of these factors should cause conifer seed to germinate in higher numbers closer to the leeward side, but this was not the case in our study.

Currently, I am not sure why germination farther away from the tree island and on the windward side would be occurring. To ensure that the elevation of our chosen tree islands did not cause this germination effect through certain tree islands having different access to water, we plan on determining each tree islands' elevation once we return to Churchill this year. Furthermore, I have placed temperature probes at each seeding site around 5 tree islands. While only 5 islands were chosen due to limited equipment, these temperature probes can allow us to ascertain if snow fall accumulates more on either side. Also, I propose that each tree island side should have soil moisture and nutrient levels determined to find if the leeward or windward sides have significantly different nutrient and water content levels. This should allow us to confirm whether leeward and windward sides significantly differ, i.e., whether they constitute microhabitats.

Unfertilized plant species nitrogen isotopes

The plant species in Churchill's boreal tundra ecotone had $\partial^{15}N$ levels within their foliage consistent with other studies. I sampled plant species participated in arbuscular (VAM), arbuscular/ectomycorrhizal (dual), arbutoid (ARB), ectomycorrhizal (ECM) or ericoid mycorrhizae (EM) (Trofymow et al., 2020; Malloch & Malloch, 1982;Teste et al., 2020). Alongside these mycorrhizal host plants, there were two non-mycorrhizal plant species, *Lesquerella artica* and *Draba alpina.* Non-mycorrhizal plants, such as *L. artica* or *D. alpina*, do not benefit or associate with mycorrhizae and instead uptake nutrients directly from the soil (Raab et al., 1996). While all plants can take up inorganic nitrogen and some simple forms of organic nitrogen, non-mycorrhizal plants are able to take up inorganic nitrogen faster than mycorrhizal plants. This ability of non-mycorrhizal plants to access other nitrogen pools than mycorrhizal plants was seen here, where non-mycorrhizal plants had the second highest $\partial^{15}N$ values within their leaves. The other plant species with the highest $\partial^{15}N$ within their tissue were arbuscular host plants and nitrogen-fixing dual mycorrhizal *Shepherdia canadensis.* Nitrogen fixing plants have been seen to have levels of $\partial^{15}N$ within their leaves close to 0, a high value comparatively to other plant species who do not have nitrogen fixing bacteria within their roots. *Shepherdia canadensis* has both mycorrhizae and nitrogen fixing bacteria in their roots where it can utilize both associations to increase the N within its leaves (Spriggs et al., 2003; Teste et al., 2020; Rousk et al., 2016; Rascher et al., 2012). Although mycorrhizae should immobilize ^{15}N, based off the ∂^{15}N within *Elymus arenarius, Equisetum variegatum* and *Potentilla palustris*, arbuscular mycorrhizae may not immobilize ^{15}N as much as arbutoid, ericoid and ectomycorrhizae (Michelsen et al., 1998; Hobbie et al., 2009; Högberg, 2011; Cui et al., 2020). In my study, the arbutoid, ericoid and ectomycorrhizal host plants had comparatively low ∂^{15}N levels within their leaves, consistent with other findings (Hobbie et al., 2009; Michelsen et al., 1998). Dual mycorrhizal host plants had the highest variation between the ∂^{15}N within their leaves, most likely as they are flexible hosts and can react to abiotic and biotic factors by forming different mycorrhizal associations (Teste et al., 2020).

Dominant plant species ∂^{15}N response to fertilizer

As urea is added into the soil from the fertilizer treatments, it is taken up by saprotrophic microbes and transformed into ammonium (Witte, 2011). Once the urea becomes ammonium, it will remain in soil microbes, leave the ecosystem, or it will be taken up by plants' roots and transferred to parts of the plant body that requires nitrogen to grow (Pardo et al., 2013). The most common tissue that plants move nitrogen to is their roots, leaves and reproductive tissue (Pardo et al., 2013). Most mycorrhizal plants had ∂^{15}N much lower than the ∂^{15}N of unfertilized soil and the fertilizer had a ∂^{15}N a little lower than the soil. Therefore, change in the ∂^{15}N of these plants would indicate a shift in the N pool the plants access when fertilizer is applied. My results show that plants that are ectomycorrhizal (*Dryas integrifolia, Picea glauca,* and *Ledum decumbens*, and *Salix reticulata,* which also as AM) and arbutoid mycorrhizal (*Arctostaphylos alpina)* have a shift in the N pool they access. This implies they are less reliant on their fungal partner to access organic N sources. On the other hand, ericoid mycorrhizal host plants did not shift their N pool, suggesting they continue to rely on organic matter as an N source. *Shepherdia canadensis,* a nitrogen fixer, and both non-mycorrhizal taxa (*Draba alpina* and *Lesquerella artica*) either increased their ∂^{15}N levels past the ∂^{15}N level of the urea or had ∂^{15}N levels higher than the soil. Whenever carbon is high in the soil and nitrogen is low, saprotrophic bacteria preferentially take up ^{14}N as they decompose organic nitrogen, leaving ∂^{15}N enriched nitrogen in the soil (Dijkstra et al., 2008). The high ∂^{15}N values in *D. alpina* and *L. artica* seen here and in other studies (Raab et al., 1996) suggest these species can utilize inorganic nitrogen sources that other plant species have trouble taking up by themselves. This could help to explain why even though *D. alpina* and *L. artica* did not significantly increase in ∂^{15}N due to fertilization, both had far higher ∂^{15}N values compared to unfertilized non-mycorrhizal plants (*Carex rupestris, D. alpina, Eleocharis palustris, Pinguicula vulgaris* and *Scirpus caespitosus).* It could be that while *D. alpina* and *L. artica* did not use the urea itself to increase in ∂^{15}N, they instead took up the ^{15}N left by saprotrophic microbes. *Shepherdia canadensis* had a significant ∂^{15}N increase due to fertilization, but its ∂^{15}N level surpassed that of the urea fertilizer. Other research in the study area shows the *S. canadensis* gets most, but not all of its N from fixation (Tydings and Markham*, In review*). It appears that *S. canadensis* can take up a ^{15}N enriched fraction the N the from the fertilizer treatments (Rascher et al., 2012).

While *Shepherdia canadensis* and the non-mycorrhizal plants acted similarly to their unfertilized counterparts, *Salix reticulata* was an interesting case. Compared to other unfertilized *Salix* species, *S. reticulata* in the fertilizer plots did have higher ∂^{15}N levels in its leaves (Hobbie et al., 2009). Given that *S. canadensis* is a nitrogen fixing plant, it is expected to have ∂^{15}N above our urea signal. The ∂^{15}N within *S. reticulata's* leaves went far higher than the urea signal, indicating that it may have accessed an alternate nutrient pool like that available to *S. canadensis* and the non-mycorrhizal plants. As *S. reticulata* is a dual (arbuscular/ectomycorrhizal) mycorrhizal host plant and has low $\partial^{15}N$ in its leaves when unfertilized, it was unexpected that the $\partial^{15}N$ levels within *S. reticulata's* leaves increased beyond the urea signal (Hobbie et al., 2009; Teste et al., 2020). As this was an expected outcome for *S. reticulata*, this will need to be investigated further. The $\partial^{15}N$ in *S. reticulata*'s leaves could have increased past the fertilizer's signal due to *Salix* switching between mycorrhizae due to the high amounts of easily-accessible nitrogen in the soil (Teste et al., 2020). Teste et al., (2020) proposes that dual mycorrhizal host plants may gain benefits from their dual status that other mycorrhizal host plants do not have. They propose that dual host plants may be able to switch between mycorrhizae based on soil nutrient availability, where grown in environments with high nutrient availability dual host plants will preferentially associate with arbuscular mycorrhizae (Teste & Laliberté, 2019). Given this additional flexibility on mycorrhizal associations based on nutrient availability, *S. reticulata* could have switched from hosting ectomycorrhizal fungi to arbuscular mycorrhizal fungi as nutrients in the soil increased, which may have influenced the $\partial^{15}N$ within its leaves. It could have also been due to environmental factors such as light availability. After studying the $\partial^{15}N$ in the leaves of several boreal plant species, Hobbie et al., (2009) noted that *Salix* leaves had reduced $\partial^{15}N$ in closed canopy forests where light availability was decreased. This could result in lowered $\partial^{15}N$ in *S. reticulata's* leaves. Either way, further study into the mycorrhizal status and density of *Salix's* roots after being grown in high nutrient soil may provide further insight into how it accesses different nitrogen pools and how this impacts the $\partial^{15}N$ within its leaves.

There were only two ectomycorrhizal host plants sampled across all the fertilizer plots: *Picea glauca* in the forest, and *Dryas integrifolia* in the tundra. Saprotrophic mycorrhizae (ericoid and ectomycorrhizae) have rhizomorphs that can extend a long distance (e.g., tens of meters) from their host (Agerer, 2001). As these rhizomorphs spread and grow, they encounter organic matter or litter and begin to create enzymes such as acid phosphatases, laccase and proteases to decompose organic matter (Courty et al., 2006). As temperatures in the boreal tundra ecotone increase, nutrient availability in the soil has been predicted to increase, which may decrease plants need to gain nutrients via mycorrhizae (Price et al., 2013; Chapin et al., 2004; Nilsson et al., 2005). This appears to have occurred in the fertilizer plots, where *P. glauca* and *D. integrifolia* both increased the ∂^{15}N within their leaves due to the addition of urea in the soil. This implies that both species took up the urea, after it was transformed into inorganic nitrogen, through their roots instead of through their mycorrhizae, potentially reducing fungal biomass and mycorrhizal fungi colonization (Urcelay et al., 2003; Nilsson et al., 2005).

In many nutrient-poor settings, like boreal and alpine environments where nitrogen is heavily limited, stress-tolerant plant species like the Ericaceae are dominant (Grime, 1979). Ericaceous plants create phenolic compounds to reinforce their tissue while conserving and storing the nutrients they gain from their roots and mycorrhizae into their tough tissues (Shevtsova et al., 2005). This tendency helps stress tolerant plants to hold onto any nutrients they gain. However, as phenolics are primarily made from carbon, stress tolerant plants that are high in these compounds have high carbon and low nitrogen in their tissues (Hättenschwiler & Vitousek, 2000). The main strategy for stress tolerant plants is to create phenolic compounds to strengthen their leaves against biotic stressors such as pathogen infestation and herbivores (Saini et al., 2024), while also storing nutrients in their leaves that they receive from their mycorrhizae (Hättenschwiler & Vitousek, 2000; Shevtsova et al., 2005). While there are numerous types of phenolic compounds, many phenolics are stored in plants vacuoles, and are used either in reaction to certain stressors (ie., pathogen infection) or are a part of a plant's defense mechanism (ie., strengthening cell walls or expressing defense related genes) (Saini et al., 2024). Even after applying fertilizer to the soil, stress-tolerant plants like *Empetrum hermaphroditum* Hagerup, do not exhibit an increase in growth rate (Shevtsova et al., 2005). Within the fertilizer plots, *V. uliginosum, L. decumbens,* and *E. nigrum* are all ericaceous plant species, and should uptake nitrogen similarly if mycorrhizal associations are accurate predictors of how plant species will take up nutrients. However, as seen with both the findings of Shevtsova et al., (2005) and the ∂^{15}N values found in the needles/leaves of *E. nigrum, L. decumbens* and *V. uliginosum,* that does not seem to be occurring. While all three plant species host ericoid mycorrhizae within their roots and are stress tolerant plants, *L. decumbens* was the only ericoid host plant that had marginally ($p = 0.052$) higher ∂^{15}N values within its leaves due to fertilization. The ∂^{15}N values within *V. uliginosum* barely changed between all fertilizer treatments, while *E. nigrum* was extremely variable, with some samples having ∂^{15}N values above the urea signal while others were below -5. This implies that both *V. uliginosum* and *E. nigrum* did not use their roots to take up the nitrogen added into the soil. While *L. decumbens* reacted similarly to *Picea glauca* and *Dryas integrifolia* given that *V. uliginosum* and *E. nigrum* did not see a change in ∂^{15}N levels, it appears that there was variation among ericoid plants in their ability to respond to increased nutrient availability.

The only arbutoid host plant I sampled was *Arctostaphylos alpina*. Arbutoid mycorrhizal fungi form similar structures to ectomycorrhizal fungi, where both form a fungal mantle and Hartig net that is able to penetrate the host plants cell walls (Mühlmann & Göbl, 2006; Kühdorf et al., 2015). One fungal species, *Leotia lubrica* (Scop.) Pers., was found to form arbutoid mycorrhizal structures and was saprotrophic in nature (Kühdorf et al., 2015; Agerer, 2001).

While *L. lubrica* is most likely not the mycorrhizal fungi that associates with *A. alpina*, as arbutoid mycorrhizae are heavily understudied, these studies help us hypothesize the ecology of arbutoid mycorrhizae in our study system. Within the fertilizer plots, *A. alpina* exhibited increased ∂^{15}N due to fertilization treatments, implying that it took up nitrogen through its roots instead of its mycorrhizae or that the mycorrhizal fungi took up nitrogen from another pool. Given the increase in ∂^{15}N due to the urea fertilizer, arbutoid mycorrhizal fungi could have reduced biomass due to fertilization similarly to ericoid and ectomycorrhizal fungi. Whether this ∂^{15}N reaction is specific to *A. alpina* in the boreal tundra ecotone, or if this occurs generally across arbutoid mycorrhizal host plants, should be the subject of further research.

My prediction that saprotrophic host plants increased the ∂^{15}N within their leaves as nitrogen availability increases appears to have been borne out for the ectomycorrhizal host plants, but not the ericoid host plants. *Ledum decumbens, Vaccinium uliginosum* and *Empetrum nigrum* did not respond well to fertilizer, with only ∂^{15}N in *L. decumbens* increasing marginally. However, instead of increasing slowly, like the ericoid mycorrhizal host plants, the ∂^{15}N within the ectomycorrhizal host plants, *Picea glauca* and *Dryas integrifolia*, increased very quickly. This implies that both species may take advantage of increasing nutrient availability as the climate crisis continues to affect boreal and subarctic regions. While both ectomycorrhizal host plants showed similar trends to one another, this was not the case for the other plant species. Instead, the ∂^{15}N values for *Ledum decumbens, Empetrum nigrum* and *Vaccinium uliginosum* were highly variable, even though they all associate with ericoid mycorrhizae and all three are stress tolerant plants. Given that plant species with similar ecology, and which associate with the same mycorrhizal syndromes did not behave the same, it appears that mycorrhizal syndromes is not an accurate predictor for how their host will take up nutrients. Instead, given that the ∂^{15}N within the ectomycorrhizal host plants leaves changed more than the ericoid host plants, it appears that the ectomycorrhizal host plants can shift between nitrogen sources more than ericoid host plants.

C:N Ratio

While the $\partial^{15}N$ within a plant's leaves helps to indicate whether they can take other pools of nitrogen and potentially bypassing their mycorrhizae, the C:N ratio values help to explain if these species will utilize the additional nitrogen and potentially increase their productivity. While there are differences between species that I will discuss later, plants generally move nutrients like carbon and nitrogen towards metabolically active organs (Zhang et al., 2020; Reich et al., 1997). Alongside the C:N ratio helping to indicate the growth rate of a plant, the C:N ratio also determines how quickly a plant's tissue will be decompose whenever it becomes litter. Given saprotrophs' N limitation in low-productivity habitats, an increasing C:N ratio will cause litter to decompose more quickly. *Empetrum nigrum* is very common in the boreal due to it being able to tolerate the low nutrient environment by using carbon to create phenolic compounds in their leave (Shevtsova et al., 2005). As phenolic levels increase in the plant's leaves, leaves will become tougher and resistant to microbial decomposition. *Empetrum nigrum* and other *Empetrum* species can exhibit lowered C:N ratio within their needles due to nitrogen fertilization (Aerts et al., 2006;Quested et al., 2003;Zhang et al., 2023), with that same trend being seen in our fertilizer plots. However, *E. nigrum* did not exhibit an increase in $\partial^{15}N$ despite a change in its C:N ratio; I believe there are two principal reasons why this could happen. First, this overall strategy of creating tough leaves and storing nutrients into them could be why the C:N ratio of *E. nigrum* lowered due to an increase in nitrogen, while not seeing an increase in $\partial^{15}N$ levels.

Instead of being able to utilize the increase in nutrients like the other species that lowered their C:N ratio, *E. nigrum* stores nutrients within the vacuoles in its leaves (Chapin, 1980; Van Wijk et al., 2003), only having a limited growth response due to increased soil nutrients, and therefore having the C:N ratio in its needles be significantly lowered (Grime, 1979). Second, given that the nitrogen taken up by *E. nigrum* was ^{14}N, *E. nigrum* may have taken up inorganic nitrogen to change the C:N ratio within its needles (Shevtsova et al., 2005). When supplied with additional inorganic nitrogen into the soil, *Empetrum hermaphroditum* and *Cassiope tetragona* (L.) D.Don*,* another ericaceous species, saw increased shoot height (Shevtsova et al., 2005) or increased root biomass (Van Wijk et al., 2003) respectively. As Dr. Markham and I supplied the fertilizer plots with organic nitrogen in the form of urea, this additional nitrogen could be coming from decomposition rates within the fertilizer plots increasing. While this would be consistent with other studies who saw increased decomposition rates due to nitrogen fertilization, this cannot be confirmed given decomposition rates were not determined (Zhang et al., 2023). However, additional study into the decomposition rates in the soil of the fertilizer plots may prove helpful in determining how additional nutrient availability in the boreal tundra ecotone affects decomposition rates. While I would have predicted *E. nigrum,* as a stress tolerant plant, would simply hoard the increased nitrogen taken from the urea and their mycorrhizae, both *Ledum decumbens* and *Vaccinium uliginosum* did not behave this way. It appears again that even similar plant species did take nitrogen up, or stored it, similarly as one another.

Arctostaphylos alpina, Empetrum nigrum, Salix reticulata, Picea glauca, and *Dryas integrifolia* had significantly lowered their C:N values in their leaves. Given it appears that many of the plant species in the fertilizer plots were able to increase the $\partial^{15}N$ in their leaves due to fertilization, this could have potentially influenced the C:N ratio within the soil. *L. decumbens* did, possibly (i.e., the p value was marginal), alter the $\partial^{15}N$ in their leaves, but did not change the C:N ratio. This may imply that *L. decumbens* took up ^{15}N from the urea and increased the total amount of nitrogen in its leaves while also taking up higher amounts of C, leaving the C:N ratio unchanged, or that while it did switch nitrogen pools to take up ^{15}N from the urea, it did not take up a significantly high amount of %N overall. *Ledum* species do not seem to respond strongly to fertilizer and do not appear to change their leaf morphology or physiology (Bubier et al., 2011). Lin et al., (2020) added between 30 – 120 kg ha^{-1} N to 16 plots located in a boreal permafrost peatland for four years. After four years, they determined the percentage cover of 8 plant species, including *Ledum palustre* L., and *Vaccinium uliginosum. L. palustre* decreased the relative cover from 12% to 4% in their medium and high nitrogen plots, with *V. uliginosum* following suit and decreasing in relative cover in high fertilization plots. It seems that both *L. palustre* and *V. uliginosum* did not respond positively to increased nutrient availability, consistent with their response in the fertilizer plots. Depending on the species, nitrogen will fractionate as it transports between different organs in the plant, with plant roots generally having higher $\partial^{15}N$ compared to their leaves (Pardo et al., 2013). This difference in ^{15}N between plant organs is primarily due to nitrogen discrimination as ammonium and nitrate are transported across the plant, with transportation and transformation of both nitrogen forms resulting in different levels of ^{15}N in plants tissues (Cui et al., 2020; Pardo et al., 2013), While finding the isotopic values within *L. decumbens* roots would help to tell the full story, *L. decumbens* may be able to take up nitrogen from the urea, just slower than other species like *P. glauca* or *D. integrifolia* and may not be able to take up enough total nitrogen from the urea to change the C:N ratio within its leaves. No matter what, the C:N ratio within the leaves of the *Ericaceous* plants does help to show the variability between their nitrogen uptake and utilization.

Arctostaphylos alpina lowered the C:N ratio within its leaves and increased the ∂^{15}N due to fertilization. Given this, *A. alpina* may be able to utilize additional nitrogen compared to other species in the fertilizer plots, differing from other studies investigating *Arctostaphylos* species when fertilized. *Arctostaphylos rubra* (Rehder & E.H.Wilson) Fernald, and *Arctostaphylos uva – ursi* (L.) Spreng, have been observed decreasing in cover % and biomass as nitrogen availability in the soil of boreal forests increased (Boonstra et al., 2017; Turkington et al., 2002; Lindgren & Sullivan, 2018). This reduced cover % and biomass could be due to competitive exclusion as other plant species increase in cover and reduce the light *A. ruba* and *A. uva - ursi* received. However, given that other *Arctostaphylos* species do not seem to respond well to fertilizer, it is interesting to see that *A. alpina* saw isotopic changes in its leaves that seemed to indicate that it responded positively to fertilizer and would be able to utilize the urea to fuel changes in its leaves. As other *Arctostaphylos* species saw a decrease in cover % and biomass due to fertilization, the cover % and biomass of *A. alpina* may be helpful to study in the future. This could help to determine if the negative response to fertilization is common among *Arctostaphylos* species, but as of right now *A. alpina* appears to be reacting positively to fertilization, lowering the C:N ratio in its leaving and increasing the ∂^{15}N within its leaves.

The non-mycorrhizal and nitrogen fixing plants throughout the plots did not see a change in the C:N ratio due to the fertilizer. These plants have other means of taking up nitrogen than other boreal species and while this may imply that *S. canadensis* does not use the urea to change its C:N ratio, it could also be that *S. canadensis* did not see a change in C:N due to using nitrogen in its leaves due to growing additional tissue. If a plant was able to use nitrogen to grow more tissue, then this may cause its C:N ratio within its leaves to remain unchanged. However, growth rates will have to be determined in the future, given that the ∂^{15}N within *S. canadensis's* leaves only increased overall by 6%.

The C:N ratio of *Salix reticulata's* leaves did not lower as fertilization increased, even though the ∂^{15}N within their leaves increased. This could be due to *S. reticulata* taking up a high amount of ^{15}N and then using the additional nitrogen from the urea to grow more leaf mass making the C:N ratio within its leaves remain unchanged. Other *Salix* species have been observed to have similar levels of variability as *S. reticulata* shown here, with *Salix spp.* being found to have variation in its mean biomass after 10 years of nitrogen fertilization (Lindgren & Sullivan, 2018). This may help to explain *S. reticulata's* leaves increasing in ∂^{15}N past the urea signal, *Salix* species may have a variable response to nitrogen fertilization. *S. reticulata* may be useful to study more extensively in the future, as it appears to be highly variable in its response to fertilization. This may be due to its dual mycorrhizal nature, a species-specific response or responding to environmental factors differently than other species sampled in the fertilizer plots.

The final two plant species sampled in the fertilizer plots were the ectomycorrhizal host plants, *Dryas integrifolia* and *Picea glauca. D. integrifolia* and *P. glauca* appear to utilize the nitrogen taken from the urea, where the N:C in their leaves increased in tandem. Commonly, plant growth increases when supplied with additional nitrogen and the C:N ratio within their tissues lowers (Ågren, 2004). When supplied with additional nitrogen, plants' growth rate increases linearly and when supplied with phosphorus, growth rates increase exponentially (Ågren, 2004). Given that *D. integrifolia* and *P. glauca* appear to have used the urea to increase the nitrogen in their leaves, their growth rate may be impacted by the increase in nutrients. *D. integrifolia* and other *Dryas* species appear varied in their response to fertilization, where some have been a decrease in cover due to fertilization (Henry et al., 1986), while others have seen

Dryas increase in %N, leaf size and plant height (Hudson et al., 2011; Welker et al., 2005). *Dryas integrifolia* in the fertilizer plots appear to be able to utilize urea fertilizer to change the physiology within its leaves and potentially increase its growth rate its leaf morphology. *Picea glauca* also appears to be responding positively to fertilization, consistent with other studies (Boonstra et al., 2008; Duan & Chang, 2015, Lang et al. 2022). The *P. glauca* seedling within the fertilizer plots may provide insight into how *P. glauca* trees in Churchill will respond to fertilization and if heightened nutrient levels will allow *P. glauca* trees to move past the treeline and onto the tundra in the future.

Chlorophyll

The chlorophyll content illustrated which plant species responded to the additional nutrients in the soil by increasing the photosynthetic capacity within their leaves. Within the boreal, an increase in nitrogen availability can potentially cause plant species to become more productive (Fyles & McGill, 1987; Zhang et al., 2020), microbes to mineralize nitrogen faster (Laungani & Knops, 2012), species composition and cover to change (Shaver et al., 2001), and reduce the density of mycorrhizae in the soil (Kranabetter & MacKenzie, 2010). However, while some plant species like *Dryas integrifolia* and *Arctostaphylos alpina* utilized the urea to increase the total nitrogen in their leaves/needles, it is unknown whether an increase in plant N content will cause higher photosynthetic capacity (Van De Weg et al., 2013). Palmroth et al. (2014) were able to determine that the photosynthetic rate of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* was not affected by an increase in nitrogen in the soil. While both species saw an increase in nitrogen in their leaves, canopy openness and light availability instead affected the chlorophyll content of both species' leaves, where nitrogen addition was only a secondary factor. They were able to find that both *Vaccinium* species did increase their overall biomass due to the fertilizer treatments, but the photosynthetic capacity did not increase in tandem with nitrogen availability. While both studies found that photosynthetic capacity is not directly related to the amount of nitrogen in plant leaves, others have found the opposite: Prager et al. (2020) studied *Betula nana, V. uliginosum*, *Rhododendron tomentosum* Harmaja*, V. vitis-idaea, Bistorta bistortoides* (Pursh) Small, *Rubus chamaemorus* L.*, Carex bigelowii* Torr. Ex Schwein., and *Eriophorum vegiantum* L., after applying 5 – 100 kg ha^{-1} of N per year. Cao et al. (2007) found similar results, where *Betula papyrifera* Marshall, leaves saw increased photosynthetic rates when additional N was applied to *B. papyrifera* seedlings. Given that nitrogen is often the limiting nutrient for plant growth, especially for tundra species, I would have expected that an increase in N would increase the photosynthetic capacity and chlorophyll content of plants' leaves. While some studies have observed that the chlorophyll and photosynthetic capacity of boreal species have a linear relationship with nutrient availability (Yang et al., 2025; Prager et al., 2020), it seems that there is equal evidence that this relationship is far more complex (Palmroth et al., 2014; Van De Weg et al., 2013).

Picea glauca, Dryas integrifolia and *Lesquerella artica* all increased the total chlorophyll content in their leaves as fertilizer level increased. Given both *P.glauca* and *D. integrifolia* increased the total amount of nitrogen present in their leaves, these species may be capable of utilizing additional nutrients to increase their photosynthetic rates. The addition of urea seems to have influenced *D. integrifolia* a great deal, as their isotopic levels (e.g., $\partial^{15}N$, C:N) along with the total chlorophyll content within their leaves all significantly changed due to increasing fertilization in the soil. This increase in chlorophyll helps to confirm that whenever nutrients become more available in the soil, *D. integrifolia* will not only be able to take it up but change their physiology to closer match plant species in high nutrient environments (Baron-Preston et al., 2024). It appears that *P. glauca* may be behaving similarly, as the total chlorophyll content in *P. glauca's* needles increased due to the fertilization treatments by 15 – 30%. Previous work in Dr. Markham's lab involved assessing the number of cones that grew on each of the *P. glauca* trees increased in the fertilizer plots. I was therefore able to compare the total chlorophyll content in each tree's needles with their cone count, and I grouped them by the fertilizer treatment that was applied to them. From this, I found that when 100 kg ha^{-1} of nitrogen and 20 kg ha^{-1} of phosphorus was applied to the soil, *P. glauca* produced a higher number of cones and had higher total chlorophyll content within their needles, consistent with other studies (Benjamin et al., 2024). Due to this, it appears that *P. glauca* trees do respond to increased levels of nutrients in the soil and can produce higher amounts of chlorophyll and higher amounts of cones. Using the amount of chlorophyll in plants leaves to estimate the photosynthetic rate of plant species, it appears that *L. artica, P. glauca* and *D. integrifolia* will increase their photosynthetic rate as the level of nutrients in the soil increases. Given that *L. artica* did not utilize the urea fertilizer to increase the total nitrogen in their leaves, it appears that both ectomycorrhizal host plants were the only plant species that utilized the urea to increase their photosynthetic capacity.

I was able to find this complex relationship between nutrient availability and chlorophyll content after assaying the total chlorophyll in the leaves of the plant species sampled in the fertilizer plots. I found that the chlorophyll of *Vaccinium uliginosum* decreased due to the fertilizer treatments. *V. uliginosum* total chlorophyll decrease as nitrogen availability increased contrasts with Yang et al. (2025) and Prager et al. (2020), where they found that nitrogen enhances chlorophyll content in *V. uliginosum* leaves. *V. uliginosum* from the fertilizer plots response also contrasts Palmroth et al. (2014) finding that other *Vaccinium* species' chlorophyll content did not relate to an increase in soil nitrogen. I did not expect *V. uliginosum* to exhibit decreased chlorophyll content within its leaves due to fertilization, as increased nutrients do not negatively affect the total chlorophyll within a plant species leaves (Yang et al., 2025). I am uncertain as to why this occurred; plants will be resampled in future fieldwork, *V. uliginosum*'s total chlorophyll content will be reassessed to determine whether lowered chlorophyll due to fertilization was a spurious result.

Specific Leaf Area

One way plants can utilize nutrients is by increasing the area of their leaves to take up higher amounts of light. This growth is commonly measured by the specific leaf area a plant exhibits (Ordoñez et al., 2009). As plants accumulate nutrients, leaves expand to take up more sunlight and therefore gain additional carbon through photosynthesis. This trend of SLA increasing due to increasing nitrogen in the leaves appears to happen across multiple environments. After examining six biomes, ranging between the alpine tundra and the tropical rain forest, Reich et al. (1999) found that as nitrogen increases in leaves, SLA increases. They also found that in each biome that, as SLA increases, the life span of the leaf decreases, and net photosynthetic rates increase. Given that nutrient availability is predicted to increase in the boreal as the climate crisis causes decomposition and nitrogen mineralization rates to heighten, boreal plants should see an increase in SLA. The SLA of boreal plant species appears to be increasing in tandem with soil nutrients: Kloeppel et al. (1998) observed *Larix occidentalis* Nutt, and *Larix lyallii* Parl., reacting to higher nitrogen availability by increasing their SLA. The specific leaf area of many plant species, even those in the tundra and boreal, appear to be increasing as nitrogen availability in the soil increases (Zhao et al., 2024). Given that some of the plant species sampled in the fertilizer plots, like *Dryas integrifolia, Picea glauca* and *Arctostaphylos alpina*, had lowered C:N ratio within their leaves due to fertilization, I would except their leaves/needles to increase in SLA. However, across all the fertilizer plots, there was not a single plant species that saw an increase in their specific leaf area due to increasing fertilization. While this could indicate that the Churchill plant species do not react to heightened nutrients by increasing their SLA, I do not believe that this is accurate. Our result is inconsistent with many other studies that have found boreal plant species to increase in SLA due to increased fertilization (Hudson et al., 2011), and some of the plant species sampled in the fertilizer plots did see increases in total chlorophyll content and NDVI due to the fertilizer treatments This all leads me to believe that instead of plant species like *D. integrifolia* that responded positively to fertilizer not increasing in SLA, it was an issue with the methodology. This issue will be corrected once Dr. Markham and I return to Churchill later this year, where we will be resampling the leaves of each plant species to look at the change in SLA as nutrients increase in the soil.

NDVI

I examined the NDVI of each forest and tundra fertilizer plot, along with the NDVI of each identifiable plant species in the plots. While I was able to collect samples from 12 plant species in the fertilizer plots, this was not possible when analyzing the NDVI of each plot. Due to issues in analyzing the orthomosaics of each fertilizer plot, only *P. glauca, Vaccinium uliginosum, Ledum decumbens, D. integrifolia* and *Shepherdia canadensis* were analyzed for NDVI. Across both the forest and the tundra, all 5 species saw an increase in NDVI due to increased fertilizer level. Even though *S. canadensis* and *V. uliginosum* did not increase their

∂^{15}N, C:N or chlorophyll values in response to fertilizer, both increased their NDVI values by 6%. This increase in NDVI could be due to changes in the density of leaves, with many plant species overlapping on top of and underneath each other. This may have caused me to accidentally analyze multiple species at once instead of analyzing just one at a time. Due to this stacking effect, plant species that did not respond well to fertilizer, like *V. uliginosum,* may have seen an inaccurate increase in NDVI. This limitation has been noted, where whenever Dr. Markham's lab finds the NDVI of each fertilizer plot again in the future, the plan is to add large markers to signal the areas where only one plant species is present. This should help to remove any inaccuracies in examining individual plant species change in NDVI.

Examining the increase in NDVI between the forest and tundra did help to show that Churchill's tundra will become greener as higher amounts of nutrients become available. Although the average NDVI of the forest was much higher than the tundra's average NDVI, it appears that the tundra saw a faster increase in NDVI due to the fertilization treatments. Given that *A. alpina* and *D. integrifolia* leaves increased in ∂^{15}N, lowered their C:N ratio and *D. integrifolia* increased the total chlorophyll content due to fertilizer, it seems that both species respond very well to fertilizer. *Dryas integrifolia* is the most abundant plant species in the tundra plots and given the increase in NDVI and total chlorophyll within *D. integrifolia's* leaves, this may help to explain the overall NDVI in the tundra. Even though the forest did not increase in NDVI as quickly as the tundra, it still seems that Churchill's boreal forest is fairly productive. Other more temperate forests dominated by *Picea abies* and *Abies alba* (Aiton) Michx., have been found to have average NDVI as high as 0.94 between 2003 and 2018, where NDVI can range between -1 and 1 (Prăvălie et al., 2022). Comparatively, while Churchill's boreal forest appears to belimited by nutrients in the soil and is still becoming more productive as fertilizer is added into the forest’s soil, increasing from 0.69 to 0.79 after three years of fertilization. As the climate crisis is affecting the boreal more every year, it may be prudent to measure the NDVI of the fertilizer plots every year to see if this productivity increase will continue, or if there is a point where the forest plots begin to see negative growth due to the nitrogen fertilizer. This would also allow for more comparison between the tundra and for more information about how Churchill’s tundra is becoming greener and more productive.

Conclusion

Increasing nutrients in the soil appears to affect the tissue of multiple boreal plant species, while only affecting the germination of *Picea glauca* and *Pinus banksiana* in the medium tundra fertilization plots. Within the fertilizer plots, each plant species had varied responses to fertilization. Non-mycorrhizal plants, *Draba alpina* and *Lesquerella artica,* did not react to heightened nutrient availability. The ectomycorrhizal host plants, *P. glauca* and *D. integrifolia,* foliage increased in ∂^{15}N, N:C, total chlorophyll content and NDVI due to fertilization. While unpredicted, these two plant species utilized the urea from the fertilizer to increase the amount of nitrogen in their tissue. As well, given both species increased the total chlorophyll in their leaves, and increased in NDVI, it seems that they are becoming more productive and photosynthetically active due to increased nitrogen availability in the soil. The increase in ∂^{15}N in *P. glauca's* and *D. integrifolia's* foliage implies reduced nitrogen is traded to the host from their mycorrhizae. While it will require more research, *P. glauca* and *D. integrifolia* may see reduced ectomycorrhizal colonization in their roots as nitrogen increased. The ericoid host plants within the plots, *Ledum decumbens, Vaccinium uliginosum,* and *Empetrum nigrum* had noticeably different responses to increased nutrients. *V. uliginosum's* leaves decreased in total chlorophyll, while *E. nigrum* did not see a change in physiology besides increasing in N. Interestingly, the ∂^{15}N within *E. nigrum's* needles was not affected by fertilization, but the C:N ratio within the same tissue significantly decreased. This implies that it was able to take up nitrogen from another source. *Empetrum nigrum* may have gotten this nitrogen from increased decomposition rates in the plots, however the decomposition and mineralization rates will have to be determined in each fertilizer plot before this can be proven. The only change in *L. decumben's* leaves was an increase in ∂^{15}N, implying it utilized the urea applied to the soil. Given the increase of $\partial^{15}N$ in *L. artica's* leaves, determining *L. artica's* growth rate when soil nutrients are increased may be useful to research in the future. Although both *V. uliginosum* and *L. decumbens* did see an increase in their NDVI values, given the methodology issues, this may not be accurate. As each ectomycorrhizal and ericoid mycorrhizal host plant did not react the same as one another, it does not seem that a plants' mycorrhizal status is an accurate indicator of how the host will take up nutrients. Different boreal plant species react to fertilizer faster than others and mycorrhizal status does not provide a clear indicator on how plants will react. An increase in nutrients in the soil did not impact germination for either *Picea glauca* or *Pinus banksiana*. In the forest and tundra, *P. banksiana* germinated significantly higher than *P. glauca.* This helps to confirm, at least during the growing season, that *P. banksiana* can survive in the boreal forest and tundra of Churchill, but how many *P. banksiana* seedlings survived Churchill's winter remains to be seen. Even though many other studies have observed tree islands to have a microhabitat on their leeward side that should benefit germination, the germination of both conifer species did not support this. Instead of germinating closer to the tree island on the leeward side, both *P. glauca* and *P. banksiana* had increased germination 10 meters away from the island on the windward side. This implies that the open tundra is somehow more beneficial to conifer germination on the tundra than anywhere around the tree islands. As this is entirely inconsistent with other studies, the tree islands should receive additional research in the future to confirm the results shown here in this thesis are accurate.

Literature Cited

AAT Bioquest, Inc. (2025). *PBS (Phosphate Buffered Saline) (1X, pH 7.4) Preparation and Recipe*. https://www.aatbio.com/resources/buffer-preparations-and-recipes/pbs-phosphate-buffered-saline

Aerts, R., Logtestijn, R. S. P. V., & Karlsson, P. S. (2006). Nitrogen supply differentially affects litter decomposition rates and nitrogen dynamics of sub-arctic bog species. *Oecologia*, *146*(4), 652–658. https://doi.org/10.1007/s00442-005-0247-5

Agerer, R. (2001). Exploration types of ectomycorrhizae. *Mycorrhiza*, *11*(2), 107–114. https://doi.org/10.1007/s005720100108

Ågren, G. I. (2004). The C: N : P stoichiometry of autotrophs – theory and observations. *Ecology Letters*, *7*(3), 185–191. https://doi.org/10.1111/j.1461-0248.2004.00567.x

Albertsen, E., Harper, K., & De Fields, D. (2014). Structure and Composition of Tree Islands and Krummholz within the Forest-Tundra Ecotone in Central and Eastern Canada. *ARCTIC*, *67*(3), 396. https://doi.org/10.14430/arctic4400

Angell, A. C., & Kielland, K. (2009). Establishment and growth of white spruce on a boreal forest floodplain: Interactions between microclimate and mammalian herbivory. *Forest Ecology and Management*, *258*(11), 2475–2480. https://doi.org/10.1016/j.foreco.2009.08.030

Asselin, H., Payette, S., Fortin, M., & Vallée, S. (2003). The northern limit of *Pinus banksiana* Lamb. in Canada: Explaining the difference between the eastern and western distributions. *Journal of Biogeography*, *30*(11), 1709–1718. https://doi.org/10.1046/j.1365-2699.2003.00935.x

Atkin, O. K., Villar, R., & Cummins, W. R. (1993). The ability of several high arctic plant species to utilize nitrate nitrogen under field conditions. *Oecologia*, *96*(2), 239–245. https://doi.org/10.1007/BF00317737

Averill, C., & Hawkes, C. V. (2016). Ectomycorrhizal fungi slow soil carbon cycling. *Ecology Letters*, *19*(8), 937–947. https://doi.org/10.1111/ele.12631

Baum, C., Toljander, Y. K., Eckhardt, K.-U., & Weih, M. (2009). The significance of host-fungus combinations in ectomycorrhizal symbioses for the chemical quality of willow foliage. *Plant and Soil*, *323*(1–2), 213–224. https://doi.org/10.1007/s11104-009-9928-x

Beck, P. S. A., Horning, N., Goetz, S. J., Loranty, M. M., & Tape, K. D. (2011). Shrub Cover on the North Slope of Alaska: A circa 2000 Baseline Map. *Arctic, Antarctic, and Alpine Research*, *43*(3), 355–363. https://doi.org/10.1657/1938-4246-43.3.355

Beck, P. S. A., Jönsson, P., Høgda, K. -A., Karlsen, S. R., Eklundh, L., & Skidmore, A. K. (2007). A ground-validated NDVI dataset for monitoring vegetation dynamics and mapping phenology in Fennoscandia and the Kola peninsula. *International Journal of Remote Sensing*, *28*(19), 4311–4330. https://doi.org/10.1080/01431160701241936

Benjamin, J. S., Roth, J. D., & Markham, J. H. (2024). Red foxes increase white spruce seed production at its northern range limit. *Basic and Applied Ecology*, *81*, 1–6. https://doi.org/10.1016/j.baae.2024.10.005

Bigras, F. J., & Colombo, S. J. (Eds.). (2001). *Conifer Cold Hardiness* (Vol. 1). Springer Netherlands. https://doi.org/10.1007/978-94-015-9650-3

Bledsoe, C., Klein, P., & Bliss, L. C. (1990). A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. *Canadian Journal of Botany*, *68*(9), 1848–1856. https://doi.org/10.1139/b90-242

Bonfils, C. J. W., Phillips, T. J., Lawrence, D. M., Cameron-Smith, P., Riley, W. J., & Subin, Z.

M. (2012). On the influence of shrub height and expansion on northern high latitude climate. *Environmental Research Letters*, *7*(1), 015503. https://doi.org/10.1088/1748-9326/7/1/015503

Bonney, M. T., Danby, R. K., & Treitz, P. M. (2018). Landscape variability of vegetation change across the forest to tundra transition of central Canada. *Remote Sensing of Environment*, *217*, 18–29. https://doi.org/10.1016/j.rse.2018.08.002

Boonstra, R., Desantis, L., Krebs, C., & Hik, D. (2008). Climate and nutrient influences on the growth of white spruce trees in the boreal forests of the Yukon. *Climate Research*, *36*, 123–130. https://doi.org/10.3354/cr00736

Boonstra, R., Krebs, C. J., & Cowcill, K. (2017). Responses of key understory plants in the boreal forests of western North America to natural versus anthropogenic nitrogen levels. *Forest Ecology and Management*, *401*, 45–54. https://doi.org/10.1016/j.foreco.2017.06.065

Britto, D. T., & Kronzucker, H. J. (2013). Ecological significance and complexity of N-source preference in plants. *Annals of Botany*, *112*(6), 957–963. https://doi.org/10.1093/aob/mct157

Bubier, J. L., Smith, R., Juutinen, S., Moore, T. R., Minocha, R., Long, S., & Minocha, S. (2011). Effects of nutrient addition on leaf chemistry, morphology, and photosynthetic capacity of three bog shrubs. *Oecologia*, *167*(2), 355–368. https://doi.org/10.1007/s00442-011-1998-9

Cameron, E. A., & Lantz, T. C. (2016). Drivers of tall shrub proliferation adjacent to the Dempster Highway, Northwest Territories, Canada. *Environmental Research Letters*, *11*(4), 045006. https://doi.org/10.1088/1748-9326/11/4/045006

Cao, B., Dang, Q.-L., & Zhang, S. (2007). Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO2] in white birch seedlings. *Tree Physiology*, *27*(6), 891–899. https://doi.org/10.1093/treephys/27.6.891

Carroll, S. B., & Bliss, L. C. (1982). Jack pine – lichen woodland on sandy soils in northern Saskatchewan and northeastern Alberta. *Canadian Journal of Botany*, *60*(11), 2270–2282. https://doi.org/10.1139/b82-278

Chapin, D. M. (1995). Physiological and Morphological Attributes of Two Colonizing Plant Species on Mount St. Helens. *American Midland Naturalist*, *133*(1), 76. https://doi.org/10.2307/2426349

Chapin, F. S., Callaghan, T. V., Bergeron, Y., Fukuda, M., Johnstone, J. F., Juday, G., & Zimov, S. A. (2004). Global Change and the Boreal Forest: Thresholds, Shifting States or Gradual Change? *AMBIO: A Journal of the Human Environment*, *33*(6), 361–365. https://doi.org/10.1579/0044-7447-33.6.361

Corrêa, A., Cruz, C., & Ferrol, N. (2015). Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: The great unknown. *Mycorrhiza*, *25*(7), 499–515. https://doi.org/10.1007/s00572-015-0627-6

Courty, P.-E., Pouysegur, R., Buée, M., & Garbaye, J. (2006). Laccase and phosphatase activities of the dominant ectomycorrhizal types in a lowland oak forest. *Soil Biology and Biochemistry*, *38*(6), 1219–1222. https://doi.org/10.1016/j.soilbio.2005.10.005

Cripps, C. L., & Eddington, L. H. (2005). Distribution of Mycorrhizal Types among Alpine Vascular Plant Families on the Beartooth Plateau, Rocky Mountains, U.S.A., in Reference to Large-Scale Patterns in Arctic–Alpine Habitats. *Arctic, Antarctic, and*

Alpine Research, *37*(2), 177–188. https://doi.org/10.1657/1523-0430(2005)037[0177:DOMTAA]2.0.CO;2

Cui, J., Lamade, E., Fourel, F., & Tcherkez, G. (2020). δ^{15} N values in plants are determined by both nitrate assimilation and circulation. *New Phytologist*, *226*(6), 1696–1707. https://doi.org/10.1111/nph.16480

Desponts, M., & Payette, S. (1993). The Holocene Dynamics of Jack Pine at Its Northern Range Limit in Quebec. *The Journal of Ecology*, *81*(4), 719. https://doi.org/10.2307/2261669

Dhillion, S. S. (1994). Ectomycorrhizae, Arbuscular Mycorrhizae, and Rhizoctonia sp. Of Alpine and Boreal Salix spp. In Norway. *Arctic and Alpine Research*, *26*(3), 304. https://doi.org/10.2307/1551943

Dial, R. J., Maher, C. T., Hewitt, R. E., & Sullivan, P. F. (2022). Sufficient conditions for rapid range expansion of a boreal conifer. *Nature*, *608*(7923), 546–551. https://doi.org/10.1038/s41586-022-05093-2

Dijkstra, P., LaViolette, C. M., Coyle, J. S., Doucett, R. R., Schwartz, E., Hart, S. C., & Hungate, B. A. (2008). ^{15} N enrichment as an integrator of the effects of C and N on microbial metabolism and ecosystem function. *Ecology Letters*, *11*(4), 389–397. https://doi.org/10.1111/j.1461-0248.2008.01154.x

Duan, M., & Chang, S. X. (2015). Responses of lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) to fertilization in some reconstructed boreal forest soils in the oil sands region. *Ecological Engineering*, *84*, 354–361. https://doi.org/10.1016/j.ecoleng.2015.09.046

Finger, R. A., Turetsky, M. R., Kielland, K., Ruess, R. W., Mack, M. C., & Euskirchen, E. S.

(2016). Effects of permafrost thaw on nitrogen availability and plant–soil interactions in a boreal Alaskan lowland. *Journal of Ecology*, *104*(6), 1542–1554. https://doi.org/10.1111/1365-2745.12639

Fitzpatrick, O. T., Blade, M., Fishback, L. A., Kershaw, G. P., Muffly, J., & Mamet, S. D. (2020). Snowpack, Tree Size, and Ecological Legacies Promote Seedling Establishment in Tree Islands at the Treeline. *Ecosystems*, *23*(8), 1714–1725. https://doi.org/10.1007/s10021-020-00499-3

Flanagan, P. W. (1986). Substrate Quality Influences on Microbial Activity and Mineral Availability in Taiga Forest Floors. In K. Van Cleve, F. S. Chapin, P. W. Flanagan, L. A. Viereck, & C. T. Dyrness (Eds.), *Forest Ecosystems in the Alaskan Taiga* (Vol. 57, pp. 138–151). Springer New York. https://doi.org/10.1007/978-1-4612-4902-3_10

Fyles, J. W., & McGill, W. B. (1987). Decomposition of boreal forest litters from central Alberta under laboratory conditions. *Canadian Journal of Forest Research*, *17*(2), 109–114. https://doi.org/10.1139/x87-021

Grime, J. P. (1973). Competitive Exclusion in Herbaceous Vegetation. *Nature*, *242*(5396), 344–347. https://doi.org/10.1038/242344a0

Grman, E., & Robinson, T. M. P. (2013). Resource availability and imbalance affect plant–mycorrhizal interactions: A field test of three hypotheses. *Ecology*, *94*(1), 62–71. https://doi.org/10.1890/12-0385.1

Haavisto, V. F., & Winston, D. A. (1974). Germination of black spruce and jack pine seed at 0.5 °C. *The Forestry Chronicle*, *50*(6), 240–240. https://doi.org/10.5558/tfc50240-6

Hansen, W. D., Foster, A., Gaglioti, B., Seidl, R., & Rammer, W. (2023). The Permafrost and

Organic LayEr module for Forest Models (POLE-FM) 1.0. *Geoscientific Model Development*, *16*(7), 2011–2036. https://doi.org/10.5194/gmd-16-2011-2023

Harsch, M. A., Hulme, P. E., McGlone, M. S., & Duncan, R. P. (2009). Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters*, *12*(10), 1040–1049. https://doi.org/10.1111/j.1461-0248.2009.01355.x

Hättenschwiler, S., & Vitousek, P. M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution*, *15*(6), 238–243. https://doi.org/10.1016/S0169-5347(00)01861-9

Henry, G. H. R., Freedman, B., & Svoboda, J. (1986). Effects of fertilization on three tundra plant communities of a polar desert oasis. *Canadian Journal of Botany*, *64*(11), 2502–2507. https://doi.org/10.1139/b86-332

Hewitt, R. E., Bennett, A. P., Breen, A. L., Hollingsworth, T. N., Taylor, D. L., Chapin, F. S., & Rupp, T. S. (2016). Getting to the root of the matter: Landscape implications of plant-fungal interactions for tree migration in Alaska. *Landscape Ecology*, *31*(4), 895–911. https://doi.org/10.1007/s10980-015-0306-1

Hobbie, E. A., & Hobbie, J. E. (2008). Natural Abundance of ^{15}N in Nitrogen-Limited Forests and Tundra Can Estimate Nitrogen Cycling Through Mycorrhizal Fungi: A Review. *Ecosystems*, *11*(5), 815–830. https://doi.org/10.1007/s10021-008-9159-7

Hobbie, J. E., Hobbie, E. A., Drossman, H., Conte, M., Weber, J. C., Shamhart, J., & Weinrobe, M. (2009). Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests:^{15} N is the key signal. *Canadian Journal of Microbiology*, *55*(1), 84–94. https://doi.org/10.1139/W08-127

Högberg, M. N., Briones, M. J. I., Keel, S. G., Metcalfe, D. B., Campbell, C., Midwood, A. J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., & Högberg, P. (2010). Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist*, *187*(2), 485–493. https://doi.org/10.1111/j.1469-8137.2010.03274.x

Högberg, P., Johannisson, C., Yarwood, S., Callesen, I., Näsholm, T., Myrold, D. D., & Högberg, M. N. (2011). Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist*, *189*(2), 515–525. https://doi.org/10.1111/j.1469-8137.2010.03485.x

Holtmeier, F.-K., & Broll, G. (1992). The Influence of Tree Islands and Microtopography on Pedoecological Conditions in the Forest-Alpine Tundra Ecotone on Niwot Ridge, Colorado Front Range, U.S.A. *Arctic and Alpine Research*, *24*(3), 216–228. https://doi.org/10.1080/00040851.1992.12002949

Hudson, J. M. G., Henry, G. H. R., & Cornwell, W. K. (2011). Taller and larger: Shifts in Arctic tundra leaf traits after 16 years of experimental warming. *Global Change Biology*, *17*(2), 1013–1021. https://doi.org/10.1111/j.1365-2486.2010.02294.x

Janusauskaite, D., & Straigyte, L. (2011). Leaf Litter Decomposition Differences between Alien and Native Maple Species. *Baltic Forestry*, *17*, 22–29.

Jarvis, P., & Linder, S. (2000). Constraints to growth of boreal forests. *Nature*, *405*(6789), 904–905. https://doi.org/10.1038/35016154

Kambo, D., & Danby, R. K. (2018). Factors influencing the establishment and growth of tree seedlings at Subarctic alpine treelines. *Ecosphere*, *9*(4), e02176. https://doi.org/10.1002/ecs2.2176

Kloeppel, B. D., Treichel, I. W., Kharuk, S., & Gower, S. T. (1998). Foliar carbon isotope discrimination in *Larix* species and sympatric evergreen conifers: A global comparison. *Oecologia*, *114*(2), 153–159. https://doi.org/10.1007/s004420050431

Kranabetter, J. M., & MacKenzie, W. H. (2010). Contrasts Among Mycorrhizal Plant Guilds in Foliar Nitrogen Concentration and δ^{15}N Along Productivity Gradients of a Boreal Forest. *Ecosystems*, *13*(1), 108–117. https://doi.org/10.1007/s10021-009-9304-y

Kranabetter, J. M., Simard, S. W., Guy, R. D., & Coates, K. D. (2010). Species patterns in foliar nitrogen concentration, nitrogen content and ^{13}C abundance for understory saplings across light gradients. *Plant and Soil*, *327*(1–2), 389–401. https://doi.org/10.1007/s11104-009-0062-6

Kühdorf, K., Münzenberger, B., Begerow, D., Gómez-Laurito, J., & Hüttl, R. F. (2015). *Leotia* cf. *Lubrica* forms arbutoid mycorrhiza with *Comarostaphylis arbutoides* (Ericaceae). *Mycorrhiza*, *25*(2), 109–120. https://doi.org/10.1007/s00572-014-0590-7

Lang, J. A., Roth, J. D., Tardif, J. C., & Markham, J. H. (2022). Red foxes enhance long-term tree growth near the Arctic treeline. *Ecosphere*, *13*(9), e4236. https://doi.org/10.1002/ecs2.4236

Lett, S., & Dorrepaal, E. (2018). Global drivers of tree seedling establishment at alpine treelines in a changing climate. *Functional Ecology*, *32*(7), 1666–1680. https://doi.org/10.1111/1365-2435.13137

Li, X., Sun, L., & Han, Y. (2024). Effects of Wildfires on Soil Organic Carbon in Boreal Permafrost Regions: A Review. *Permafrost and Periglacial Processes*, *35*(4), 493–503. https://doi.org/10.1002/ppp.2247

Lin, G., Zeng, D.-H., & Mao, R. (2020). Traits and their plasticity determine responses of plant performance and community functional property to nitrogen enrichment in a boreal peatland. *Plant and Soil*, *449*(1–2), 151–167. https://doi.org/10.1007/s11104-020-04478-4

Lindgren, P. M. F., & Sullivan, T. P. (2018). Influence of repeated fertilization on forage production for native mammalian herbivores in young lodgepole pine forests. *Forest Ecology and Management*, *417*, 265–280. https://doi.org/10.1016/j.foreco.2018.03.002

Liu, R., He, Y., Zhou, G., Shao, J., Zhou, L., Zhou, H., Li, N., Song, B., Liang, C., Yan, E., Chen, X., Wang, X., Wang, M., Bai, S. H., Zhou, X., & Phillips, R. P. (2021). Mycorrhizal effects on decomposition and soil CO_2 flux depend on changes in nitrogen availability during forest succession. *Journal of Ecology*, *109*(11), 3929–3943. https://doi.org/10.1111/1365-2745.13770

Loranger, H., Zotz, G., & Bader, M. Y. (2016). Early establishment of trees at the alpine treeline: Idiosyncratic species responses to temperature-moisture interactions. *AoB PLANTS*, *8*, plw053. https://doi.org/10.1093/aobpla/plw053

Malloch, D., & Malloch, B. (1982). The mycorrhizal status of boreal plants: Additional species from northeastern Ontario. *Canadian Journal of Botany*, *60*(7), 1035–1040. https://doi.org/10.1139/b82-131

Mamet, S. D., Cairns, D. M., Brook, R. K., & Kershaw, G. P. (2015). Modeling the spatial distribution of subarctic forest in northern Manitoba using GIS-based terrain and climate data. *Physical Geography*, *36*(2), 93–112. https://doi.org/10.1080/02723646.2014.994253

Mamet, S. D., & Kershaw, G. P. (2012). Subarctic and alpine tree line dynamics during the last 400 years in north-western and central Canada. *Journal of Biogeography*, *39*(5), 855–868. https://doi.org/10.1111/j.1365-2699.2011.02642.x

Mamet, S. D., & Kershaw, G. P. (2013). Multi-scale Analysis of Environmental Conditions and Conifer Seedling Distribution Across the Treeline Ecotone of Northern Manitoba, Canada. *Ecosystems*, *16*(2), 295–309. https://doi.org/10.1007/s10021-012-9614-3

Michelsen, A., Quarmby, C., Sleep, D., & Jonasson, S. (1998). Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. *Oecologia*, *115*(3), 406–418. https://doi.org/10.1007/s004420050535

Milbau, A., Vandeplas, N., Kockelbergh, F., & Nijs, I. (2017). Both seed germination and seedling mortality increase with experimental warming and fertilization in a subarctic tundra. *AoB PLANTS*, *9*(5). https://doi.org/10.1093/aobpla/plx040

Moreau, D., Bardgett, R. D., Finlay, R. D., Jones, D. L., & Philippot, L. (2019). A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, *33*(4), 540–552. https://doi.org/10.1111/1365-2435.13303

Mucha, J., Peay, K. G., Smith, D. P., Reich, P. B., Stefański, A., & Hobbie, S. E. (2018). Effect of Simulated Climate Warming on the Ectomycorrhizal Fungal Community of Boreal and Temperate Host Species Growing Near Their Shared Ecotonal Range Limits. *Microbial Ecology*, *75*(2), 348–363. https://doi.org/10.1007/s00248-017-1044-5

Mühlmann, O., & Göbl, F. (2006). Mycorrhiza of the host-specific *Lactarius deterrimus* on the roots of *Picea abies* and *Arctostaphylos uva-ursi*. *Mycorrhiza*, *16*(4), 245–250. https://doi.org/10.1007/s00572-006-0038-9

Mullen, R. W. (2015). Nutrient Cycling in Soils: Nitrogen. In J. L. Hatfield & T. J. Sauer (Eds.), *Soil Management: Building a Stable Base for Agriculture* (pp. 67–78). Soil Science Society of America. https://doi.org/10.2136/2011.soilmanagement.c5

Munier, A., Hermanutz, L., Jacobs, J. D., & Lewis, K. (2010). The interacting effects of temperature, ground disturbance, and herbivory on seedling establishment: Implications for treeline advance with climate warming. *Plant Ecology*, *210*(1), 19–30. https://doi.org/10.1007/s11258-010-9724-y

Mycorrhizal Symbiosis. (2008). Elsevier. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S. G., Campbell, C., Hurry, V., Linder, S., & Högberg, M. N. (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytologist*, *198*(1), 214–221. https://doi.org/10.1111/nph.12139

Newsham, K. K., Upson, R., & Read, D. J. (2009). Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecology*, *2*(1), 10–20. https://doi.org/10.1016/j.funeco.2008.10.005

Nilsson, L. O., Giesler, R., Bååth, E., & Wallander, H. (2005). Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. *New Phytologist*, *165*(2), 613–622. https://doi.org/10.1111/j.1469-8137.2004.01223.x

Nilsson, L. O., & Wallander, H. (2003). Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist*, *158*(2), 409–416. https://doi.org/10.1046/j.1469-8137.2003.00728.x

Obu, J. (2021). How Much of the Earth's Surface is Underlain by Permafrost? *Journal of Geophysical Research: Earth Surface*, *126*(5), e2021JF006123. https://doi.org/10.1029/2021JF006123

Ordoñez, J. C., Van Bodegom, P. M., Witte, J. M., Wright, I. J., Reich, P. B., & Aerts, R. (2009).

A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography*, *18*(2), 137–149. https://doi.org/10.1111/j.1466-8238.2008.00441.x

Orwin, K. H., Kirschbaum, M. U. F., St John, M. G., & Dickie, I. A. (2011). Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: A model-based assessment: Organic nutrient uptake enhances soil C. *Ecology Letters*, *14*(5), 493–502. https://doi.org/10.1111/j.1461-0248.2011.01611.x

Palmroth, S., Holm Bach, L., Nordin, A., & Palmqvist, K. (2014). Nitrogen-addition effects on leaf traits and photosynthetic carbon gain of boreal forest understory shrubs. *Oecologia*, *175*(2), 457–470. https://doi.org/10.1007/s00442-014-2923-9

Pardo, L. H., Semaoune, P., Schaberg, P. G., Eagar, C., & Sebilo, M. (2013). Patterns in δ^{15}N in roots, stems, and leaves of sugar maple and American beech seedlings, saplings, and mature trees. *Biogeochemistry*, *112*(1–3), 275–291. https://doi.org/10.1007/s10533-012-9724-1

Payette, S., Delwaide, A., Couillard, P.-L., & Pilon, V. (2017). Disjunct jack pine (*Pinus banksiana*) populations of the boreal forest in eastern Canada: Expanding, declining, or stable?

Botany, *95*(7), 697–707. https://doi.org/10.1139/cjb-2016-0319

Prager, C. M., Boelman, N. T., Eitel, J. U. H., Gersony, J. T., Greaves, H. E., Heskel, M. A., Magney, T. S., Menge, D. N. L., Naeem, S., Shen, C., Vierling, L. A., & Griffin, K. L. (2020). A mechanism of expansion: Arctic deciduous shrubs capitalize on warming-induced nutrient availability. *Oecologia*, *192*(3), 671–685. https://doi.org/10.1007/s00442-019-04586-8

Prăvălie, R., Sîrodoev, I., Nita, I.-A., Patriche, C., Dumitraşcu, M., Roşca, B., Tişcovschi, A., Bandoc, G., Săvulescu, I., Mănoiu, V., & Birsan, M.-V. (2022). NDVI-based ecological dynamics of forest vegetation and its relationship to climate change in Romania during 1987–2018. *Ecological Indicators*, *136*, 108629. https://doi.org/10.1016/j.ecolind.2022.108629

Prescott, C. E., Maynard, D. G., & Laiho, R. (2000). Humus in northern forests: Friend or foe? *Forest Ecology and Management*, *133*(1–2), 23–36. https://doi.org/10.1016/S0378-1127(99)00295-9

Price, D. T., Alfaro, R. I., Brown, K. J., Flannigan, M. D., Fleming, R. A., Hogg, E. H., Girardin, M. P., Lakusta, T., Johnston, M., McKenney, D. W., Pedlar, J. H., Stratton, T., Sturrock, R. N., Thompson, I. D., Trofymow, J. A., & Venier, L. A. (2013). Anticipating the consequences of climate change for Canada's boreal forest ecosystems. *Environmental Reviews*, *21*(4), 322–365. https://doi.org/10.1139/er-2013-0042

Quested, H. M., Cornelissen, J. H. C., Press, M. C., Callaghan, T. V., Aerts, R., Trosien, F., Riemann, P., Gwynn-Jones, D., Kondratchuk, A., & Jonasson, S. E. (2003). DECOMPOSITION OF SUB-ARCTIC PLANTS WITH DIFFERING NITROGEN ECONOMIES: A FUNCTIONAL ROLE FOR HEMIPARASITES. *Ecology*, *84*(12), 3209–3221. https://doi.org/10.1890/02-0426

Raab, T. K., Lipson, D. A., & Monson, R. K. (1996). Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: Implications for the alpine nitrogen cycle. *Oecologia*, *108*(3), 488–494. https://doi.org/10.1007/BF00333725

Rascher, K. G., Hellmann, C., Máguas, C., & Werner, C. (2012). Community scale ^{15}N isoscapes: Tracing the spatial impact of an exotic N_2 -fixing invader. *Ecology Letters*, *15*(5), 484–491. https://doi.org/10.1111/j.1461-0248.2012.01761.x

Razgulin, S. M. (2022). Mycorrhizal Complexes and Their Role in the Ecology of Boreal Forests (Review). *Biology Bulletin*, *49*(6), 704–712. https://doi.org/10.1134/S1062359022060140

Read, D. J., & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist*, *157*(3), 475–492. https://doi.org/10.1046/j.1469-8137.2003.00704.x

Reddy, G. S. V., Bokka, S. K. R., Chellem, S. R., Kalpana, K., Indrani, K., Bindu, T. P. L. H., & Navya, D. S. (2024). Mutualistic Relationships between Plants and Mycorrhizal Fungi Impacts on Ecosystem Functioning: A Review. *UTTAR PRADESH JOURNAL OF ZOOLOGY*, *45*(13), 242–261. https://doi.org/10.56557/upjoz/2024/v45i134151

Reich, P. B., Ellsworth, D. S., Walters, M. B., Vose, J. M., Gresham, C., Volin, J. C., & Bowman, W. D. (1999). GENERALITY OF LEAF TRAIT RELATIONSHIPS: A TEST ACROSS SIX BIOMES. *Ecology*, *80*(6), 1955–1969. https://doi.org/10.1890/0012-9658(1999)080[1955:GOLTRA]2.0.CO;2

Reich, P. B., Walters, M. B., & Ellsworth, D. S. (1997). From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences*, *94*(25), 13730–13734. https://doi.org/10.1073/pnas.94.25.13730

Repáč, I. (2011). Ectomycorrhizal Inoculum and Inoculation Techniques. In M. Rai & A. Varma (Eds.), *Diversity and Biotechnology of Ectomycorrhizae* (Vol. 25, pp. 43–63). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-15196-5_3

Rew, L. J., McDougall, K. L., Alexander, J. M., Daehler, C. C., Essl, F., Haider, S., Kueffer, C., Lenoir, J., Milbau, A., Nuñez, M. A., Pauchard, A., & Rabitsch, W. (2020). Moving up and over: Redistribution of plants in alpine, Arctic, and Antarctic ecosystems under global change. *Arctic, Antarctic, and Alpine Research*, *52*(1), 651–665. https://doi.org/10.1080/15230430.2020.1845919

Ropars, P., & Boudreau, S. (2012). Shrub expansion at the forest–tundra ecotone: Spatial heterogeneity linked to local topography. *Environmental Research Letters*, *7*(1), 015501. https://doi.org/10.1088/1748-9326/7/1/015501

Rousk, K., Sorensen, P. L., & Michelsen, A. (2016). Nitrogen Transfer from Four Nitrogen-Fixer Associations to Plants and Soils. *Ecosystems*, *19*(8), 1491–1504. https://doi.org/10.1007/s10021-016-0018-7

Saini, N., Anmol, A., Kumar, S., Wani, A. W., Bakshi, M., & Dhiman, Z. (2024). Exploring phenolic compounds as natural stress alleviators in plants- a comprehensive review. *Physiological and Molecular Plant Pathology*, *133*, 102383. https://doi.org/10.1016/j.pmpp.2024.102383

Sapkota, S., Salem, M., Jahed, K. R., Artlip, T. S., & Sherif, S. M. (2023). From endodormancy to ecodormancy: The transcriptional landscape of apple floral buds. *Frontiers in Plant Science*, *14*, 1194244. https://doi.org/10.3389/fpls.2023.1194244

Schulte-Uebbing, L., & De Vries, W. (2018). Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. *Global Change Biology*, *24*(2). https://doi.org/10.1111/gcb.13862

Scott, P. A., Hansell, R. I. C., & Erickson, W. R. (1993). Influences of Wind and Snow on Northern Tree-Line Environments at Churchill, Manitoba, Canada. *Arctic*, *46*(4), 316–323. JSTOR.

Shaver, G. R., Bret-Harte, M. S., Jones, M. H., Johnstone, J., Gough, L., Laundre, J., & Chapin, F. S. (2001). SPECIES COMPOSITION INTERACTS WITH FERTILIZER TO CONTROL LONG-TERM CHANGE IN TUNDRA PRODUCTIVITY. *Ecology*, *82*(11), 3163–3181. https://doi.org/10.1890/0012-9658(2001)082[3163:SCIWFT]2.0.CO;2

Shevtsova, A., Nilsson, M., Gallet, C., Zackrisson, O., & Jäderlund, A. (2005). Effects of long-term alleviation of nutrient limitation on shoot growth and foliar phenolics of *Empetrum hermaphroditum*. *Oikos*, *111*(3), 445–458. https://doi.org/10.1111/j.0030-1299.2005.13524.x

Shimomura, N., Matsuda, M., Ariyoshi, K., & Aimi, T. (2012). Development of mycelial slurries containing surfactant for cultivation of the edible ectomycorrhizal mushroom *Rhizopogon roseolus* (syn. *Rhizopogon rubescens*). *Botany*, *90*(9), 839–844. https://doi.org/10.1139/b2012-054

Sierra, C. A., Malghani, S., & Loescher, H. W. (2017). Interactions among temperature, moisture, and oxygen concentrations in controlling decomposition rates in a boreal forest soil. *Biogeosciences*, *14*(3), 703–710. https://doi.org/10.5194/bg-14-703-2017

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis* (3rd ed). Academic Press.

Spriggs, A. C., Stock, W. D., & Dakora, F. D. (2003). Influence of mycorrhizal associations on foliar δ^{15}N values of legume and non-legume shrubs and trees in the fynbos of South Africa: Implications for estimating N_2 fixation using the ^{15}N natural abundance method. *Plant and Soil*, *255*(2), 495–502. https://doi.org/10.1023/A:1026044831178

Sutinen, S., Partanen, J., Vihera-Aarnio, A., & Hakkinen, R. (2009). Anatomy and morphology in developing vegetative buds on detached Norway spruce branches in controlled conditions before bud burst. *Tree Physiology*, *29*(11), 1457–1465. https://doi.org/10.1093/treephys/tpp078

Tamm, C. O. (1991). *Nitrogen in Terrestrial Ecosystems* (Vol. 81). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-75168-4

(Ted) Hogg, E. H., & Schwarz, A. G. (1997). Regeneration of planted conifers across climatic moisture gradients on the Canadian prairies: Implications for distribution and climate change. *Journal of Biogeography*, *24*(4), 527–534. https://doi.org/10.1111/j.1365-2699.1997.00138.x

Tedersoo, L., Bahram, M., Toots, M., Diédhiou, A. G., Henkel, T. W., Kjøller, R., Morris, M. H., Nara, K., Nouhra, E., Peay, K. G., Põlme, S., Ryberg, M., Smith, M. E., & Kõljalg, U. (2012). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology*, *21*(17), 4160–4170. https://doi.org/10.1111/j.1365-294X.2012.05602.x

Teste, F. P., Jones, M. D., & Dickie, I. A. (2020). Dual-mycorrhizal plants: Their ecology and relevance. *New Phytologist*, *225*(5), 1835–1851. https://doi.org/10.1111/nph.16190

Teste, F. P., & Laliberté, E. (2019). Plasticity in root symbioses following shifts in soil nutrient availability during long-term ecosystem development. *Journal of Ecology*, *107*(2), 633–649. https://doi.org/10.1111/1365-2745.13103

Timoney, K. P., & Mamet, S. (2020). No treeline advance over the last 50 years in subarctic western and central Canada and the problem of vegetation misclassification in remotely sensed data. *Écoscience*, *27*(2), 93–106. https://doi.org/10.1080/11956860.2019.1698258

Treu, R., Laursen, G. A., Stephenson, S. L., Landolt, J. C., & Densmore, R. (1995). Mycorrhizae from Denali National Park and Preserve, Alaska. *Mycorrhiza*, *6*(1), 21–29. https://doi.org/10.1007/s005720050101

Trofymow, J. A., Shay, P.-E., Myrholm, C. L., Tomm, B., Bérubé, J. A., & Ramsfield, T. D. (2020). Fungi associated with tree species at an Alberta oil sands reclamation area, as determined by sporocarp assessments and high-throughput DNA sequencing. *Applied Soil Ecology*, *147*, 103359. https://doi.org/10.1016/j.apsoil.2019.09.009

Turkington, R., John, E., Watson, S., & Seccombe-Hett, P. (2002). The effects of fertilization and herbivory on the herbaceous vegetation of the boreal forest in north-western Canada: A 10-year study. *Journal of Ecology*, *90*(2), 325–337. https://doi.org/10.1046/j.1365-2745.2001.00666.x

Ueyama, M., Tahara, N., Nagano, H., Makita, N., Iwata, H., & Harazono, Y. (2018). Leaf- and ecosystem-scale photosynthetic parameters for the overstory and understory of boreal forests in interior Alaska. *Journal of Agricultural Meteorology*, *74*(2), 79–86. https://doi.org/10.2480/agrmet.D-17-00031

Urcelay, C., Bret-Harte, M. S., Díaz, S., & Chapin, F. S. (2003). Mycorrhizal colonization mediated by species interactions in arctic tundra. *Oecologia*, *137*(3), 399–404. https://doi.org/10.1007/s00442-003-1349-6

Vaario, L.-M., Guerin-Laguette, A., Matsushita, N., Suzuki, K., & Lapeyrie, F. (2002). Saprobic potential of *Tricholoma matsutake*: Growth over pine bark treated with surfactants. *M ycorrhiza*, *12*(1), 1–5. https://doi.org/10.1007/s00572-001-0144-7

Van De Weg, M. J., Shaver, G. R., & Salmon, V. G. (2013). Contrasting effects of long term versus short-term nitrogen addition on photosynthesis and respiration in the Arctic. *Plant Ecology*, *214*(10), 1273–1286. https://doi.org/10.1007/s11258-013-0250-6

Van Der Heijden, M. G. A., Martin, F. M., Selosse, M., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, *205*(4), 1406–1423. https://doi.org/10.1111/nph.13288

Van Der Heijden, M. G. A., Streitwolf-Engel, R., Riedl, R., Siegrist, S., Neudecker, A., Ineichen, K., Boller, T., Wiemken, A., & Sanders, I. R. (2006). The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist*, *172*(4), 739–752. https://doi.org/10.1111/j.1469-8137.2006.01862.x

Vanhala, P., Karhu, K., Tuomi, M., Björklöf, K., Fritze, H., & Liski, J. (2008). Temperature sensitivity of soil organic matter decomposition in southern and northern areas of the boreal forest zone. *Soil Biology and Biochemistry*, *40*(7), 1758–1764. https://doi.org/10.1016/j.soilbio.2008.02.021

Wallenda, T., & Kottke, I. (1998). Nitrogen deposition and ectomycorrhizas. *New Phytologist*, *139*(1), 169–187. https://doi.org/10.1046/j.1469-8137.1998.00176.x

Wallin, E. (2018). *From growth cessation to bud burst—Conifer seedling development in response to nursery culture and environmental stimuli*. School for Forest Management, Swedish University of Agricultural Sciences.

Wang, Z. M., & Lechowicz, M. J. (1998). Effect of sowing date on the germination and establishment of black spruce and jack pine under simulated field conditions. *Écoscience*, *5*(1), 95–99. https://doi.org/10.1080/11956860.1998.11682438

Welker, J. M., Fahnestock, J. T., Sullivan, P. F., & Chimner, R. A. (2005). Leaf mineral nutrition of Arctic plants in response to warming and deeper snow in northern Alaska. *Oikos*, *109*(1), 167–177. https://doi.org/10.1111/j.0030-1299.2005.13264.x

Wilmking, M., Juday, G. P., Barber, V. A., & Zald, H. S. J. (2004). Recent climate warming forces contrasting growth responses of white spruce at treeline in Alaska through temperature thresholds. *Global Change Biology*, *10*(10), 1724–1736. https://doi.org/10.1111/j.1365-2486.2004.00826.x

Wintermans, J. F. G. M., & De Mots, A. (1965). Spectrophotometric characteristics of chlorophylls *a* and *b* and their phenophytins in ethanol. *Biochimica et Biophysica Acta (BBA) - Biophysics Including Photosynthesis*, *109*(2), 448–453. https://doi.org/10.1016/0926-6585(65)90170-6

Witte, C.-P. (2011). Urea metabolism in plants. *Plant Science*, *180*(3), 431–438. https://doi.org/10.1016/j.plantsci.2010.11.010

Yang, H., Wu, Y., Zhang, C., Lyu, L., Wu, W., Huang, Z., & Li, W. (2025). Growth and Physiological Traits of Blueberry Seedlings in Response to Different Nitrogen Forms. *Plants*, *14*(10), 1444. https://doi.org/10.3390/plants14101444

Yogabaanu, U., Weber, J.-F. F., Convey, P., Rizman-Idid, M., & Alias, S. A. (2017). Antimicrobial properties and the influence of temperature on secondary metabolite production in cold environment soil fungi. *Polar Science*, *14*, 60–67. https://doi.org/10.1016/j.polar.2017.09.005

Zhang, J., He, N., Liu, C., Xu, L., Chen, Z., Li, Y., Wang, R., Yu, G., Sun, W., Xiao, C., Chen, H. Y. H., & Reich, P. B. (2020). Variation and evolution of C:N ratio among different organs enable plants to adapt to N-limited environments. *Global Change Biology*, *26*(4), 2534–2543. https://doi.org/10.1111/gcb.14973

Zhang, X.-F., Zhong, N.-H., Li, R., Shi, F.-X., & Mao, R. (2023). Nitrogen addition mediates monospecific and mixed litter decomposition in a boreal peatland. *CATENA*, *225*, 107051. https://doi.org/10.1016/j.catena.2023.107051

Zhao, X., Liu, Z., & Jin, G. (2024). Effects of nitrogen addition and leaf age on needle traits and the relationships among traits in *Pinus koraiensis*. *Environmental and Experimental Botany*, *223*, 105795. https://doi.org/10.1016/j.envexpbot.2024.105795

Zhu, Q., & Zhuang, Q. (2013). Modeling the effects of organic nitrogen uptake by plants on the carbon cycling of boreal forest and tundra ecosystems. *Biogeosciences*, *10*(12), 7943–7955. https://doi.org/10.5194/bg-10-7943-2013