Nitrogen Form Uptake Capacities by Arbuscular Mycorrhizae and Ectomycorrhizae

By

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Abstract

Plant growth and survival are affected by the nutrients available in the environment. Nitrogen (N) is most often the limiting nutrient in terrestrial ecosystems, particularly in temperate and boreal forests, such as those on Vancouver Island. To overcome the challenge of limited nutrient availability, plants have evolved symbiotic relationships with fungi, called mycorrhizae. While research on the importance of mycorrhizal symbioses for N uptake by plants continues to grow, we have a limited understanding of the mechanisms of N uptake and transfer by mycorrhizae. This knowledge is crucial to fully understand N uptake and assimilation by plants. This study aimed to determine the influence of soil N availability on conifer growth and foliar N content, and on the N form preferences and sporocarp N content of associated mycorrhizae. Inorganic and organic soil N production was determined for two sites, Fairy Lake and San Juan, near Port Renfrew British Columbia, under pure plantations of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), Sitka spruce (*Picea sitchensis* [Bong.] Carr.), western redcedar (*Thuja plicata* Donn ex D. Don in Lamb) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.). Ammonium, nitrate and amino acid production contrasted between the sites, with relatively higher N production in San Juan compared to Fairy Lake. This indicated differences in soil N cycling, most likely due to differences in moisture and topography. In general, conifer species did not affect inorganic and organic soil N production. Growth of conifers increased with increasing N availability, and differed between species, with Douglas-fir and Sitka spruce having the greatest growth and western redcedar having the least growth. Foliar %N and δ^{15}N were found to differ among the conifer species, and western redcedar had the lowest foliar N concentrations. While site quality was not reflected in foliar %N, foliar δ^{15}N was found to increase with increasing δ^{15}N of the forest floor. Ectomycorrhizal (ECM) sporocarps reflected site quality, with greater N concentrations but lower δ^{15}N values on the higher N site. Sporocarp ^{15}N concentrations were higher than foliar ^{15}N concentrations, suggesting N isotope fractionation by mycorrhizae. Finally, site N availability was not related to the rates of N form uptake by ECM genera. Both ECM and arbuscular mycorrhizae (AM) did not have substantial nitrate uptake, despite a greater supply of nitrate. Ammonium was found to be taken up at higher rates than nitrate in the ECM and AM roots, suggesting a preference for ammonium, possibly due to ammonium being energetically cheaper to metabolize and suppressing nitrate transporters in mycorrhizal fungi. Differences in proportions of N form uptake and sporocarp N content among ECM genera were seen, indicating potential niche formation based on functional traits such as N form uptake and mycelial morphology. Knowing how mycorrhizae respond to different N forms and rates of N supply will not only increase our knowledge of N dynamics in mycorrhizal symbioses, but will help predict the effects environmental changes, such as disturbance and N deposition, may have on these systems.

Table of Contents

List of Tables

List of Figures

Appendix

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Chapter 1 - Introduction

1.1 Nitrogen

Plant growth and survival is often limited by a lack of nutrients in the environment. In terrestrial ecosystems, especially forests in temperate and boreal climates, nitrogen is usually the limiting nutrient (Sylvia *et al*., 1999; Chalot and Plassard, 2011; Courty *et al*., 2015). Nitrogen (N) is the most abundant mineral element in plant tissues and makes up 2% of total plant dry matter (Miller and Cramer, 2004). It is a key nutrient that is required throughout the plant life cycle, as it is needed for the synthesis of proteins, nucleic acids, coenzymes and many secondary plant compounds (Miller and Cramer, 2004).

The main source of N on Earth is from the atmosphere as dinitrogen gas (N_2), which makes up 78% of the atmosphere (Morot-Gaudry and Touraine, 2001). Other forms of N, such as ammonia (NH_3) and nitrogen oxides (N_2O, NO_2 and NO) are also emitted into the atmosphere by industrial smoke, forest fires, volcanic activity and denitrification, but in small amounts (Morot-Gaudry and Touraine, 2001). However, most plants are only able to access and assimilate N from the soil, which contains a very small fraction of the total N on Earth (0.00024%) (Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). Atmospheric N_2 enters the soil through different forms of N fixation. One form of N fixation is through lightning and ultraviolet radiation, which uses O_2 or ozone to oxidize N_2, and forms nitrate (NO_3^-) and nitrite (NO_2^-) in the soil (Morot-Gaudry and Touraine, 2001). Humans also directly add N into the environment through N fertilizers to increase crop yields, but excessive fertilizer application has led to negative environmental effects, such as the eutrophication of ground and surface water from leaching of NO_3^- and atmospheric pollution by the release of ammonia (NH_3) and N_2O (Figure 1.1) (Miller and Cramer, 2004).

One of the biggest sources of N in the soil is through biological N fixation by free-living and endosymbiotic prokaryotes, called diazotrophs, that convert N_2 to ammonium (NH_4^+) with the enzyme nitrogenase (Jackson *et al*., 2008). Examples of free-living N fixing bacteria include *Clostridium*, *Azotobacter* and *Klebsiella* (Trinchant, Drevon and Rigaud, 2001). Some diazotrophs are able to form symbiotic relationships with specific plant species, where they exchange assimilated N for carbon (C) (Trinchant, Drevon and Rigaud, 2001). One such

diazotroph is the cyanobacterium *Nostoc w*hich forms associations with many lichens (*Collema* and *Peltigera*) and some fungal and plant species (Trinchant, Drevon and Rigaud, 2001). Other symbiotic diazotrophs form root nodules with their host plant, where N fixation occurs (Trinchant, Drevon and Rigaud, 2001). For example, *Frankia* is an actinomycete bacterium that associates with woody angiosperms such as *Alnus*, *Casuarina* and *Myrica* (Trinchant, Drevon and Rigaud, 2001). Also, bacteria in the family Rhizobiaceae (*Rhizobium*, *Sinorhizobium*, *Bradyrhizobium* and *Azorhizobium*) form symbiotic relationships with leguminous plants (Trinchant, Drevon and Rigaud, 2001).

The NH_4^+ produced by free-living diazotrophs directly enters the soil once the microbe dies, while the N assimilated by symbiotic diazotrophs only enters the soil through excretion and/or once host plants or animals die and are decomposed by micro-organisms (Morot-Gaudry and Touraine, 2001). Soil organic N accumulates through the decomposition of plant, animal, and microbial biomass by a wide variety of micro-organisms, such as bacteria and fungi (Sylvia *et al*., 1999). This decomposition process leads to the release of numerous organic N compounds, from peptides, proteins, free amino acids and nucleic acids to vitamins, antibiotics, and metabolic intermediates (Sylvia *et al*., 1999). Through mineralization by microbes, these organic N compounds get converted into inorganic N compounds (Sylvia *et al*., 1999). While the pool of inorganic N in the soil is much smaller compared to the organic N pool, inorganic N compounds are very important as they act as substrates, metabolic intermediates and electron acceptors (Sylvia *et al*., 1999). The forms of inorganic N include NH_4^+, hydroxylamine (NH_2OH), N_2, N_2O, NO, NO_2^- and NO_3^-, with the largest pools of inorganic N in the soil being NH_4^+ and NO_3^- (Sylvia *et al*., 1999).

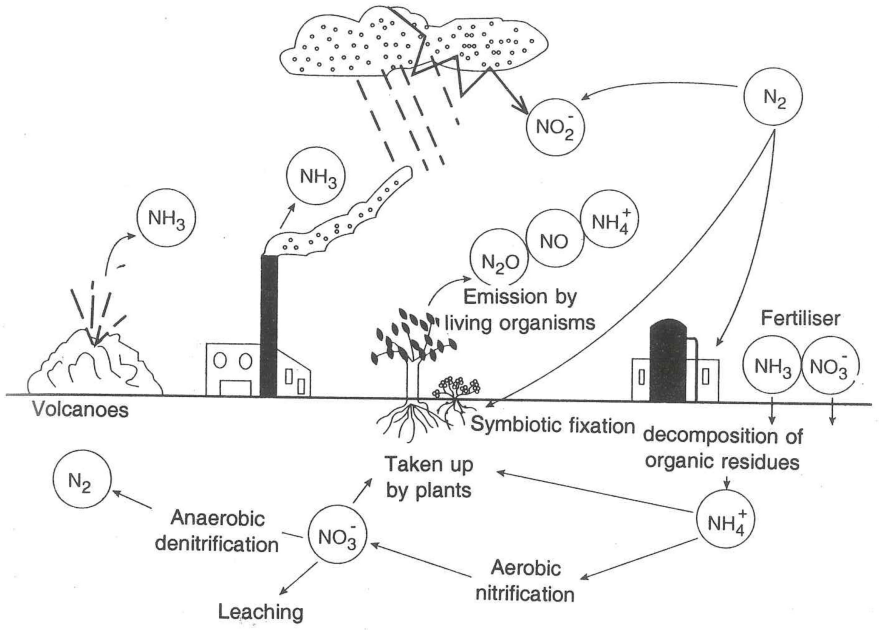


Figure 1.1 – The Terrestrial Nitrogen Cycle (diagram from Morot-Gaudry and Touraine, 2001).

1.2 Nitrogen Mineralization/Immobilization

N mineralization is the conversion of organic N into inorganic N, mainly in the forms of NH_4^+ and NO_3^-, by specific microbes in the soil (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). More specifically, ammonification is used to describe the transformation of organic N to NH_4^+ (Sylvia *et al*., 1999). However, the reverse reaction, known as immobilization, can also occur, where NH_4^+ is assimilated by microbes and converted back into organic N (Sylvia *et al*., 1999). Immobilization also happens with NO_3^-, but NO_3^- must first be reduced to NH_4^+ before it can be incorporated into microbial cells (Sylvia *et al*., 1999).

1.2.1 Ammonification/ NH_4^+ Mineralization

Heterotrophic microbes decompose organic N compounds for energy and carbon, through ammonification, and NH_4^+ is released as a by-product (Sylvia *et al*., 1999). Organic N is converted into NH_4^+ by extracellular and intracellular microbial enzymes (Sylvia *et al*., 1999). Extracellular enzymes break down organic N polymers and the resulting monomers cross the microbial cell membrane and are further metabolized, producing NH_4^+, which is released into the soil (Sylvia *et al*., 1999). Major extracellular enzymes produced by microorganisms breakdown proteins, aminopolysaccharides (from microbial cell walls), and nucleic acids (Sylvia

et al., 1999). Proteins are broken down into peptides or individual amino acids by proteinase, protease and peptidase enzymes (Sylvia *et al*., 1999). Compounds in microbial cell walls are broken down by corresponding extracellular enzymes, for examples, chitin forms fungal cell walls and insect exoskeletons and is broken down by chitinase and chitobiase enzymes (Sylvia *et al*., 1999). Nucleic acids are broken down into individual nucleotides by ribonucleases and deoxyribonucleases (Sylvia *et al*., 1999). These smaller N compounds are then translocated into the microbial cells where they are further degraded by intracellular enzymes and NH_4^+ is released (Sylvia *et al*., 1999). Amino acids in the microbial cells are degraded based on their amide or amine functional groups and NH_4^+ is released (Sylvia *et al*., 1999). For example, the amide groups of asparagine and glutamine are cleaved by asparaginase and glutaminase enzymes (Sylvia *et al*., 1999). Amino acid N, on the other hand, is released by amino acid dehydrogenases and amino acid oxidases in a process called deamination (Sylvia *et al*., 1999).

1.2.2 Immobilization/Assimilation

Microbes and other organisms assimilate NH_4^+ into amino acids by two enzymatic pathways: glutamate dehydrogenase and glutamine synthetase (GDH/GS pathway) and glutamine synthetase-glutamate synthase (GS/GOGAT pathway) (Sylvia *et al*., 1999; Chalot and Plassard, 2011). In most soils, the NH_4^+ concentration is low, so the GS/GOGAT pathway is used (Sylvia *et al*., 1999). The GDH/GS pathway seems to dominate in mycorrhizal fungi, while the GS/GOGAT pathway seems to dominate in plants (Chalot and Plassard, 2011). Generally, there is net immobilization of NH_4^+ if the availability of N is limiting, but if not, net mineralization occurs (Sylvia *et al*., 1999). Also, in most soils, the productivity of heterotrophic microorganisms is limited mainly by the amount of carbon available, therefore, a specific C/N ratio determines whether N is mineralized or immobilized (Sylvia *et al*., 1999).

Aside from mineralization/immobilization, NH_4^+ can be bound to the cation exchange sites of soil particles or fixed to the lattice of clay minerals (NH_4^+ fixation) due to its positive charge (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001). Also, NH_4^+ can react chemically with organic compounds or be volatized at high pH (Sylvia *et al*., 1999). NH_4^+ in the soil has

three biological fates. It can be taken up by plants, assimilated by microbes and/or oxidized to nitrate by nitrifying microorganisms (Sylvia *et al*., 1999).

1.2.3 Nitrification

Autotrophic nitrification is the microbial production of NO_3^- from the oxidation of NH_4^+ (Sylvia *et al*., 1999). This is a two-step, two organism process where inorganic N is used as an energy source for nitrifying bacteria (Sylvia *et al*., 1999). Step 1 is ammonia oxidation, where NH_4^+ is converted to NO_2^- by ammonia-oxidizing bacteria of the "*Nitroso*-" genera (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). There are five genera of ammonia-oxidizers, with *Nitrosomonas* being the best characterized and most well studied (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). Ammonia oxidation is also mediated by ammonia-oxidizing archaea in the phylum *Thaumarcheota* (Ke *et al*., 2015). In Step 2, nitrite oxidation, nitrite-oxidizing bacteria convert NO_2^- to NO_3^- (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). Nitrite-oxidizing bacteria are more phylogenetically diverse than ammonia oxidizers, with *Nitrobacter* species being among the most dominant nitrite-oxidizers (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001). Heterotrophic bacteria and fungi, and methane-oxidizing bacteria in the soil are also known to oxidize NH_4^+ and organic N into NO_2^- or NO_3^- (Sylvia *et al*., 1999).

The nitrification process is negatively affected by low soil pH (pH<5), anaerobic conditions, lack of soil water and temperatures below 5°C and above 40°C (Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). NO_3^- is also lost through leaching, as clay particles in the soil are negatively charged (Morot-Gaudry and Touraine, 2001).

1.2.4 Denitrification

Denitrification by anaerobic heterotrophic bacteria converts NO_3^- into nitrogen gases (N_2, N_2O, NO and NO_2) by becoming an electron acceptor in place of O_2 via oxidative phosphorylation (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). Denitrification happens when O_2 is limited, the concentration of NO_3^- and soil moisture

are high, carbohydrates in the soil are plentiful and temperatures are warm (Miller and Cramer, 2004).

The soil N availability for plants is dependent on the balance between mineralization, nitrification and denitrification rates (Miller and Cramer, 2004). Due to the biological nature of the N cycle, the availability of NH_4^+ and NO_3^- varies seasonally, and the location and form of N in the soil profile can vary due to leaching, soil water and temperature (Miller and Cramer, 2004).

1.3 Plant Nitrogen Uptake and Assimilation

In almost all ecosystems, plants (including conifer species) take up inorganic N mainly in the form of NH_4^+ and NO_3^- ions (Morot-Gaudry and Touraine, 2001; Jackson *et al*., 2008; Courty *et al*., 2015). Plant roots are also able to absorb organic N as small amino molecules like amino acids and urea, but this is thought to play a major role in plant N nutrition only on very N-poor sites and in cold environments where N mineralization is limited, such as boreal and arctic ecosystems (Morot-Gaudry and Touraine, 2001; Jackson *et al*., 2008; Courty *et al*., 2015). However, recent studies have shown that plants in many different biomes on Earth, including temperate climates, have greater capacity to take up organic N than was previously thought, particularly dissolved organic N (Bennett and Prescott, 2004; Finzi and Berthrong, 2005; Jones et al., 2005; Moran-Zuloaga *et al*. 2015).

NH_4^+ and NO_3^- in the soil move toward plant roots through mass flow and diffusion (Miller and Cramer, 2004; Courty *et al*., 2015). NO_3^- is actively transported across the plasma membrane of epidermal and cortical root cells, where net uptake of NO_3^- is based on the balance between active influx and passive efflux (Miller and Cramer, 2004). The uptake of NO_3^- is coupled with the movement of protons down an electrochemical gradient, dependent on the H^+-ATPases that maintain the proton gradient across the plasma membrane (Miller and Cramer, 2004). NH_4^+ is often the preferred N source because of its lower energy cost to assimilate compared to NO_3^- (Courty *et al*., 2015). To deal with the heterogeneous and dynamic levels of NH_4^+ and NO_3^- in the soil, which can range from less than 100µM to more than 10mM in soil solution, plant roots have N transporter proteins with different affinities (Xu *et al*., 2012). High-

affinity transport systems (HATS) in roots are able to assimilate NH_4^+ and NO_3^- at low concentrations in the soil, between 1µM and 1mM, while low-affinity transport systems (LATS) are able to assimilate NH_4^+ and NO_3^- at high concentration, greater than 0.5mM (Jackson *et al*., 2008).

The amount of N taken up by roots in soil solution only accounts for a small portion of the total N that is assimilated by most plants (Courty *et al*., 2015). This is known as the "plant" or "direct" pathway, where nutrients are directly taken up from the soil through the root epidermal cells and root hairs (Bücking *et al*., 2012; Lanfranco *et al*., 2016). The majority of N that plants receive in natural environments is from mutualistic symbiotic associations with mycorrhizal fungi, known as the mycorrhizal pathway (Bücking *et al*., 2012; Courty *et al*., 2015; Lanfranco *et al*., 2016).

1.4 Mycorrhizal Symbiosis

Fungi are heterotrophic eukaryotes that play a key role in many microbiological and ecological processes, from influencing soil fertility, decomposition and the cycling of minerals and organic matter to promoting plant health and nutrition (Finlay, 2008). They require an external source of carbon for energy and cellular synthesis, as they are unable to photosynthesize (Finlay, 2008). Fungi have evolved three strategies to obtain carbon, by living as saprotrophs, necrotrophs or biotrophs (Finlay, 2008). Saprotrophs obtain their nutrients from dead organic matter, while necrotrophic and biotrophic fungi obtain nutrients from a living host (Gupta *et al*., 2000). Necrotrophs kill their host cells while biotrophs grow in living host cells (Gupta *et al*., 2000). Biotrophic fungi may have a deleterious, neutral or symbiotic relationship with their host. One of the most ancient and widespread forms of fungal symbiosis with plants, and arguably the most important symbiosis on Earth, is the mycorrhizal association (Finlay, 2008; Bücking *et al*., 2012).

The term "mykorrhiza" was first used by A.B. Frank in 1885 to describe modified root structures in trees, but the term has since been used to describe a variety of mutualistic, symbiotic associations between fungi and plant roots (Frank, 2005 (English translation); Finlay, 2008; Smith and Read, 2008). Fossil records show that mycorrhizal interactions evolved 400 to

460 million years ago and these interactions played a major role in the colonization of land by plants (Finlay, 2008; Bücking *et al*., 2012). Mycorrhizal fungi form mutualistic associations with over 90% of all plant species (Lanfranco *et al*., 2016). These relationships are mutually beneficial for both partners and consist of a bidirectional exchange of resources (Bücking *et al*., 2012). The mycorrhizal fungus provides the plant with nutrients, such as N and phosphorus (P), and increases the abiotic (drought, salinity, heavy metals) and biotic (root pathogens) stress resistance of the plant (Bücking *et al*., 2012). In return, the plant provides the fungus with 4 to 20% of its photosynthetically derived carbon, which the fungus allocates to growing mycelium and developing spores or fruiting bodies (Bücking *et al*., 2012; Courty *et al*., 2015). These interactions have a major influence on plant nutrient use efficiency in natural ecosystems, which are usually characterized as nutrient limited, especially with respect to N and P (Courty *et al*., 2015). Mycorrhizae are able to increase the acquisition of nutrients through their extraradical mycelium (mycelium growing in the soil), which is a physical extension of the root system, and thereby increase the surface area over which nutrients can be absorbed (Finlay, 2008; Courty *et al*., 2015).

There are three main categories of mycorrhizal symbiosis based on morphology and the plant and fungal species involved: endomycorrhizae, ectomycorrhizae and ectendomycorrhizae (Finlay, 2008). The main distinction between ectomycorrhizae and the other two categories is that, here, the fungal structure does not penetrate root cells of the host (Gupta *et al*., 2000) (Figure 1.2). Endomycorrhizae can be broken down into the subgroups: Arbuscular, Ericoid and Orchidaceous mycorrhizae (Finlay, 2008). Arbuscular mycorrhizae (AM) are the most ancient mycorrhizal relationship, where arbuscular Glomalean fungi are thought to have formed the first mycorrhizal association with plants around 460 million years ago (Redecker *et al*., 2000; Finlay, 2008). AM symbiosis is the most common mycorrhizal interaction. AM fungi in the phylum Glomeromycota associate with approximately 80% of all known plant species, including agriculturally important crops such as soybeans, corn, rice and wheat (Bijl *et al*., 2011; Bücking *et al*., 2012; Lanfranco *et al*., 2016). Only 250 species of AM fungi have been described but meta-sequencing of soil samples suggest this number is much higher (Bijl *et al*., 2011; Lanfranco *et al*., 2016). Ectomycorrhizal (ECM) relationships are formed between fungi from the phyla

Basidiomycota, Ascomycota and Zygomycota, and long-lived perennial plants and trees (Finlay, 2008). Approximately 10,000 fungal species and 8,000 plant species form these relationships (Finlay, 2008). These plant species are usually trees and shrubs from cool, temperate, boreal or montane forests, as well as arctic shrubs, chaparral vegetation and species in Dipterocarpaceae and Caesalpinioideae in tropical forests (Finlay, 2008). While only 3% of seed plants form ECM associations, including families such as Betulaceae, Fagaceae, Pinaceae, and Salicaceae, they have a global importance as they contain the dominant plant species in many forest ecosystems and have economic value as the main producers of timber (Gupta *et al*., 2000; Smith and Read, 2008; Bücking *et al*., 2012). Ectomycorrhizal fungi do not penetrate the host's root cells but form intercellular hyphae called a Hartig net and a mantle or sheath around each root (discussed further below) (Finlay, 2008; Bücking *et al*., 2012). Ectendomycorrhizal relationships have characteristics of both endomycorrhizae and ectomycorrhizae, where there is fungal penetration of the plant root cell wall and a Hartig net, but a mantle may or may not be present (Gupta *et al*., 2000; Finlay, 2008).

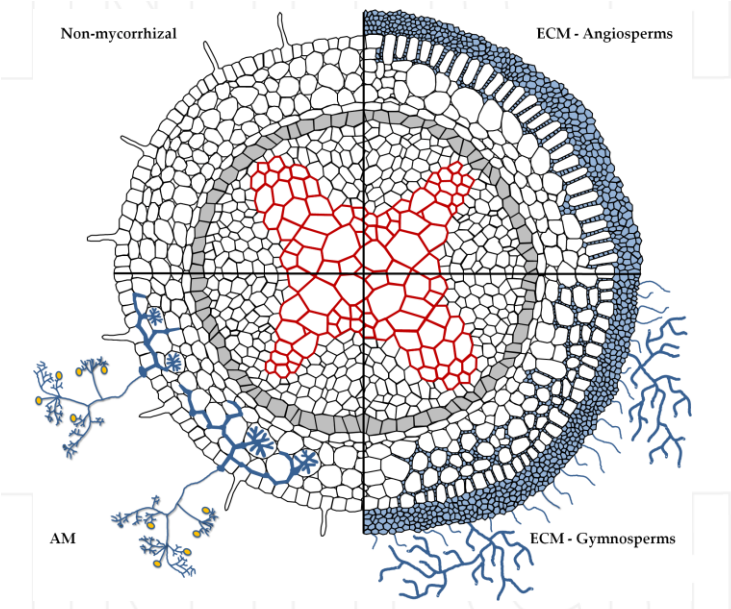


Figure 1.2 – Root cross section depicting the differences in hyphal penetration and structural characteristics between arbuscular mycorrhizae (AM) and ectomycorrhizae (ECM) (figure from Bücking *et al*., 2012).

1.4.1 Arbuscular Mycorrhizae versus Ectomycorrhizae

AM fungi are obligate biotrophs that require the host to complete their life cycle and produce spores (Bücking *et al*., 2012). AM fungal spores are able to germinate without the presence of a host, relying on stores of triacylglyceride and glycogen as energy (Bijl *et al*., 2011; Bücking *et al*., 2012). The spores produce a hyphal germ tube that extends several centimeters to search for plant roots and if no root is encountered, the hyphal extension stops (Bijl *et al*., 2011; Bücking *et al*., 2012). In the presence of a root, the hypha begins branching, signalled by strigolactones released by root exudates (Bijl *et al*., 2011; Bücking *et al*., 2012). Once the fungal hyphae reach the root, an appressorium, called a hyphopodium, is formed at the site where the fungus makes contact with the root (Bijl *et al*., 2011; Bücking *et al*., 2012). The hyphopodium is a flattened extension of the fungal hypha that penetrates the root via a penetration apparatus and guides the hypha through the epidermal cell, by invagination of the cell membrane, towards the root cortex and into the cortical cell (Bijl *et al*., 2011; Bücking *et al*., 2012). Here, in the cortical cell, extensive branching of the hypha into a bush-like structure occurs, called an arbuscule, and the interface for nutrient exchange between the fungus and plant is created (Bijl *et al*., 2011; Bücking *et al*., 2012). The arbuscule does not enter the plant cytoplasm by a plant-derived membrane called the periarbuscular membrane (PAM), which creates a periarbuscular space between the plant and fungus (Bijl *et al*., 2011; Bücking *et al*., 2012). The periarbuscular space is where the exchange of resources occurs between the plant and fungus (Bijl *et al*., 2011). The PAM contains mycorrhizae-induced transporters that enable the plant to take up nutrients from the mycorrhizal interface (Bijl *et al*., 2011; Bücking *et al*., 2012).

The long standing view has been that the AM association is unimportant in the uptake and transfer of N from the soil to plants and is only important for P uptake, due to the higher mobility of inorganic N compared to inorganic P and the inaccessibility of organic N for AM fungi (Smith and Smith, 2011). Negative, neutral and positive effects of AM associations on plant N nutrition and uptake have been found (Review by Corrêa *et al*., 2015). While the ability of AM fungi to improve the N nutrition of host plants is widespread within Glomeromycota, there is high intraspecific diversity and it is often context dependent (Munkvold *et al*., 2004; Bücking and Kalfe, 2015; Mensah *et al*., 2015). However, it is established that N can be

transferred through AM fungal hyphae from the soil to the host plant, as mycorrhiza-inducible NH_4^+ and NO_3^- transporters in the plant have been found, which allow the uptake of N from the mycorrhizal interface (Bücking and Kalfe, 2015; Correa *et al*., 2015). NH_4^+ and NO_3^- can be taken up by the extraradical mycelium (ERM) of the AM fungi via NH_4^+ and NO_3^- transporters, however, the preferred form is NH_4^+ as it is energetically less costly than NO_3^- and amino acids (Lanfranco *et al*., 2016).

NH_4^+ is taken up from the soil by NH_4^+ transporters (AMT) in the ERM. Only one AMT gene, GintAMT, an AMT of the AM fungus *Rhizophagus irregularis* (previously *Glomus intraradices*) has so far been characterized (Smith and Smith, 2011; Lanfranco *et al*., 2011; Bücking and Kalfe, 2015). High expression levels of GintAMT1 in the ERM indicate that this transporter is mainly responsible for NH_4^+ uptake by the fungal hyphae from the soil, while higher expression of GintAMT2 in the intraradical mycelium helps with re-uptake of NH_4^+ by the fungus from the symbiotic interface (Lanfranco *et al*., 2011; Bücking and Kalfe, 2015). Inside the ERM, NH_4^+ and NO_3^- compounds are converted into amino acids, mainly arginine, and translocated to the intercellular hyphae and to the PAM, where amino acids are converted back into NH_4^+ by arginase and urease and transferred from fungus to plant (Smith and Smith, 2011; Lanfranco *et al*., 2016). Tian *et al.* (2010) found that an external supply of NO_3^- stimulated a NO_3^- transporter in the ERM of *Rhizophagus irregularis*. However, this NO_3^- transporter was repressed when internal levels of NH_4^+ and downstream metabolite glutamine were increased (Tian *et al.* 2010; Bücking and Kalfe, 2015). Suppressed NO_3^- transporter expression by the presence of other N sources is known as N catabolite repression and is seen in many organisms, including the control of NH_4^+ transporters in other fungi (Bücking and Kalfe, 2015). While NH_4^+ seems to be the form of N preferred by AM fungi, studies have found AM fungi to be involved in organic N acquisition, previously thought to be a feature only of ECM (Hawkins *et al.* 2000; Hodge *et al.,* 2001; Leigh *et al*. 2009, Lanfranco *et al*., 2011). Amino acid permease (AAP) transporters have been identified in AM fungi, contributing to organic N uptake (Lanfranco *et al*., 2016). Cappellazzo *et al.* (2008) identified an AAP (GmosAAP1) from the AM fungus *Funneliformis mosseae*, which was found to be upregulated in the ERM when exposed to organic N, indicating a first step in amino acid assimilation from the soil. Carbon is transported

from the host to the fungus through monosaccharide transporters (MST2) in the arbuscular membrane of the fungus (Bücking *et al*., 2012; Lanfranco *et al*., 2016). The transporter not only takes up glucose, but other monosaccharides like xylose, indicating the fungus' ability to use cell wall sugars as a carbon source (Bücking *et al*., 2012; Lanfranco *et al*., 2016).

Like AM fungi, ECM fungi are able to live in plant roots forming mutual symbiotic relationships (Bücking *et al*., 2012). ECM fungi have lost the ability to degrade plant cell wall polysaccharides (such as cellulose and pectin), and can only penetrate between cells into the root cortex, whereas AM fungi are able to penetrate between and through root cell walls (Chalot and Plassard, 2011; Bücking *et al*., 2012). Unlike AM, that are only able to scavenge for nutrients, ECM are able to both take up nutrients from the soil and release nutrients from organic matter through hydrolysis (Chalot and Plassard, 2011).

ECM symbiosis causes morphological changes in the root structure, from extensive branching of lateral roots due to the production of many meristems, to the inhibition of root hair formation and the enlargement of cortical cells (Bücking *et al*., 2012). ECM fungi and hosts also release compounds to signal one another, although the chemicals differ from those used in the AM symbiosis (Bücking *et al*., 2012). ECM fungi produce hormones, such as auxins, cytokinins, abscisic acid and ethylene, which cause changes to root morphology, while the root exudes compounds, such as rutin and zeatin, which stimulate hyphal growth and branching towards the root (Bücking *et al*., 2012). The ECM symbiosis has three components: the hyphal sheath or mantle, the Hartig net and ERM. The hyphal sheath or mantle encloses the entire root and is structurally very diverse, forming a thin, loose assemblage of hyphae to a thick and multilayered structure. The surface can be smooth and compact or rough with many emerging hyphal strands. The mantle is involved in nutrient storage and transfers assimilated nutrients to the plant. The Hartig net is where the transfer of nutrients between the fungus and plant occurs; the interface between both partners. The Hartig net is formed through hyphal penetration between root cortex cells (intracellular). The ERM (also found in AM fungi) acts as an extension of the root system, having either individual hyphae growing into the soil or aggregates of hyphae growing in parallel, called rhizomorphs, connecting both the soil and the

sporocarps of the fungus (Smith and Read, 2008; Bijl *et al*., 2011; Bücking *et al*., 2012). In ECM, nutrients are exchanged simultaneously across the interface, which includes the plasma membranes and cell walls of both partners and the matrix between them (Bücking *et al*., 2012).

The importance of AM associations for P uptake is well known and the evidence for their involvement in N uptake is growing. ECM associations, on the other hand, are known to play a major role in the uptake of inorganic N and the release and uptake of P and N from organic sources (Chalot and Plassard, 2011). Similar to AM, nutrient transporters can be found throughout the ECM association (Bücking *et al*., 2012). ECM fungi, like their AM counterparts, have high-affinity P transporters in the ERM that allow for P assimilation (Bücking *et al*., 2012). NH_4^+ transporters (AMT) are also found in the fungal plasma membrane and contribute to either the uptake of NH_4^+ or prevention of NH_4^+ leakage (Chalot and Plassard, 2011). High-affinity AMT are upregulated in the ERM and downregulated in the Hartig net, indicating a high capability of the ERM for NH_4^+ uptake, while the Hartig net acts more as a source of NH_4^+ for the plant, as this downregulation reduces the re-absorption capabilities of the fungus (Chalot and Plassard, 2011; Bücking *et al*., 2012). The presence and upregulation of high affinity NH_4^+ importers in plant roots also aids in NH_4^+ transfer from the fungus to the plant (Bucking *et al*., 2012). Before NO_3^- is assimilated by nitrate reductase (NR) and nitrite reductase (NiR) enzymes, it is taken up in ECM associations through an energy-dependent process by specific plasma membrane NO_3^- transporters (NT). Many NTs belong to the major facilitator superfamily, specifically in the nitrate/nitrite porter family, which can be found in both prokaryotes and eukaryotes (Chalot and Plassard, 2011; Montanini *et al*., 2002; Courty *et al*., 2015). NRT2 is a well described ECM fungal NT identified from *Hebeloma cylindrosporum* and *Tuber borchii* (Chalot and Plassard, 2011; Montanini *et al*., 2002). With sufficient carbon from the plant, NRT2 genes and NTs are stimulated by external NO_3^- and N starvation but down-regulated by NH_4^+ and glutamine (Courty *et al*., 2015).

ECM fungi contain extracellular proteases that cleave proteins in organic matter (Chalot and Plassard, 2011). Mycorrhizal tissues contain fungal amino acid transporters that allow the uptake of amino acids from the soil and prevent amino acid loss by hyphal leakage (Chalot and Plassard, 2011). A high affinity general amino acid transporter, GAP1, was identified in *Amanita*

muscaria and *Hebeloma cylindrosporm* (Chalot and Plassard, 2011). All 20 amino acids found in proteins were found to bind to GAP1, indicating its broad spectrum capabilities (Chalot and Plassard, 2011). The preference of ECM fungi for particular N forms is debated. The majority of the 68 ECM fungal species studied by Nygren et al. (2008) were reported to prefer NH_4^+ as an inorganic N source, with the ability to grow on NO_3^- being widely distributed among the different species. Other studies found certain ECM species to prefer NO_3^- over NH_4^+ (Scheromm *et al.*, 1990; Montanini *et al*., 2002; Courty *et al*., 2015). Some studies show ECM fungi to have a preference for NH_4^+ over NO_3^- *in vitro* (Rangel-Castro *et al*., 2002; Guidot *et al.,* 2005) and in the field (Clemmensen *et al*., 2008). This suggests that ECM fungi may be adapted to the available N forms in the soil, which could allow the fungi to compete with other soil microbes (Chalot and Plassard, 2011). Strong competition exists in the soil for available N sources, where soil microbes other than ECM fungi are usually the first sinks for added N (Chalot and Plassard, 2011).

1.5 Site Description

The study sites in this thesis are a part of the B.C. Ministry of Forests, Lands and Natural Resource Operations' Experimental Project 571 (EP 571) and are located near Port Renfrew (latitude 48° 33-36' N; longitude 124° 19-21' W; elevation 90-250m) on the west coast of Vancouver Island which are one of three locations in this project (Berch *et al*., 2001). EP 571 is a species-espacement trial that was established in 1962 to study the stand dynamics of pure plantations of four tree species, Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), Sitka spruce (*Picea sitchensis* [Bong.] Carr.), western redcedar (*Thuja plicata* Donn ex D. Don in Lamb) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) (Omule and Krumlik, 1987; Berch *et al*., 2001). Initially supporting old-growth forests of western hemlock, western redcedar, amabilis fir and some Douglas-fir and Sitka Spruce, the sites were logged between 1958 and 1960 and slash burned in 1961 (Omule and Krumlik, 1987; Omule, 1988). Following the burning, in 1962 24 plots, around 700m^2 in area, were established at each site in all three locations with 81 seedlings planted in each plot, in a randomized complete block design (Omule and Krumlik, 1987; Klinka *et al*., 1996; Prescott *et al*., 2000). Each location contains plots with trees at

spacings of 2.7 x 2.7m, 3.7 x 3.7m or 4.6 x 4.6m (Omule and Krumlik, 1987; Prescott *et al*., 2000). Each spacing treatment is replicated twice for each of four tree species (Douglas-fir, Sitka spruce, western redcedar and western hemlock) (Omule and Krumlik, 1987).

This thesis focuses on the four tree species planted at the smallest spacings, to reduce competition by understorey vegetation. The 2.7 x 2.7m spacing plots near Port Renfrew are separated into three sites with eight plots each. The three sites, Fairy Lake (48° 35'N, 124° 19'W), San Juan (48° 35'N, 124° 12'W) and WC 1000 (48° 33'N, 124° 21'W), are topographically very distinct from one another. Fairy Lake is located midslope and faces south, WC 1000 is also on a slope but faces north and San Juan is at a valley bottom (Omule, 1988). These sites also differ in their understory vegetation, San Juan and WC 1000 are dominated by salmonberry (*Rubus spectabilis* Pursh) and sword fern (*Polystichum munitum* [Kaulf.] Presl.) while Fairy Lake is dominated by salal (*Gaultheria shallon* Pursh) and red huckleberry (*Vaccinium parvifolium* Smith) (Prescott et al., 2000). All three sites are on the windward side of the Vancouver Island Mountains located within the Submontane Very Wet Maritime Coastal Western Hemlock zone (CWHvm1) of the Biogeoclimatic Ecosystem Classification system in British Columbia (Klinka *et al*., 1994; Prescott *et al*., 2000).

Mean annual temperature in Port Renfrew is 8.8°C and mean annual precipitation is 3943mm (including 62mm of snow), with 197 frost free days per year (Prescott *et al*., 2000). The soils on the three sites are characterized as Ferro-Humic or Humo-Ferric Podzols and Leptomoders, Mormoders, and Vermimulls as the humus forms (Prescott *et al*., 2000).

1.6 Biogeoclimatic Ecosystem Classification (BEC) in British Columbia

Biogeoclimatic Ecosystem Classification (BEC) is a system of ecological classification that groups ecosystems at three levels of integration (regional, local, and chronological) and by three major elements: climate, vegetation and site (Pojar, Klinka and Meidinger, 1987; Green and Klinka, 1994). The BEC system is based on the climax vegetation from later successional stages (Green and Klinka, 1994). Understory vegetation is a very useful indicator of site quality and successional development and a predictor of the potential natural vegetation of that site (Pojar, Klinka and Meidinger, 1987). The BEC system also uses site quality, along with vegetation and climate, to classify ecosystems. The basic elements of site quality include climate, soil moisture regime (SMR) and soil nutrient regime (SNR) (Green and Klinka, 1994). SMR is the average annual amount of soil water available to plants, with nine classifications, from the driest soil being "very xeric" (0) to the wettest being "hydric" (8) (Pojar, Meidinger and Klinka, 1991; Green and Klinka, 1994). SNR is the amount of essential soil nutrients available to plants, particularly nitrogen for forest ecosystems (Green and Klinka, 1994). SNR has five classes, "very poor", with low amount of N and other nutrients and slow turnover of organic matter, to "very rich", with high amount of available N and other nutrients and fast turnover of organic matter (Green and Klinka, 1994). An edaptopic grid, with the ranges for relative SMR and SNR, is used to display the relationships between the different sites that occur in that regional climate (Pojar, Meidinger and Klinka, 1991). Vegetation analyses are undertaken to identify sites and indicator plants are used to infer relative SMR and SNR (Green and Klinka, 1994).

The sites in this study are in the Submontane Very Wet Maritime Coastal Western Hemlock biogeoclimatic zone (CWHvm1). CWHvm1 is the most widespread BEC unit in the Vancouver Forest Region (Green and Klinka, 1994). This zone occurs along the windward side of Vancouver Island, from Jordan River to Port Hardy, and on both sides of the Island, north of Kelsey Bay (Green and Klinka, 1994). On the mainland, it occurs along the windward slopes of the Coast Mountains (Green and Klinka, 1994). On Vancouver Island, it contains an elevational range from sea level to 600m (Green and Klinka, 1994). The climate of the CWHvm1 is wet and

humid, with cool summers and mild winters, with very little snow (Green and Klinka, 1994). The growing season is long and precipitation is high (Green and Klinka, 1994). Forests are dominated by western hemlock, amabilis fir and, to a lesser extent, western redcedar (Green and Klinka, 1994). The understory vegetation is well developed, with the shrub layer dominated by red huckleberry and Alaskan blueberry and the moss layer dominated by step moss (*Hylocomium splendens)* and lanky moss (*Rhytidiadelphus loreus)* (Pojar, Meidinger and Klinka, 1991; Green and Klinka, 1994). Herbs are scarce and other plant species such as deer fern *(Blechnum spicant)*, five-leaved bramble (*Rubus pedatus*), bunchberry (*Cornus canadensis*) and queen's cup (*Clintonia uniflora*) are less common (Green and Klinka, 1994).

1.7 Conifer Species Studied

1.7.1 Douglas-fir

Douglas-fir (*Pseudotsuga menziesii*) is one of the most valuable timber trees in the world and is a dominant tree species in the forests of Western North America (Hermann and Lavender, 1990). Douglas-fir belongs to the *Pinaceae* family, and, despite its name, is not a true fir. There are two varieties of Douglas-fir, coastal Douglas-fir (*P. menziesii* (Mirb.) Franco var. *menziesii*) and Rocky Mountain or blue Douglas-fir (*P. menziesii* var. *glauca* (Beissn.) Franco) (Hermann and Lavender, 1990). The coastal Douglas-fir has a native range 2200km in length, from west-central B.C. to central California (Hermann and Lavender, 1990). The Rocky Mountain variety has a larger range of 4500km, stretching from the Rocky Mountains in Canada to central Mexico (Hermann and Lavender, 1990). Douglas-fir can thrive under a variety of climates, from the maritime climate of the coastal Pacific Northwest, characterized by mild, wet winters and cool, dry summers with a long frost free season to the more continental climate of the interior, characterized by colder and longer winters with more snow, hot summers, and a shorter frost free season (Hermann and Lavender, 1990). The coastal Douglas-fir grows best on well-aerated soils with a pH of 5 to 6 but does not perform well on poorly drained or compacted soils (Hermann and Lavender, 1990). The main limiting factors of Douglas-fir growth are temperature in the northern range and moisture in the south (Hermann and Lavender, 1990). Douglas-fir forms mycorrhizal associations with ectomycorrhizal fungi.

1.7.2 Sitka Spruce

Sitka Spruce (*Picea sitchensis*) is the largest spruce species in the world and is commercially valuable for lumber and pulp (Harris, 1990). Sitka spruce belongs to the family *Pinaceae* and is a coastal species, needing humid conditions for growth (Harris, 1990). It has a long but narrow range along the north Pacific coast of North America, from Alaska to California, where the widest part of the range extends 210km inland in southeast Alaska and northern B.C. (Harris, 1990). Sitka spruce only grows in a maritime climate, where the winters are mild, summers cool and frequent rain, fog and moist air provide ample moisture year around (Harris, 1990). Sitka spruce requires high levels of available calcium, magnesium and phosphorus and grows best on deep, moist well-aerated and well drained soils, doing poorly on swampy sites (Harris, 1990). Sitka spruce is frequently found on alluvial soils along streams, or on soils with high organic material, with a pH of 4.0 to 5.7 (Harris, 1990). Sitka spruce forms mycorrhizal associations with ectomycorrhizal fungi.

1.7.3 Western hemlock

Western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) is an important tree species for the forestry industry as it is an “all-purpose raw material”, used for fiber and construction (Packee, 1990). Western hemlock belongs to the family *Pinaceae* and grows best in humid areas (Packee, 1990). Western hemlock grows along the Pacific coast of North America, a distance of 3200km from central California to Alaska, and is a dominant tree species in coastal B.C. (Packee, 1990). It prefers a mild, humid climate with abundant fog and precipitation (Packee, 1990). Western hemlock is a very versatile species as it grows on soil from all bedrock types and all major landforms within its range (Packee, 1990). Height decreases with increasing soil clay content and soil bulk density, due to decreased soil aeration and the inability of the roots to penetrate compacted soil (Packee, 1990). Western hemlock forms mycorrhizal associations with ectomycorrhizal fungi.

1.7.4 Western redcedar

Western redcedar (*Thuja plicata*) is in the family Cupressaceae. It has very valuable wood that is resistant to decay and insects, allowing the trees to live for 800 - 1,000 years (Minore, 1990). Western redcedar has a native range along the Pacific coast from the middle of California to the southeastern point of Alaska, where optimal growth occurs at the latitudinal centre (Minore, 1990). The smaller interior range extends from the southwestern point of the Continental Divide in B.C. through to western Montana and northern Idaho (Minore, 1990). In B.C., western redcedar's elevational range is from sea level to 1190 m (Minore, 1990). Coastal western redcedar grows in a maritime climate, where annual precipitation ranges from 890mm to 6600mm (Minore, 1990). It is commonly found on forested swamps but can also be found on sites too dry for western hemlock, due to its stronger root penetration (Minore, 1990). Western redcedar can tolerate stagnant winter water tables, around 15cm below the soil surface (Minore, 1990). It can also tolerate a wide range of soil properties and is found on all landforms, soil textures and parent materials on Vancouver Island (Minore, 1990). Western redcedar stands increase the surface soil cation exchange capacity, pH and amount of exchangeable calcium (Minore, 1990). Western redcedar forms mycorrhizal associations with arbuscular mycorrhizal fungi.

1.8 Study Objective

This study focused on the interrelationships between soil N form availability, tree species performance and N form uptake by mycorrhizae. The overall objective of this study was to determine the inorganic and organic N levels in the soil under four conifer species on two sites expected to differ in N availability, and to relate these measures to growth of the tree species and the N form preferences of the mycorrhizal tree roots. Chapter Two presents the characterization of soil N concentration and production under four conifer species planted on two sites. Chapter Three relates tree growth, foliar and ectomycorrhizal sporocarp N concentrations and natural ^{15}N abundance on the two sites to soil N levels. Chapter Four focuses on N form uptake capacity by AM and ECM on sites with contrasting N availability using

microelectrode ion flux measurements. Chapter Five presents an overall conclusion. Hypotheses for each set of experiments are presented in the respective chapters.

The mycorrhizal symbiosis between fungi and plants is arguably one of the most important plant associations, however, much about this symbiosis is not well understood. We know little about the N form preferences of mycorrhizal fungi and whether differences in N form uptake by mycorrhizal species relate to their N environment. This thesis aims to address the question of mycorrhizal adaptation to the soil N environment.

Chapter 2 – Soil Nitrogen Availability

2.1 Introduction

The availability of N and the N forms in a soil are dependent on the N cycle in that environment. Soil N cycling and transformation processes, such as the mineralization of organic N to NH_4^+ and nitrification of NH_4^+ to NO_3^-, are mainly mediated by communities of soil microorganisms, as affected by the environment (Ribbons *et al*., 2016). The balance between gross and net rates of mineralization and nitrification can indicate the turnover and immobilization rates of NH_4^+ and NO_3^-, which are often rapid in forest soil (Ribbons *et al*., 2016). The N cycle, and the microorganisms that are involved are very dynamic and are affected by many factors.

2.1.1 Factors affecting the N cycle and N production

Microbial population

For each stage of the N cycle, specific microorganisms are needed to mediate the conversion of N into successive forms. For nitrification, autotrophic (and even some heterotrophic) nitrifying bacteria must be present in the soil (Sylvia *et al*., 1999). Nitrifiers are present in most soils but they may be present at levels too low to produce significant amounts of NO_3^-. For example, most woodland soils have low available NO_3^- because they have relatively few nitrifying bacteria, however, a disturbance may increase NH_4^+ levels thereby increasing NO_3^- and nitrifier populations (Sylvia *et al*., 1999; Kronzucker *et al*., 1997).

Soil aeration

Nitrifiers are almost exclusively aerobic and require high concentrations/fluxes of oxygen in the soil for nitrification to occur (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). Nitrification is optimal when soil pore space is approximately 60% water-filled (Sylvia *et al*., 1999). As oxygen becomes limiting, autotrophic nitrifiers produce more NO and N_2O (Sylvia *et al*., 1999). The concentration of oxygen in the soil is also the most important controlling factor for denitrifying bacteria (Sylvia *et al*., 1999; Miller and Cramer, 2004). Denitrification is an anaerobic process, where oxygen inhibits bacterial enzyme activity (Sylvia *et al*., 1999; Miller and Cramer, 2004).

Substrate availability

After oxygen availability, substrate availability is the next most important regulating factor for the different stages of the N cycle (Sylvia *et al*., 1999). In most soils, the productivity of heterotrophic microorganisms in ammonification is limited mainly by the amount of carbon (C) available. The C/N ratio determines whether N is mineralized or immobilized (Sylvia *et al*., 1999). For many denitrifying bacteria, denitrification rates are also limited by C, as well as NO_3^- availability (Sylvia *et al*., 1999). Whether C or NO_3^- is more limiting is dependent on soil type, plant community or management practices, however, NO_3^- is limiting in most soils as net N mineralization and net NO_3^- production is small (Sylvia *et al*., 1999). For nitrification, NH_4^+ availability is the next most limiting factor after oxygen (Sylvia *et al*., 1999). Since nitrification is dominated by autotrophic nitrifiers, CO_2 concentrations also affect nitrifier activity (Sylvia *et al*., 1999). Higher CO_2 in the soil compared to the atmosphere is beneficial for these bacteria as long as O_2 does not become limiting (Sylvia *et al*., 1999).

Other soil and environmental factors

Factors such as soil pH, temperature, water potential and salinity can all affect the microorganisms in the N cycle (Sylvia *et al*., 1999). Generally, autotrophic nitrifiers are neutrophilic and nitrification rates decrease in soils with pH 4.5 or less (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). At a high soil pH, NO_2^- can accumulate because there is a greater inhibition of nitrite-oxidizers compared to ammonium-oxidizers (Sylvia *et al*., 1999). However, high rates of nitrification and high NO_3^- concentrations have been observed in some acid soils (pH<4.5), which could be due to acidophilic autotrophic nitrifiers, heterotrophic nitrifiers and alkaline microsites (Sylvia *et al*., 1999). Denitrifiers also have optimal activity at near neutral soil pH (Sylvia *et al*., 1999). Temperature affects most biological processes and, in general, biological processes increase as temperature increases until a maximum threshold is reached, after which activity begins to decrease (Sylvia *et al*., 1999). For example, nitrification decreases at temperatures below 5°C and above 40°C (Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004). Lack soil water also inhibit nitrification and denitrification (Morot-Gaudry and Touraine, 2001; Miller and Cramer, 2004).

Allelochemical inhibitors

Specific secondary metabolites, such as tannins and polyphenols, can inhibit nitrification (Sylvia *et al*., 1999). These inhibitors are usually not the primary reason for low NO_3^- concentrations in soils, as competition by plant uptake and microbial immobilization have greater effects on the NO_3^- pool (Sylvia *et al*., 1999).

2.1.2 The Effects of Tree Species on Soils

Tree species influence the chemical and biological processes and properties of the soil beneath them, particularly the forest floor (Ribbons *et al*., 2016). Tree species directly influence the soil through leaf litter input and the subsequent formation of the forest floor, and through root litter inputs and the alteration of the soil structure (Ribbons *et al*., 2016). Also, tree species affect rates of litter decomposition, nutrient release, C turnover and soil respiration (Ribbons *et al*., 2016). Differences in N-cycling processes, and resulting availability of N forms have been found under different tree species (Ste-Marie and Paré, 1999; Malchair and Carnol, 2009; Ribbons *et al.*, 2016), as well as differences in microbial communities (Leckie *et al*., 2004; Prescott and Grayston, 2013; Prescott and Vesterdal, 2013). However, the influence of tree species on soils may be context dependent (Prescott and Vesterdal, 2013).

Prescott and Vesterdal (2005) described the effects tree species have on soil chemistry in B.C., characterizing Douglas-fir forest floors as having intermediate pH and calcium (Ca) and phosphorus (P) concentrations relative to other B.C. conifer species. High bacterial:fungal ratio, net N mineralization, nitrification and N concentrations were also found in these forest floors, due to high N concentrations and low lignin:N ratio of Douglas-fir needle litter (Prescott and Vesterdal, 2005). Sitka spruce forest floors have moderately high net N mineralization rates and a low bacterial:fungal ratio compared to other conifer species (Prescott and Vesterdal, 2005). Intermediate to high concentrations of N, P, Ca, and potassium (K) were found in Sitka spruce litter and forest floors, with faster decomposition of needle litter compared to cedar, hemlock and Douglas-fir (Prescott and Vesterdal, 2005). Western hemlock forest floors and needle litter have lower pH and Ca levels, relative to other species (Prescott and Vesterdal, 2005). Compared to western redcedar and Douglas-fir, western hemlock has intermediate rates of net N

mineralization and N concentration of the forest floor and needle litter, as well as intermediate rates of litter decay (Prescott and Vesterdal, 2005). Western hemlock forest floors are also dominated by NH_4^+ and have a low bacteria:fungal ratio (Prescott and Vesterdal, 2005). Western redcedar forest floors have high pH, concentration of Ca, bacterial:fungal ratio and proportion of NO_3^- compared to other conifers (Prescott and Vesterdal, 2005). However, western redcedar has the lowest rates of litter decomposition and net N mineralization, and therefore N concentrations are usually not greater than under the other three species (Prescott and Vesterdal, 2005).

2.1.3 The Effects of Mycorrhizae on Soil N Processes

The dominant view of soil decomposition and N cycling processes historically focused on two separate functional groups of soil fungi, those that were decomposers (e.g. saprotrophic fungi) involved in the breakdown of organic matter and subsequent release of nutrients, and those that were fungal mutualists (i.e. mycorrhizae) that absorbed mineral ions that were released from the decomposition process (Read and Perez-Moreno, 2003). However, research over the last few decades has shown that these mycorrhizal fungal mutualists are not only able to scavenge nutrients, but can degrade organic substrates as well, leading to the classification of different mycorrhizal types (ECM, AM, ericoid, etc.) based on their abilities to mobilize nutrients from polymers (Read and Perez-Moreno, 2003).

In forest ecosystems, almost all tree species are associated with AM or ECM fungi (Phillips *et al*., 2013). Due to differences in aboveground and belowground traits and processes, AM and ECM plants contribute to variations in the biogeochemical makeup and nutrient profiles of the ecosystems they dominate (Phillips *et al*., 2013). For example, ECM fungi, but not AM fungi, produce hydrolytic and oxidative extracellular enzymes that allow them to access organic nutrient pools unavailable to AM fungi (Phillips *et al*., 2013; Lin *et al*., 2017). These enzymes degrade soil organic matter allowing ECM fungi to take up N from chitin, protein and phenol-protein compounds and P from inositol phosphates (Phillips *et al*., 2013). Also, ECM fungi can weather minerals by releasing organic chelators and hydrogen ions to increase P and calcium availability (Phillips *et al*., 2013). This difference between AM and ECM fungi also

affects the decomposition rates, and resulting nutrient release from AM and ECM plant leaf litter (Phillips *et al*., 2013; Lin *et al*., 2017). Litter of AM plants decomposes rapidly compared to ECM plant litter, as AM fungi scavenge for nutrients released by saprotrophic microbes while ECM fungi can degrade their own plant litter (Phillips *et al*., 2013; Lin *et al*., 2017; Read and Perez-Moreno, 2003). These differences create a pattern of mineral versus organic N cycling, where, in general, AM dominated forest soils have higher inorganic N (NH_4^+ and NO_3^-) concentrations (higher inorganic N: organic N ratios), net N mineralization and net nitrification rates than ECM dominated forest soils (Lin *et al*., 2017). This pattern indicates a mineral (inorganic) N economy in AM trees and an organic N economy in ECM trees (Phillips *et al*., 2013; Lin *et al*., 2017).

2.1.4 Range of nitrogen in temperate forest soils

While N is considered to be the limiting nutrient to tree growth in temperate forests (Sylvia *et al*., 1999; Chalot and Plassard, 2011; Courty *et al*., 2015), the availability of inorganic and organic N varies widely depending on topography, soil type, vegetation, precipitation and many other chemical, physical and biological factors. Temperate rainforests of Tasmania were found to have 31.1 µmol NH_4^+/g soil and 0.6 µmol NO_3^-/g soil (Adams *et al*., 1989) and cold-temperate forests in Connecticut have been shown to contain 1.71 – 25.8 µmol total inorganic N/g soil (Berthrong and Finzi, 2006). In Oregon, Douglas-fir forests were found to have 2.59 µmol NH_4^+/g soil and 0.54 µmol NO_3^-/g soil while western hemlock and Sitka spruce forests had 4.53 µmol NH_4^+/g soil and 0.08 µmol NO_3^-/g soil (Stark and Hart, 1997). Temperate wet conifer forests in Ireland have been found to contain 1.4-2.8 µmol amino acids/g of mineral soil (Jones *et al*., 2002). This range in inorganic and organic N concentrations can also be seen in soils of British Columbia, where temperate conifer forests have been shown to have 6-42 µmol NH_4^+/g soil, 21-50 µmol NO_3^-/g soil and 0.1-0.5 µmol amino acids/g soil (Prescott *et al*., 2000; Hannam and Prescott, 2003).

2.1.5 Characteristics of EP571 Port Renfrew

This study focuses on two sites in the EP571 espacement trial, Fairy Lake (48° 35'N, 124° 19'W) and San Juan (48° 35'N, 124° 12'W), both near Port Renfrew, B.C. Fairy Lake and San Juan are topographically distinct from one another. Fairy Lake is located mid-slope, on a water-shedding site, with an elevation of 200-280m, a south-southwest aspect and a slope gradient of 10-60% (Omule, 1988; Prescott *et al*., 2000). San Juan is in a valley bottom, and is a water-receiving site, with an elevation of 65-85m (Omule, 1988; Prescott *et al*., 2000). The moisture and nutrient regime of the soils in Fairy Lake is characterized as slightly dry and very poor to medium (Klinka *et al*., 1996; Prescott *et al*., 2000). The soil in San Juan ranges from fresh to very moist and from poor to very rich, depending on the plot (Klinka *et al*., 1996; Prescott *et al*., 2000). The dominant humus form in Fairy Lake is Leptomoder-Mormoder and Vermimull-Leptomoder in San Juan (Prescott *et al*., 2000). These sites also differ in their understory vegetation, San Juan is dominated by salmonberry (*Rubus spectabilis* Pursh), sword fern (*Polystichum munitum* [Kaulf.] Presl.) and lady fern (*Athyrium filix-femina* (L.) Roth), and to a lesser extent, deer fern (*Blechnum spicant* (L.) Sm.), salal (*Gaultheria shallon* Pursh) and three leaf foamflower (*Tiarella trifoliata* L.) (Klinka *et al*., 1996; Prescott *et al*., 2000). Fairy Lake is dominated by salal and red huckleberry (*Vaccinium parvifolium* Smith), and to a lesser extent, deer fern, Alaska blueberry (*Vaccinium alaskaense* Howell*)*, sword fern and salmonberry (Klinka *et al*., 1996; Prescott *et al*., 2000). Mean annual temperature in Port Renfrew is 8.8°C and mean annual precipitation is 3943mm (including 62mm of snow), with 197 frost free days per year (Prescott *et al*., 2000). These sites are on the windward side of the Vancouver Island Mountains located within the Submontane Very Wet Maritime Coastal Western Hemlock zone (CWHvm1) of the Biogeoclimatic Ecosystem Classification (BEC) system in British Columbia (Klinka *et al*., 1994; Prescott *et al*., 2000).

2.1.6 Previous studies of EP571

In previous studies, Prescott *et al*. (2000) and Ribbons *et al*. (2016), examined forest floor chemistry, nutrient concentrations and rates of N mineralization and nitrification among the sites and four conifer species (Douglas-fir, western redcedar, western hemlock and Sitka

spruce) in the EP571 species-espacement trial. Prescott *et al*. (2000) found no significant differences in initial NO_3^--N and NH_4^+-N concentrations in the forest floor between San Juan and Fairy Lake, but found higher N mineralization rates, nitrification rates and concentrations of P and K in San Juan. Of the four sites sampled in EP571, nutrient concentrations in the forest floor differed among the four tree species, but no significant site*species interactions were found (Prescott *et al*., 2000).

Ribbons *et al*. (2016) found no significant differences in pH and total C and N concentrations of the forest floor between Fairy Lake and San Juan, among the four tree species, or in site*species interactions. Gross ammonification (N mineralization) rates and gross NH_4^+ consumption were found to be significantly higher in the forest floor at San Juan compared to Fairy Lake and greatest under western redcedar. Rates of gross nitrification and NO_3^- consumption were found to be greatest in the forest floor in Fairy Lake compared to San Juan, but no significant differences were seen among the tree species overall. The site*species interaction was found to be significant (Ribbons *et al*., 2016).

2.1.7 Study Objective

The objective of this study was to characterize the N forms and production in the two contrasting sites, Fairy Lake and San Juan, through point samples and buried bag analyses. The purpose of characterizing N on these sites was to relate the results to later work on mycorrhizal N form uptake. Although earlier studies examined N form production on these sites, I wanted to expand on these studies by using different methodologies, examining the mineral soil along with the forest floor, characterizing organic N production and to confirm differences in NH_4^+ and NO_3^- production between the sites, as contradictory results were found. Based on these previous soil studies, and on differences in topography, moisture and understory vegetation, I hypothesized that the soil in San Juan would have greater NH_4^+, NO_3^- and amino acid production than in Fairy Lake. I hypothesized that there would be differences in N forms and production in the soil under the four different trees species, Douglas-fir, Sitka spruce, western redcedar and western hemlock. I hypothesized that ECM Douglas-fir and Sitka spruce plots might have greater N production, and potentially greater inorganic N concentrations than the

AM western redcedar plots. Lastly, I hypothesized that sites of greater productivity as indicated by the BEC gradient would exhibit greater NH_4^+, NO_3^- and amino acid production.

2.2 Materials and Methods

2.2.1 Site Description

The study area was located near Port Renfrew, B.C. and is part of the B.C Ministry of Forests, Lands and Natural Resource Operations' Experimental Project (EP) 571 espacement trial planted in 1960 (Omule, 1988). Three sites from the EP571 trial were used for the study: Fairy Lake (48° 35'N, 124° 19'W, 200-280m elevation), San Juan (48° 35'N, 124° 12'W, 65-85m elevation) and WC1000 (48° 33'N, 124° 21'W), however, Fairy Lake and San Juan were the main focus of this study. Each site contained 24, 700m^2 plots with trees of four species planted at three spacings, in a randomized complete block design. The 2.7m spacing plots were used for this study. All three sites consisted of two plots each of Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW) at 2.7 m spacing (Table 2.1).

Table 2.1 – Plot number and corresponding tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and BEC site series numbers for the 2.7 x 2.7m spacing plots in the three sites in the EP 571 trial near Port Renfrew, B.C.

Site	Plot number	Tree Species	BEC Site Series number
Fairy Lake (FL)	18	FD	01
	22	HW	03
	23	CW	03
	24	HW	03
	25	FD	06
	32	CW	05/01
	36	SS	05/01
	38	SS	05/01
San Juan (SJ)	41	CW	07
	42	FD	09
	43	SS	07
	44	DF	09
	45	HW	09
	46	SS	09
	47	HW	09
	48	CW	09
WC 1000	2	HW	
	3	FD	
	4	CW	
	5	SS	
	6	CW	
	7	FD	
	13	SS	
	16	HW	

2.2.2 Data Collection and Preparation

Inorganic (NO_3^--N and NH_4^+-N) and organic (amino acid-N) nitrogen (N) concentrations in the soil were determined through the buried bag method, Plant Root Simulator (PRS®) probes and point samples were taken in May 2015. The buried bag method determined the gross inorganic and organic N production during a 6-week incubation period. The PRS® probes determined the available NH_4^+-N and NO_3^--N ions during a 6-month period. The point samples determined inorganic and organic N concentrations available at a specific point in time, time 0

(T0), which was the start of the 6-week incubation period. Additional point samples were also taken in July 2015 for soil physical and chemical analyses: bulk density, N mineralization, pH, exchangeable cations, cation exchange capacity (CEC), %N, %C, δ^{15}N and % moisture content.

2.2.2.1 May Point Sampling (T0)

From May 14-22, 2015, three forest floor (FF) and mineral soil (MS) samples were taken from each of the eight plots in Fairy Lake and San Juan (96 samples from 16 plots in total). The six samples were taken in a systematic pattern; one from the top left corner of the plot, one from the middle and one from the bottom right corner. At each location in the plot, large twigs, leaves and debris were brushed from the surface and a 10cm diameter PVC ring was placed on top of the forest floor. With a soil knife and using the PVC ring as a tracer, a 10cm diameter circle of forest floor was removed and placed into a plastic bag and tied. Then, using a stony soil auger, a 20cm deep core of mineral soil was removed and placed into a separate bag and tied. The samples were put on ice in a cooler and brought back to a 4°C fridge.

2.2.2.2 Buried Bag Method (T6)

From May 14-22, 2015, five forest floor and mineral soil samples were buried in each of the eight plots in Fairy Lake and San Juan (160 samples from 16 plots in total). The samples were buried in a systematic pattern; one forest floor and mineral soil sample was buried roughly 3m from each of the four corners of each plot, and one in the middle. Before the samples were taken, large twigs, leaves and debris were brushed from the surface and a 10cm diameter PVC ring was placed on top of the forest floor. With a soil knife and using the PVC ring as a tracer, a 10cm diameter circle of forest floor, ranging from 1.5 to 5cm in thickness, was removed and placed into a polyethylene bag (grocery store produce bags) and tied. A 20cm deep core of mineral soil was removed, using a stony soil auger, and placed into a separate polyethylene bag and tied. The mineral soil bag was placed back into the hole, with the forest floor bag placed on top. The hole was then covered with leaves and branches and flagged. The buried bags were removed after 6 weeks (T6) (June 22nd-29th, 2015), placed on ice in a cooler and brought back to a 4°C fridge.

The soil samples from the T0 point sampling and buried bags were all chemically extracted fresh within 24-48 hours of removal from the sites. Prior to extraction, all soils were sieved in a 0.5mm strainer to remove large debris. For the T0 point samples, all three forest floor samples per plot were mixed together by hand and two 4g subsamples were weighed out and 40mL of 2M KCl solution were added to each (32 samples in total). The samples were then shaken for one hour in a benchtop reciprocal shaker and centrifuged at 3500 rpm for 15 minutes at 20°C, followed by storage in a -20°C freezer. An additional 4g subsample was removed to determine moisture content. This same procedure was done for T0 mineral soil samples (32 samples in total). For the buried bag samples, a similar protocol was followed but each sample was processed individually. Each forest floor and mineral soil sample was mixed by hand separately and one 4g subsample was taken from each (10 samples per plot). 40mL of 2M KCl was added to each subsample, solutions were shaken for one hour and centrifuged at 3500 rpm for 15 minutes at 20°C, followed by storage in a -20°C freezer (160 samples in total).

2.2.2.3 July Point Sampling

From July 6-7, 2015, five forest floor and mineral soil samples were taken from each of the eight plots in Fairy Lake and San Juan (160 samples from 16 plots in total). The samples were taken in a systematic pattern; one forest floor and mineral soil sample was taken from the midpoint between every corner and one from the middle of the plot. Similar to the May point samples, large twigs, leaves and debris were brushed from the surface and a 10cm diameter PVC ring was placed on top of the forest floor at each location in the plot. With a soil knife and using the PVC ring as a tracer, a 10cm diameter circle of forest floor was removed and placed into a plastic bag and tied. Then, using a stony soil auger, a 20cm deep core of mineral soil was removed and placed into a separate bag and tied. The samples were put on ice in a cooler and brought back to a 4°C fridge. Within 24-48 hours of collection, the samples were laid out in a greenhouse to air dry for approximately two weeks (July 8-21, 2015). After the samples were air dried, all forest floor and mineral soil samples were separately weighed, lightly crushed with a hammer and rolling pin to break down large soil chunks, and sieved in a 2mm sieve pan. The

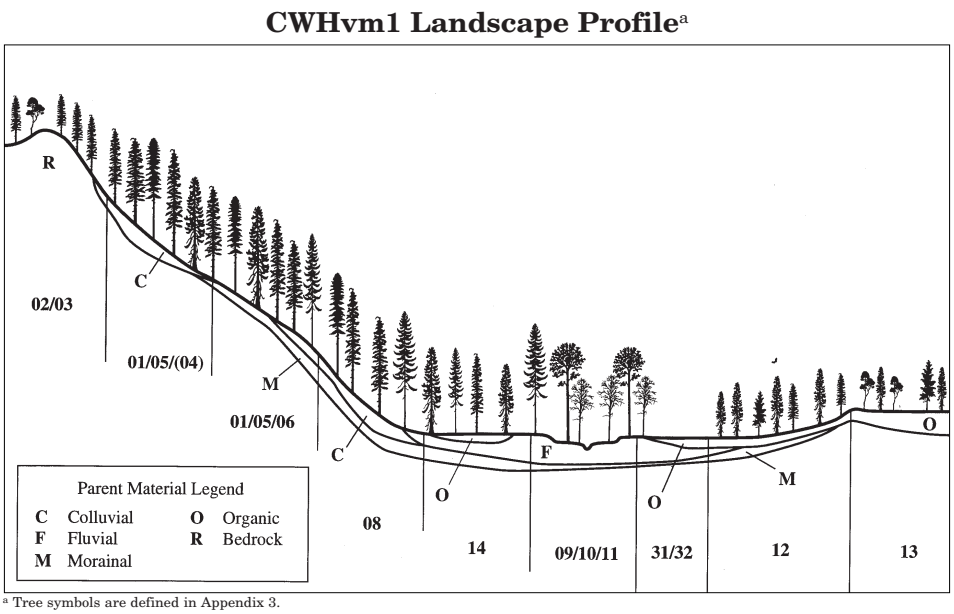
samples were weighed again in order to determine the bulk density of the soils. The remaining samples were stored in a cool dry place for use in later analyses (N mineralization, pH, exchangeable cations, CEC, %N, %C and moisture content).

2.2.2.4 Plant Root Simulator (PRS®) probes

PRS® probes measure soil ion supply *in situ* through ion capture with positively and negatively charged ion resin membranes. From May 14-28, 2015, eight NH_4^+-N and eight NO_3^--N PRS® probes (15cm x 3cm x 0.5cm) from Western Ag Innovations were placed into each of the eight plots in Fairy Lake, San Juan and WC 1000 (384 probes on 24 plots in total). The NH_4^+-N and NO_3^--N probes were paired and four of these pairs were placed in a zig-zag pattern on each half of every plot. At each location in the plot, the forest floor was removed and both types of probe were inserted into the mineral soil side by side, on a diagonal until the top of the probes were covered. Depending on the soil texture and rock content, a soil knife was used to create an opening for the probes. The probes were then flagged and covered with soil and leaves. The PRS® probes were removed six months later (November 13, 2015), cleaned in distilled water and shipped to Western Ag Innovations for analysis within the week. Both types of probes (NH_4^+-N and NO_3^--N) were analyzed in groups of four, giving two data points per ion in each plot.

2.2.2.5 BEC Site Series

While in the field, BEC site series for all plots in Fairy Lake and San Juan were determined using the Field Guide for Site Identification and Interpretation for the Vancouver Forest Region (Green and Klinka, 1994). Based on a general survey of topography (Figure 2.1), vegetation (Figure 2.2) and soil moisture and nutrient regimes (Figure 2.3), each plot was given a specific site series in the CWHvm1 BEC unit. BEC site series: 03, 01, 05/01, 06, 07 and 09 were determined, indicating a gradient of increasing N richness (Table 2.1).



[a] Tree symbols are defined in Appendix 3.

Figure 2.1 – BEC site series based on the landscape profile in the CWHvm1 zone, from the Field Guide for Site Identification and Interpretation for the Vancouver Forest Region (Green and Klinka, 1994).

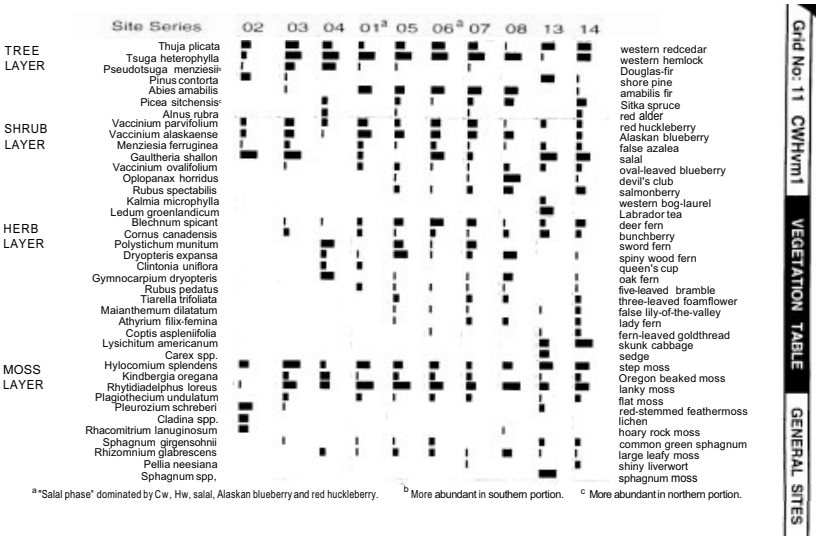


Figure 2.2 – BEC site series based on the dominant vegetation in the CWHvm1 zone, from the Field Guide for Site Identification and Interpretation for the Vancouver Forest Region (Green and Klinka, 1994).

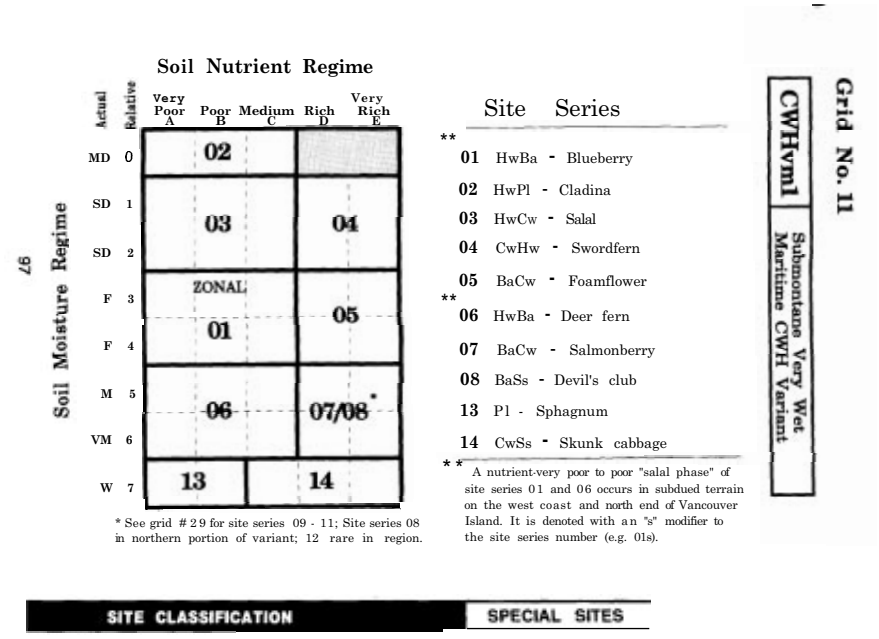


Figure 2.3 – BEC site series based on the soil moisture and nutrient regime in the CWHvm1 zone, from the Field Guide for Site Identification and Interpretation for the Vancouver Forest Region (Green and Klinka, 1994).

2.2.3 Analyses

2.2.3.1 Inorganic N (NH_4^+-N and $N0_3^-$-N)

The Astoria Pacific Segmented Flow Analyzer was used to determine the NH_4^+-N and NO_3^--N concentrations in the 2M KCl buried bag and T0 extracts. All frozen samples were thawed, shaken for 15 minutes in a benchtop reciprocal shaker and centrifuged at 3500 rpm for 15 minutes. The resulting supernatant was then removed for analysis, 2M KCl was used as a blank and standards were made following the protocol at the Pacific Forestry Centre, Canadian Forest Service, Victoria, B.C (Bremmer, 1965; Carter, 1993).

2.2.3.2 Organic N (Amino Acid-N)

The 2M KCl buried bag and T0 extracts were also used to determine the free amino acid-N present in the soil. All frozen samples were thawed, shaken for 15 minutes in a benchtop reciprocal shaker and centrifuged at 3500 rpm for 15 minutes. The resulting supernatant was then analyzed using a standard ninhydrin method and measured on a UV-visible spectrophotometer at 570nm for the total N concentration (µg N/mL) at the Pacific Forestry Centre (Amato and Ladd, 1988; Joergensen and Brookes, 1990; Swift and Bignell, 2001). Since ninhydrin also reacts with NH_4^+-N in the solution, the NH_4^+-N levels determined from the segmented flow analyzer were subtracted from the total N concentrations to give the amino acid-N concentrations in each sample.

2.2.3.3 Mineralizable N

Soil samples from the July point sampling were used to determine mineralizable N by anaerobic incubation (Bremmer, J.M. 1996). Three grams of mineral soil and 0.75g of forest floor were incubated in MilliQ water in an incubation chamber at 30°C in the dark for two weeks. After two weeks, the samples were removed, extracted with 3M KCl solution and shaken in a reciprocal shaker for two hours. Once mixed, the extracts were centrifuged at 3000 rpm for 30 minutes. The resulting supernatant was then removed and analyzed for NH_4^+-N in the Astoria Pacific Segmented Flow Analyzer at the Pacific Forestry Centre.

2.2.3.4 Plant Root Simulator (PRS®) probes

Once removed from the field and cleaned with distilled water to remove excess soil, the PRS® probes were mailed to Western Ag Innovations in Saskatoon, Saskatchewan and analyzed for NH_4^+-N and $NO3^-$-N (µg/10cm^2/6 months), in all three sites (Fairy Lake, San Juan and WC 1000), colorimetrically using an automated flow injection analysis system. The probes in Fairy Lake and San Juan were also analyzed for calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), sulphur (S), lead (B), aluminum (Al) and cadmium (Cd) using inductively-coupled plasma spectrometry.

2.2.3.5 N concentrations

The July point samples were used to determine the N concentrations (%) of the soils in Fairy Lake and San Juan. The mineral soil samples were ground into a fine powder in a Wig-L-bug grinder and the forest floor samples were ground in a mortar and pestle using liquid nitrogen. The ground samples were put in a 55°C oven overnight. 50mg of mineral soil and 20mg of forest floor samples were packaged in tin capsules and analyzed in a Flash 1112 Series Combustion Elemental Analyzer.

2.2.3.6 Total N

Total N was calculated by multiplying soil %N by the mass per unit area of the forest floor and mineral soil of each plot. Forest floor and mineral soil mass per area (kg/m^2) was calculated by multiplying the area of soil sample taken, the depth of the soil sample core and the specific bulk density of the forest floor and mineral soil at each plot. The separate forest floor and mineral soil values were added together and the final values were then scaled up to a per hectare basis (multiplied by 10,000).

2.2.3.7 $\delta^{15}N$

Soil $\delta^{15}N$ was measured using the July point samples. 6 ± 0.1mg of ground forest floor and 8 ± 0.1mg of ground mineral soil were packaged in tin capsules and analyzed on a V Advantage Isotope-Ratio Mass Spectrometer at the Pacific Forestry Centre.

2.2.3.8 pH

The July point soil samples were used to determine the pH of the soils. Using a modified protocol by Kalra and Maynard (1991), 20mL of distilled water was added to 5g of forest floor and mineral soil samples (4:1 water to soil ratio). A few additional milliliters of water were added to some samples as the soil slurry was too thick for the pH probe. The samples were stirred 4-5 times over 30 minutes, left unstirred for 30 minutes, and then analyzed with an Accumet Research AR50 pH meter.

2.2.3.9 Bulk density

The bulk density of the soils was determined using the air-dried soils from the July point samples. Bulk density was calculated by determining the volume of the soil core and dividing it by the weight of the air-dried soil core (volume of soil removed/soil weight).

2.2.3.10 Exchangeable Cations and Cation Exchange Capacity (CEC)

The July point soil samples were used to determine the exchangeable cations and cation exchange capacity (CEC) of the soils. The five forest floor samples and five mineral soil samples from each plot were bulked into one forest floor sample and one mineral soil sample per plot. The bulked samples were mixed thoroughly by hand and 0.5g of forest floor and 2.5g of mineral soil were used. Each sample (32 samples in total) was vacuum extracted with 1M NH_4Cl and analyzed in an Inductively Coupled Plasma Optical Emission Spectrometer to determine the exchangeable cations in the soils. The samples that were extracted with NH_4Cl were then re-extracted with 10% NaCl and analyzed in the Astoria Pacific Segmented Flow Analyzer to determine the CEC of the soils (Kalra and Maynard, 1991).

2.2.3.11 Moisture Content

The May fresh point soil samples were used to determine the percent moisture content of the soils. A 4g subsample of mineral soil and forest floor was taken from each plot and oven dried for two days at 55°C. The percent moisture content, as a percent of dry soil weight, was determined using the standard formula: ((fresh soil weight – oven dry soil weight)/oven dry soil weight) x 100.

2.2.4 Statistical Analysis

For all data, assumptions of normality and homogeneity of variance were determined using the Kolmogorov-Smirnov test and Levene's test, as data was not normally distributed. NH_4^+-N, NO_3^--N and amino acid-N concentrations were analyzed for each of T6 forest floor (T6FF), T6 mineral soil (T6MS), T6 forest floor + mineral soil (T6FF+MS), T0 forest floor (T0FF),

T0 mineral soil (T0MS), T0 forest floor + mineral soil (T0FF+MS) and Net (T6 – T0) forest floor + mineral soil (NetFF+MS), using a nested mixed effects Analysis of Variance (ANOVA) model and Least Significant Difference (LSD) post hoc analysis for means tests. The nested ANOVA model contained three factors: site, tree species and plot. There was an interaction between site and tree species and plot was nested in site, with replicate as the error term. All NH_4^+-N, NO_3^--N and amino acid-N data were log transformed to meet the assumptions of normality (lognormal) (McClave and Sincich, 2009).

A nested ANOVA and LSD post hoc were used to analyze total N for T0FF, T0MS and T0FF+MS and δ^{15}N for T0FF and T0MS. Total N data was log transformed to meet the assumptions of normality (lognormal) (McClave and Sincich, 2009).

A nested ANOVA and LSD post hoc were used to analyze NH_4^+-N and NO_3^--N PRS® probe data and mineralizable N data (A2.20-A2.28). Mineralizable N data was log transformed to meet the assumptions of normality (lognormal) (McClave and Sincich, 2009).

A one-way ANOVA and LSD post hoc were used to find a relationship between BEC Site Series numbers: 03, 01, 05/01, 06, 07 and 09, indicating a gradient of increasing N richness, and T6FF+MS and NetFF+MS NH_4^+-N, NO_3^--N and amino acid-N concentrations (McClave and Sincich, 2009).

One-way ANOVAs were used to analyze soil pH, bulk density and % moisture content by site and tree species. All ANOVA models were balanced and a significance of $\alpha \leq 0.05$ was used (McClave and Sincich, 2009). Statistical analyses were conducted in SAS® (Statistical Analysis System, Cary, NC, USA).

2.3 Results

2.3.1 Temperature and Precipitation

The mean annual temperature in Port Renfrew B.C. in 2015 was 10.3°C ± 4.0, with monthly means of 11.5°C ± 2.5 in May, 14.1°C ± 3.1 in June and 16.1°C ± 3.0 in July. Total rainfall in 2015 was 2310.6 mm, with 25.0mm in May, 9.8mm in June and 27.8mm in July. Temperature and precipitation data was taken from monthly and yearly means from the Vancouver Island School-Based Weather Station Network (http://www.victoriaweather.ca/).

2.3.2 Chemical and Physical Analyses

On average, the pH of the mineral soil in San Juan (4.41 ± 0.09) was higher than in Fairy Lake (3.99 ± 0.08) ($p<0.0001$). Similar trends were seen in the forest floor (Table A2.1 and A2.2). The mineral soil in San Juan was found to have a greater bulk density (589.24 ± 32.68 kg/m^3) than that in Fairy Lake (362.65 ± 30.84 kg/m^3) ($p<0.0001$), with similar trends seen in the forest floor (Table A2.4 and A2.5). In July, the forest floor and mineral soil in Fairy Lake had a greater moisture content (128.36 ± 15.16% and 34.95 ± 8.17%, respectively) than in San Juan (80.27 ± 9.88% and 13.38 ± 3.82 %, respectively).

2.3.3 Characterizing the Soil N

2.3.3.1 T0, T6 and Net NH_4^+-N concentrations

At T0, on average, higher NH_4^+-N concentrations were measured in the FF, MS and FF+MS in San Juan compared to Fairy Lake (Table 2.2 and Table A2.6). Overall, there were significant differences (Table 2.2) in NH_4^+-N among the different tree species (Table A2.7), with western hemlock and western cedar, on avareage, having low T0 NH_4^+-N concentrations. Site*species interactions (Table A2.8) and plot within each site for T0FF, T0MS and T0FF+MS (Figure A2.1) were also significant.

Table 2.2 – P-values from nested ANOVAs for T6, T0 and net forest floor (FF) and mineral soil (MS) NH_4^+-N concentrations (using log transformed data).

Source of Variation	T0FF	T0MS	T0FF+MS	T6FF	T6MS	T6FF+MS	Net (FF+MS)
Site	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0017**
Species	**0.0114**	**0.0011**	**0.0060**	**0.0015**	0.0588	**0.0190**	**0.0035**
Site*Species	**<0.0001**	**<0.0001**	**<0.0001**	0.0658	**0.0427**	**0.0209**	**0.0236**
Plot(Site)	**0.0007**	**<0.0001**	**<0.0001**	0.1170	**0.0359**	**0.0191**	0.1447

The gross NH_4^+-N production over 6 weeks in the FF, MS, and FF+MS was greater in San Juan compared to Fairy Lake (Table 2.2 and Table 2.3). Differences in gross NH_4^+-N production among the four tree species were also seen for the FF and FF+MS (Table 2.2). On average, the western redcedar, Sitka spruce and Douglas-fir plots had the greatest NH_4^+-N production, while the western hemlock plots had the lowest NH_4^+-N production (Table 2.4). T6MS and T6FF+MS had significant site*species interactions (Table 2.2), where the hemlock plots had low gross

NH_4^+-N production in Fairy Lake and in San Juan, along with spruce (Table 2.5). There were also significant differences among the plots within each site for T6MS and T6FF+MS NH_4^+-N concentrations (Table 2.2 and Figure 2.4).

The trends for net FF+MS NH_4^+-N production were similar to gross FF+MS NH_4^+-N production (Figure A2.2), with site, species and site*species having significant effects (Table 2.2). San Juan had more net NH_4^+-N production than Fairy Lake (4.39 ± 1.12 kg/ha and 0.98 ± 0.25 kg/ha, respectively) and hemlock was found to have lower net NH_4^+-N production compared to the other tree species (Figure A2.2).

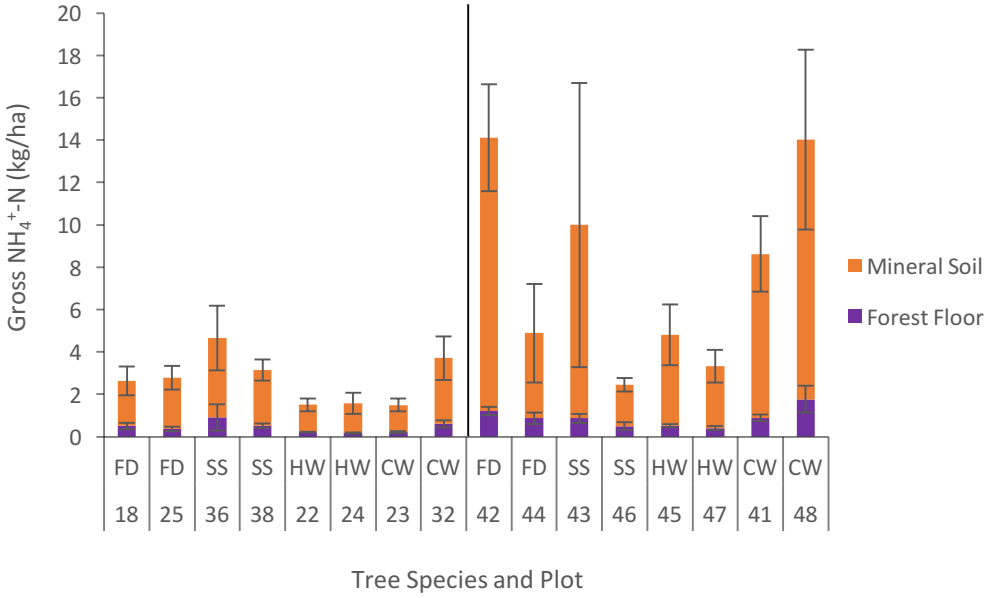


Figure 2.4 - Mean ± S.E. gross (T6) forest floor and mineral soil NH_4^+-N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

Table 2.3 - Mean ± S.E. grossNH_4^+-N (kg/ha) concentrations and significant means differences for T6 forest floor, mineral soil and forest floor + mineral soil by site (Fairy Lake (FL) and San Juan (SJ)).

Site	Gross Forest Floor		Gross Mineral Soil		Gross FF+MS	
FL	b	0.44 ± 0.09	b	2.24 ± 0.28	b	2.68 ± 0.31
SJ	a	0.87 ± 0.11	a	6.90 ± 1.20	a	7.78 ± 1.24

Table 2.4 - Mean ± S.E. gross NH_4^+-N (kg/ha) concentrations and significant means differences for T6 forest floor and forest floor + mineral soil by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW).

Tree Species	Gross Forest Floor		Gross FF+MS	
CW	a	0.87 ± 0.20	a	6.96 ± 1.60
FD	a	0.74 ± 0.11	a	6.10 ± 1.36
HW	b	0.33 ± 0.04	b	2.80 ± 0.52
SS	a	0.70 ± 0.16	a	5.06 ± 1.71

Table 2.5 - Mean ± S.E. gross mineral soil and forest floor + mineral soil NH_4^+-N (kg/ha) concentrations, p-values from nested ANOVAs and significant means differences for site*species interactions.

Fairy Lake	T6 Mineral Soil (p=0.0792)		T6 FF+MS (p=0.0162)	
CW	ab	2.19 ± 0.58	ab	2.60 ± 0.68
FD	ab	2.26 ± 0.42	a	2.70 ± 0.46
HW	b	1.34 ± 0.28	b	1.53 ± 0.27
SS	a	3.18 ± 0.78	a	3.90 ± 0.76
San Juan	T6 Mineral Soil (p=0.0542)		T6 FF+MS (p=0.0327)	
CW	a	9.99 ± 2.30	a	11.32 ± 2.46
FD	ab	8.46 ± 2.19	ab	9.50 ± 2.25
HW	b	3.61 ± 0.80	b	4.07 ± 0.85
SS	b	5.54 ± 3.38	b	6.22 ± 3.38

2.3.3.2 T0, T6 and Net NO_3^--N (kg/ha) concentrations

The NO_3^--N concentrations in T0FF, T0MS and T0FF+MS were significantly greater in San Juan than in Fairy Lake (Table 2.6 and Table A2.9). There were significant differences (Table 2.6) in T0FF, T0MS and T0FF+MS NO_3^--N concentrations among the tree species, with the hemlock plots having high NO_3^--N concentrations and cedar and Douglas-fir having low concentrations, overall (Table A2.10). The site*species interactions (Table A2.11) and plot differences within each site (Figure A2.3) for T0FF, T0MS and T0FF+MS were also significant.

Table 2.6 - P-values from nested ANOVA for T6, T0 and net forest floor and mineral soil NO_3^--N concentrations (using log transformed data).

Source of Variation	T0FF	T0MS	T0FF+MS	T6FF	T6MS	T6FF+MS	Net (FF+MS)
Site	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Species	**<0.0001**	**<0.0001**	**<0.0001**	0.4104	0.1621	0.1849	0.1254
Site*Species	**<0.0001**	**<0.0001**	**<0.0001**	0.4056	**0.0337**	**0.0379**	0.0550
Plot(Site)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

There were significant differences in gross NO_3^--N production in T6FF, T6MS and T6FF+MS between the two sites (Table 2.6), where San Juan soils had higher NO_3^--N production than Fairy Lake soils for all three variables (Table 2.7). While T6MS and T6FF+MS had significant overall site*species interactions (Table 2.6), significant differences were not seen in NO_3^--N production in the soil under the different species within each site ($p>0.05$). Significant variability in T6FF, T6MS and T6FF+MS gross NO_3^--N production were also seen among the different plots within each site (Table 2.6 and Figure 2.5).

The trends for net NO_3^--N production were very similar to gross NO_3^--N production and differences were significant for site and plot(site) (Table 2.6). San Juan had more net NO_3^--N production than Fairy Lake (24.06 ± 4.81 kg/ha and 0.14 ± 0.14 kg/ha, respectively). Significant variability in net FF+MS NO_3^--N production was also seen among the different plots within each site (Table 2.6 and Figure A2.4).

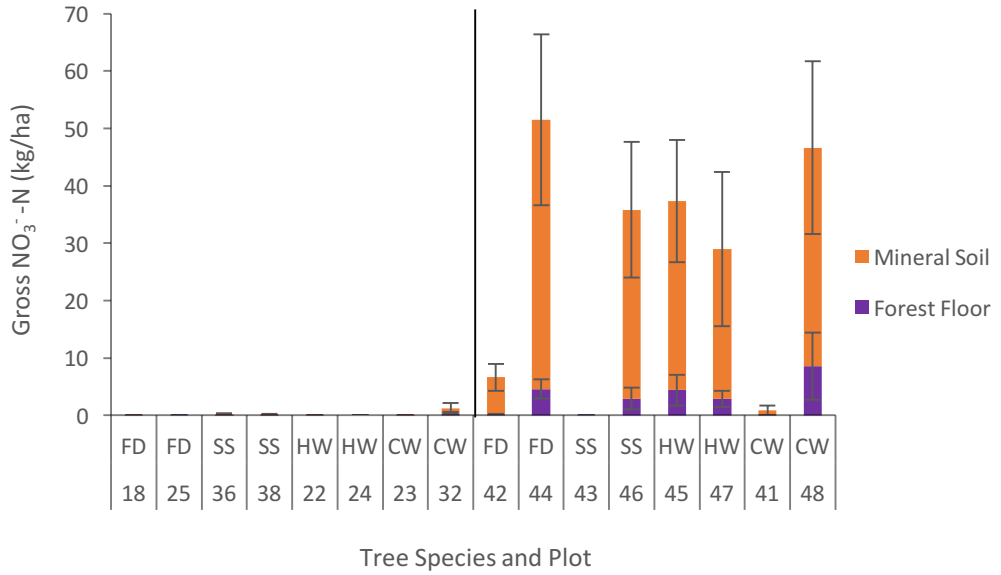


Figure 2.5 – Mean ± S.E. gross (T6) forest floor and mineral soil NO_3^--N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

Table 2.7 – Mean ± S.E. NO_3^--N (kg/ha) concentrations for T6 forest floor, mineral soil and forest floor + mineral soil by site (Fairy Lake (FL) and San Juan (SJ)).

Site	T6 Forest Floor		T6 Mineral Soil		T6 FF+MS	
FL	b	0.04 ± 0.03	b	0.17 ± 0.12	b	0.21 ± 0.15
SJ	a	2.95 ± 0.91	a	23.02 ± 4.33	a	25.97 ± 4.97

2.3.3.3 – T0, T6 and Net amino acid-N concentrations

The amino acid-N concentrations in T0FF, T0MS and T0FF+MS were significantly greater in San Juan than in Fairy Lake soils (Table 2.8 and Table A2.12). There were only significant differences in amino acid-N levels among the different tree species in the T0FF fraction (Table 2.8), where, the Douglas-fir plots had the greatest amino acid-N concentrations, with the cedar and spruce plots being intermediate and hemlock plots having the lowest amino acid-N concentrations (Table A2.13). The site*species interaction (Table A2.14) and plot differences within each site (Figure A2.5) were found to be significant for T0FF, T0MS and T0FF+MS.

Table 2.8 - P-values from nested ANOVA for T6, T0 and net forest floor and mineral soil amino acid-N concentrations (using log transformed data).

Source of Variation	T0FF	T0MS	T0FF+MS	T6FF	T6MS	T6FF+MS	Net (FF+MS)
Site	**<0.0001**	**<0.0001**	**<0.0001**	**0.0053**	**<0.0001**	**<0.0001**	**<0.0001**
Species	**<0.0001**	0.3777	0.2861	0.4221	0.2767	0.2159	0.2201
Site*Species	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.6560	0.2316	0.3518
Plot(Site)	**<0.0001**	**<0.0001**	**<0.0001**	0.0589	**0.0005**	**0.0002**	**0.0010**

There were significant differences in gross amino acid-N production between the two sites in the FF, MS and FF+MS (Table 2.8), showing higher amino acid-N production in San Juan compared to Fairy Lake (Table 2.9). No significant species differences were found but a significant site*species interaction was seen in T6FF (Table 2.8). In Fairy Lake, the soils under Sitka spruce had high amino acid-N production and the soils under western redcedar were low, on average (Table 2.10). In San Juan, western redcedar, on average, had the highest amino acid-N production, while the spruce plots were very variable (Figure 2.6). Significant variability was also seen in amino acid-N production in T6MS and T6FF+MS among the different plots within sites (Table 2.8 and Figure 2.6).

The trends for net FF+MS amino acid-N production were similar to gross FF+MS amino acid-N, and differences were only significant for site and plot(site) (Table 2.8 and Figure A2.6). The soils in San Juan had significantly higher net amino acid-N production (24.44 ± 2.35 kg/ha) compared to Fairy Lake (15.39 ± 1.17 kg/ha).

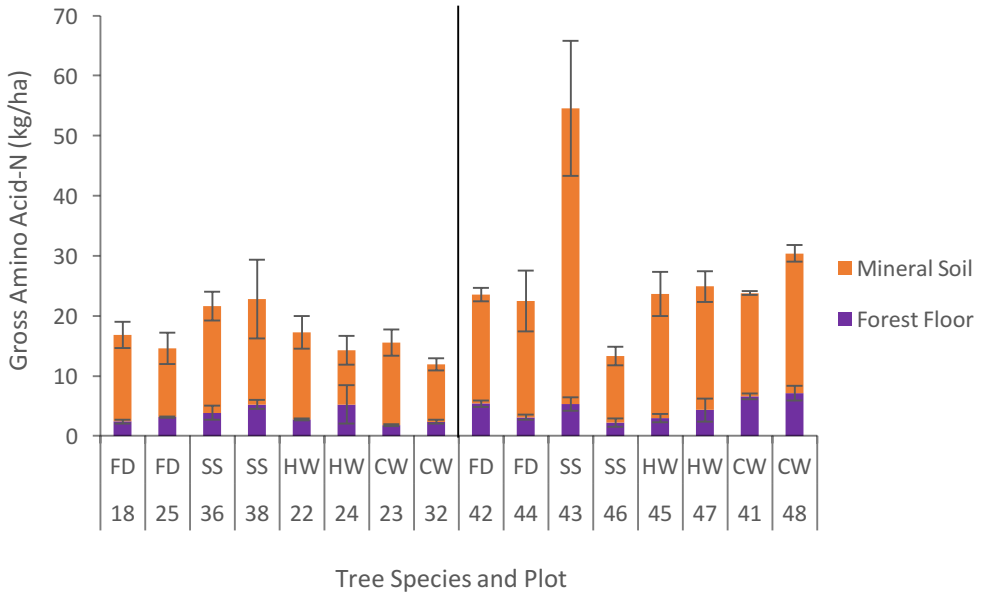


Figure 2.6 - Mean ± S.E. gross (T6) forest floor and mineral soil amino acid-N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

Table 2.9 – Mean ± S.E. amino acid-N (kg/ha) concentrations and significant means differences for T6 forest floor, mineral soil and forest floor + mineral soil by site (Fairy Lake (FL) and San Juan (SJ)).

Site	T6 Forest Floor		T6 Mineral Soil		T6 FF+MS	
FL	b	3.35 ± 0.44	b	13.51 ± 1.12	b	16.86 ± 1.18
SJ	a	4.63 ± 0.42	a	22.46 ± 2.28	a	27.09 ± 2.40

Table 2.10 - Mean ± S.E. T6 forest floor amino acid-N (kg/ha) concentrations, p-values from nested ANOVA and significant means differences for site*species interactions.

Tree Species	T6 Forest Floor Fairy Lake (p=0.0152)		T6 Forest Floor San Juan (p=0.0032)	
CW	b	2.09 ± 0.19	a	6.86 ± 0.64
FD	ab	2.78 ± 0.19	b	4.24 ± 0.51
HW	ab	3.99 ± 1.57	b	3.63 ± 0.98
SS	a	4.55 ± 0.68	b	3.78 ± 0.79

2.3.3.4 T0 total N (kg/ha) concentrations

At T0, significant differences in total N concentrations between soils from the two sites were seen in the forest floor, mineral soil and forest floor + mineral soil (Table 2.11), with San Juan having higher total N concentrations than Fairy Lake for all three variables (Figure 2.7).

Significant differences in T0FF total N were also found among plots within each site (Table 2.11).

Table 2.11 - P-values from nested ANOVAs for T0 forest floor, mineral soil and forest floor + mineral soil total N concentrations (using log transformed data).

Source of Variation	T0 Forest Floor	T0 Mineral Soil	T0 FF+MS
Site	**<0.0001**	**0.0013**	**0.0003**
Species	0.0561	0.9861	0.9472
Site*Species	0.2079	0.1718	0.1594
Plot(Site)	**<0.0001**	0.3730	0.2487

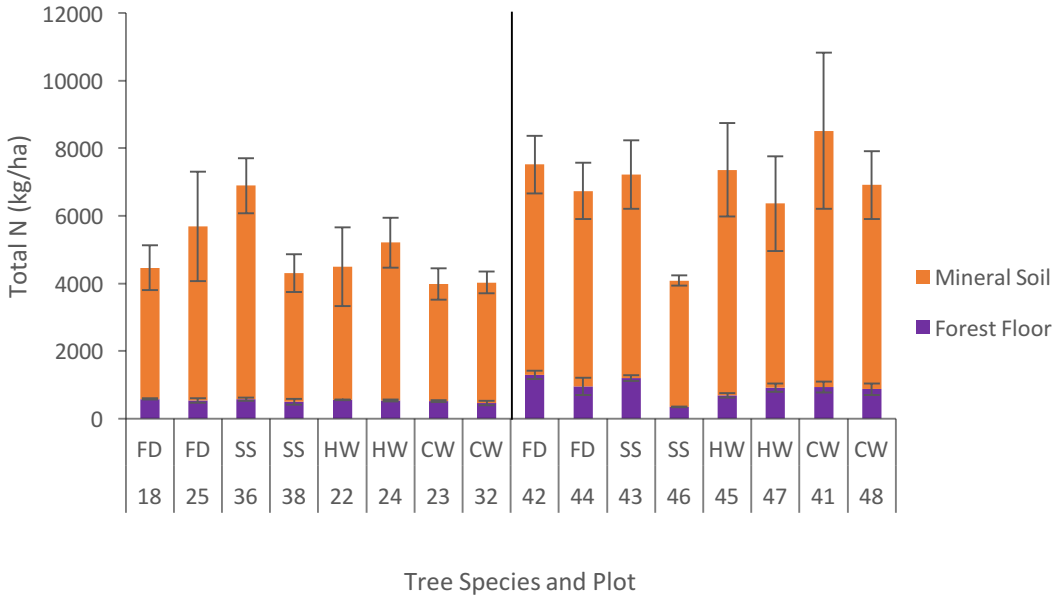


Figure 2.7 – Mean ± S.E. T0 forest floor and mineral soil total N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

2.3.3.5 – T0 δ^{15}N (‰) concentrations

At T0, there was a significant site*species interaction in forest floor δ^{15}N (Table 2.12). In Fairy Lake, the soils under Douglas-fir and western redcedar had among the highest δ^{15}N levels, while in San Juan, there were no significant differences among the four tree species (Table 2.13). Significant differences were also seen in T0FF δ^{15}N among the different plots within each site (Table 2.12 and Figure 2.8).

Table 2.12 - P-values from two-way nested ANOVA for T0 forest floor and mineral soil δ^{15}N (‰) values.

Source of Variation	T0 Forest Floor	T0 Mineral Soil
Site	0.4030	0.8590
Species	0.3721	0.8436
Site*Species	**0.0307**	0.7176
Plot(Site)	**<0.0001**	0.6247

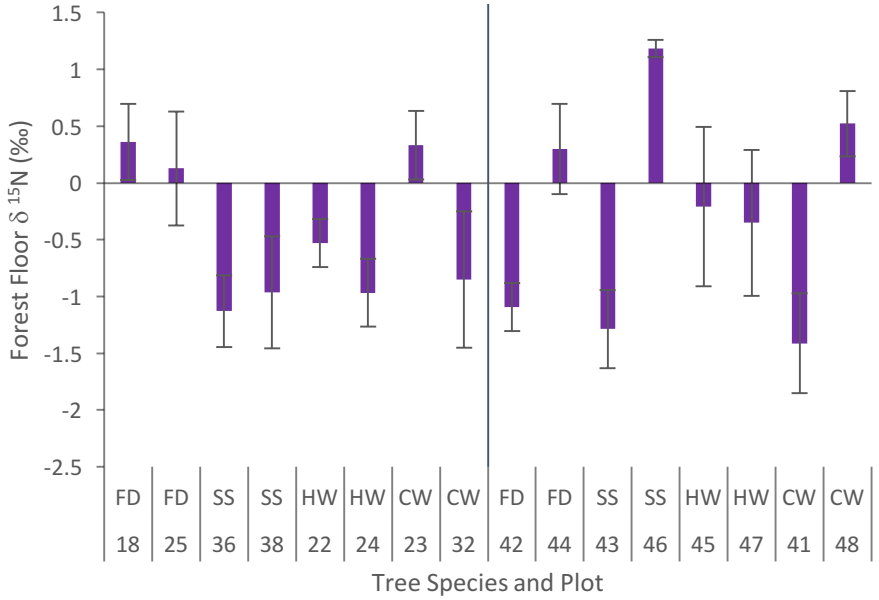


Figure 2.8 – Mean ± S.E. T0 forest floor δ^{15}N (‰) values by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

Table 2.13 – Mean ± S.E. T0 forest floor δ^{15}N (‰) values, p-values from nested ANOVA and significant means differences for site*species interactions.

Tree Species	T0 Forest Floor Fairy Lake (p=0.0158)		T0 Forest Floor San Juan (p=0.7793)	
CW	ab	-0.2590 ± 0.3731	a	-0.4450 ± 0.4057
FD	a	0.2567 ± 0.2746	a	-0.3980 ± 0.3145
HW	b	-0.7480 ± 0.1874	a	-0.2889 ± 0.4442
SS	b	-1.0470 ± 0.2777	a	-0.0520 ± 0.4434

2.3.4 BEC Site Series with NH_4^+-N, NO_3^--N and amino acid-N concentrations

Significant differences in gross FF+MS NH_4^+-N, NO_3^--N and amino acid-N production were seen among the plots from different BEC Site Series numbers (p=0.0001, p<0.0001 and

p=<0.0001, respectively). Plots with BEC values 09, 07 and 05/01 had high gross NH_4^+-N production and 03 had low gross NH_4^+-N production ($p<0.05$, Figure 2.9). The plots with BEC value 09 had the highest gross NO_3^--N production, while 07 had the highest gross amino acid-N production ($p<0.05$, Figure 2.9). Similar trends were also seen with net FF+MS NH_4^+-N, NO_3^--N and amino acid-N production (Figure A2.7).

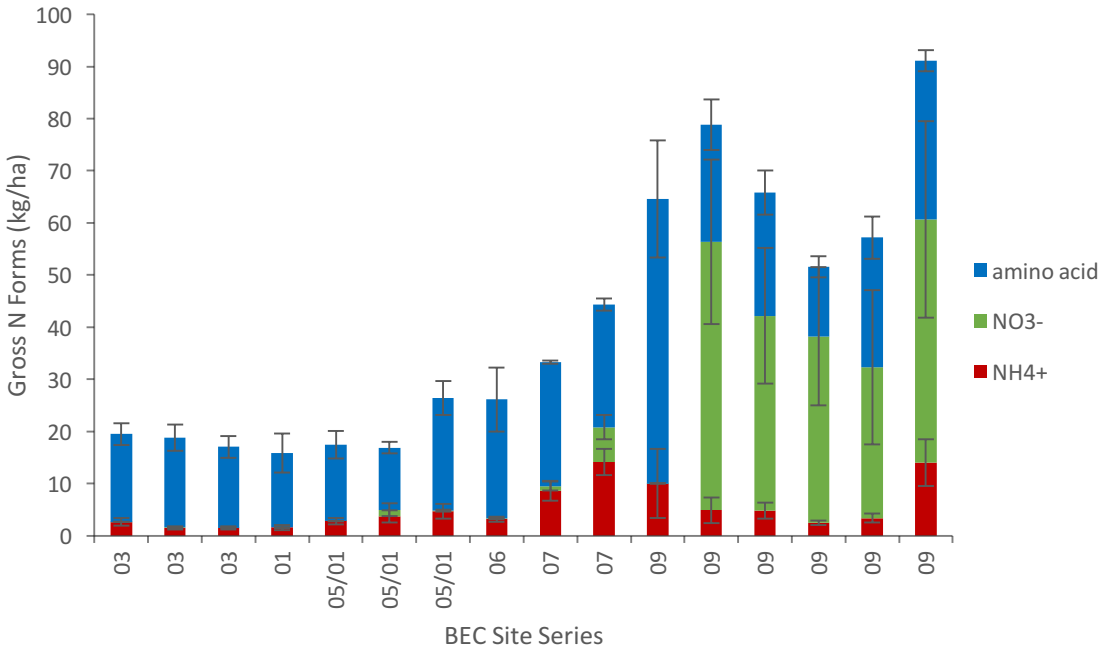


Figure 2.9 – Mean gross (T6) forest floor + mineral soil NH_4^+-N, NO_3^--N and amino acid-N concentrations (kg/ha) for all 16 plots at Fairy Lake and San Juan by BEC site series.

2.4 Discussion

Overall, greater initial and gross NH_4^+-N, NO_3^--N and amino acid-N concentrations were seen in the forest floor and mineral soil in San Juan compared to Fairy Lake. Prescott *et al.* (2000) also examined N concentration and mineralization in these sites and while they found no difference in initial NH_4^+-N and NO_3^--N concentrations between the forest floor in San Juan and Fairy Lake, they did find greater ammonification and nitrification rates in San Juan. While Ribbons *et al*. (2016) found greater gross ammonification in the forest floor at San Juan, greater gross nitrification was seen in the forest floor at Fairy Lake. In my study, the total N concentrations in the forest floor and mineral soil were greater in San Juan compared to Fairy Lake, but no difference in soil $\delta^{15}N$ was found between the sites. Both Ribbons *et al*. (2016) and Prescott *et al.* (2000) found no difference in total N concentrations between the two sites.

My two study sites had very distinct topographies. San Juan was at a valley bottom while Fairy Lake was located midslope. Prescott *et al.* (2000) concluded that moisture and nutrient status of the sites, due to differences in slope position, were the major influences on N mineralization and nitrification. This was also reflected in the contrasting understory vegetation between the sites. San Juan was dominated by sword fern, salmonberry and threeleaf foamflower which are indicative of water-receiving, N rich sites. On the other hand, Fairy Lake was dominated by salal and red huckleberry, which are indicative of drier, N-poor sites. Salal and red huckleberry are ericaceous shrubs that produce tannins, which interfere with N mineralization in the soil, thus, contributing to poorer sites (Prescott and Vesterdal, 2005). While overall we found Fairy Lake and San Juan to be contrasting sites, within each site, there was variability in N transformation between the plots of the same species, which was particularly noticeable in San Juan. This could reflect variation in nutrient concentrations among the plots within a site, where some plots were not as productive as others, which could also explain Ribbons *et al*. (2016) finding of greater gross nitrate production in Fairy Lake. This could also reflect an issue with the *in situ* buried bag method, where by chance, pockets of high nutrient concentrations in the soil were analyzed, skewing the productivity of that plot.

While no significant difference in mineral soil NH_4^+-N production was seen among the species in my study, on average, low initial and gross NH_4^+-N concentrations were found in the forest floor in the western hemlock plots compared to the other tree species. Western hemlock forest floors and needle litter are found to have lower pH relative to other tree species (Prescott and Vesterdal, 2005), which may explain the lower NH_4^+-N concentrations and production in the hemlock forest floors in my study. Overall, the Douglas-fir, Sitka spruce and western redcedar plots all had high forest floor NH_4^+-N production. Prescott and Vesterdal (2005) described Sitka spruce forest floors in B.C. as having moderately high net N mineralization rates. Both Ribbons *et al*. (2016) and Prescott *et al.* (2000) found high NH_4^+-N production in the cedar forest floors. This is consistent with the findings by Harmer and Alexander (1986) but differs from Prescott and Preston (1994) and Prescott *et al.* (1995) who

found low N mineralization in cedar forest floors. Prescott *et al.* (2000), Prescott and Preston (1994) and Prescott (1996) also found Douglas-fir forest floors to have high NH_4^+-N production. However, these studies also found high nitrification rates in Douglas-fir forest floors but I found no significant difference in forest floor and mineral soil NO_3^--N production among the species, similar to Ribbons *et al*. (2016). On average, the forest floor and mineral soil in the hemlock plots had the greatest initial NO_3^--N concentrations, with cedar and Douglas-fir having the lowest concentrations. Douglas-fir and western redcedar are often characterized as preferring NO_3^- over NH_4^+ (Krajina, 1969; Turner *et al*., 1993; D'Amore *et al*., 2009; Boczulak *et al.,* 2014), which could explain lower initial NO_3^--N concentrations compared to hemlock and spruce.

Overall, the forest floor in the Douglas-fir plots had the greatest initial amino acid-N concentrations, with the cedar and spruce plots being intermediate and hemlock plots having the lowest initial amino acid-N concentrations. No significant differences among the species were found in initial amino acid-N concentrations in the mineral soil, or in amino acid-N production in the forest floor and mineral soil. Hannam and Prescott (2003) found differences in soluble organic nitrogen concentrations between high levels in spruce-fir forests and low levels in cedar-hemlock forests on contrasting sites in B.C.

Differences in N concentrations and transformation between my study and those by Prescott *et al.* (2000) and Ribbons *et al*. (2016) were found, even though the same sites were used. This could be attributed to many factors, such as different sampling times and different soil analysis methods. Prescott *et al.* (2000) took soil samples for initial nutrient concentrations and N transformation rates in the fall and winter while Ribbons *et al*. (2016) took them in the summer. Seasonal differences have a major influence on initial N concentrations, and subsequently N mineralization, especially in areas like Port Renfrew where there are distinct high and low rainfall periods. I sampled soil for initial N concentrations in May 2015 and left buried bag incubations for N production from May until June 2015. Late spring to the end of summer receives the lowest rainfall in Port Renfrew and 2015 was a particularly dry year, with one of the lowest summer rainfalls in almost a decade (precipitation data from the Vancouver

Island School-Based Weather Station Network). A lack of soil water affects ammonification and nitrification because it inhibits nitrifying and ammonifying microbes by lowering intracellular water potential and reducing hydration and activity of enzymes (Stanford and Epstein, 1973; Stark and Firestone, 1995; Sahrawat, 2008). Low water content in the soil also restricts substrate supply to microbes, especially with N forms like NH_4^+ that are easily fixed to soil clay minerals (Stark and Firestone, 1995; Sahrawat, 2008). Low precipitation during my sampling period could have contributed to lower N concentrations and differences in NH_4^+ and NO_3^- production compared to those seen in Prescott *et al.* (2000) and Ribbons *et al*. (2016). All three studies used different soil analysis methods, however, which is more likely to account for differences in results. My study used *in situ* buried bags, Prescott *et al.* (2000) used laboratory incubations and Ribbons *et al*. (2016) used ^{15}N pool-dilution methods for N mineralization and nitrification. These different analyses could have easily given different N values, since *in situ* incubations can often reflect contrasting weather and microclimates, such as differences in canopy closure, among sites (Klinka *et al*., 1996). Laboratory incubations, on the other hand, can bypass this discrepancy by maintaining similar environmental factors like temperature and water, but buried bags may be able to give a more realistic picture of the actual nutrient production in different sites.

Organizing the plots by BEC site series showed a corresponding gradient of N richness, irrespective of species. The 09 plots on my sites were N-rich plots and were floodplains on high bench, valley bottom sites. These plots were dominated by salmonberry and sword fern, which indicate nutrient rich and wet sites. The soil N data supported this, as the 09 sites had the highest NO_3^--N production and high NH_4^+-N production. The 05/01 and 07 plots also had high NH_4^+-N production and the 07 plots had the highest amino acid-N production. The 05/01 to 07 plots are also nutrient rich sites, ranging from mesic, mid-slope positions to a lower slope position. They have understorey vegetation dominated by blueberry, foamflower and salmonberry, representing fresh to very moist soils on medium to nutrient rich sites. The soil N data also supported the plots characterized as poorer sites, as 03 plots were found to have low NH_4^+-N production. These plots are found on crests and upper slopes, where water easily leaves

the area, producing drier soils. Hardier vegetation types, including ericaceous shrubs like salal, are more prevalent here because they can thrive on low nutrient and water sites with their ericoid mycorrhizal associations.

Due to differences in aboveground and belowground traits and processes, AM and ECM plants contribute to variations in the biogeochemical makeup and nutrient profiles of the ecosystems they dominate (Phillips *et al*., 2013). Read (1991) hypothesized that, on a global scale, differences between mycorrhizal types occurs along a gradient of organic to inorganic N cycling. Phillips *et al*. (2013) recently updated this hypothesis by suggesting a 'Mycorrhizal-Associated Nutrient Economy' framework, which associated inorganic N economies with AM trees and organic N economies with ECM trees. Lin *et al.* (2017) synthesized data from 100 sites across the globe where AM and ECM forests co-occurred on the same sites. Overall, they found that inorganic N concentrations, net N mineralization and nitrification rates were higher in AM than ECM forests, supporting the inorganic N economy in AM forests and organic N economy in ECM forests. This N cycling pattern was not seen in my study, where the AM western redcedar plots did not have distinctively higher NH_4^+ and NO_3^- production and lower amino acid concentrations compared to the ECM Douglas-fir, western hemlock and Sitka spruce plots. Patterns of N cycling between AM and ECM forests have commonly been observed among plants across latitudinal gradients (Phillips *et al*., 2013). Larger spatial distances between forests in different biomes, such as boreal versus tropical forests, may show greater differences in AM and ECM N cycling. My study was localized to one region and plots were located in close proximity, which may have diminished any differences in AM and ECM nutrient cycling.

2.5 Conclusion

When conducting an experiment on a study site, it is critical to first understand and characterize the site. The purpose of this study was to determine the soil quality of the two sites and under the different tree species, through analyzing N form availability. This baseline information can then be used to interpret results of other experiments on these sites. The soil N data and the characterization of the sites will be used in the following two chapters, where

Chapter 3 will discuss soil N forms in relation to tree growth, foliar N and sporocarp N and Chapter 4 will discuss N form uptake by AM and ECM communities in sites contrasting in N.

Overall, I found differences in soil quality between the two sites, with San Juan having greater initial and gross NH_4^+-N, NO_3^--N and amino acid-N concentrations in the forest floor and mineral soil compared to Fairy Lake. This contrast was most likely due to differences in topography and moisture between the two sites, which was reflected in the composition of the understorey vegetation. Total N concentrations in the forest floor were also greater in San Juan, but no difference in soil $\delta^{15}N$ was found between the sites. While the initial N concentrations differed among the forest floor and mineral soil samples under each tree species, N production, for the most part, did not. There was no significant difference in mineral soil NH_4^+-N production and forest floor and mineral soil NO_3^--N and amino acid-N production among the species, suggesting that tree species have less of an effect on N production than environmental factors like moisture and slope. When plots in each site were organized by BEC site series numbers, indicating a gradient of increasing soil nutrients and moisture, the gradient was reflected in an increase in NH_4^+, NO_3^- and amino acid production, supporting the BEC classification system. Finally, different N economies in the soil under AM versus ECM trees have been suggested, however, this was not seen in my study. This could potentially be due to the close proximity of the AM and ECM trees and the relative similarity of sites, compared to global studies.

Chapter 3 - Relating Tree and Fungal Sporocarp Characteristics to Soil Nitrogen Availability

3.1 Introduction

Plants are sessile organisms that must acclimate and adapt to their local environment, as they are unable to relocate to optimal habitats. In any given ecosystem, many biotic and abiotic factors affect plant growth and survival, from animal pests to a lack of water and nutrients. Plants have evolved physiological and molecular mechanisms to deal with unfavorable conditions. In temperate forest ecosystems, such as those on Vancouver Island, nitrogen (N) is usually the limiting nutrient to tree growth and survival, indicated by positive growth responses to N fertilization (Binkley *et al*., 1995, Chappell *et al*., 1991; Wu, 2011).

N is the most abundant mineral element in plant tissues, making up ~2% of total plant dry matter (Miller and Cramer, 2004). In plants, carbon (C) and N metabolism are tightly regulated to sustain optimal growth and development (Zheng, 2009). Physiological and biochemical studies show plant photosynthetic output to be negatively affected by N deficiency, and photosynthesis recovers when N is resupplied (Coruzzi and Zhou, 2001; Coruzzi and Bush, 2001). C compounds produced through photosynthesis, such as sucrose and glucose, provide the energy and C-skeletons for N assimilation (Zheng, 2009). Inorganic and organic N compounds are synthesized into amino acids, by the incorporation of NH_4^+ into C-skeletons, and the resulting proteins are the key building blocks of the cell (Zheng, 2009). Nucleic acids, coenzymes and many secondary plant compounds, along with proteins, are produced from N throughout the life of a plant (Miller and Cramer, 2004). Therefore, both C and N are critical for cellular function and an adequate supply of both elements is needed for plant growth, development and completion of the life cycle (Zheng, 2009).

3.1.1 N form preferences of four conifer species and tree growth

Plants show plasticity in the N forms that they take up, but they may show preferences for specific inorganic or organic N forms, with increased uptake or growth when that N form is supplied (Boczulak *et al.,* 2014; Britto and Kronzucker, 2013). The N form preferences of plant species are thought to be related to the N forms most available in native soils and these

preferences may be used as a predictive tool for plant fitness in a given ecosystem (Boczulak *et al.,* 2014).

Douglas-fir often grows in dry, warm soils and on disturbed sites where NO_3^- concentrations are relatively high (Boczulak *et al.,* 2014). While Krajina (1969) reported that Douglas-fir prefers NO_3^- and grows best in soils with high nitrification rates, many studies have found a preference for NH_4^+ in this species (Bledsoe 1976; van den Driessche, 1971; Gijsman, 1991; Turner *et al*., 1993). Others have found that Douglas-fir has a preference for NO_3^- in solution culture (Bigg and Daniel, 1968; Krajina *et al*., 1973) and in many soil conditions (van den Driessche, 1978; Boczulak *et al.,* 2014). Douglas-fir has a broad ecological amplitude so there may be considerable genetic variability in nitrogen metabolism (Turner *et al*., 1993).

Sitka spruce grows naturally in moist, cool soils where NH_4^+ is abundant (Boczulak *et al.,* 2014). Numerous studies of different spruce species have found a preference for, and increased growth with, NH_4^+ (Ingested and Molin, 1960; Marschner *et al*., 1991; Lumme, 1994; Kronzucker *et al*., 1997). This trend has been observed for Sitka spruce, specifically (Nelson and Shelby, 1974; Boczulak *et al.,* 2014).

Western redcedar has a widespread distribution and is able to grow on soils with low N and P concentrations, in part, by resorbing a higher percentage of foliar N compared to other conifer species (Antos *et al*., 2016). While western redcedar is tolerant of a wide range of soil properties and N conditions, studies have found a preference for NO_3^- (Krajina, 1969; Krajina *et al*., 1973; Turner *et al*., 1993; D'Amore *et al*., 2009; Bennett and Prescott, 2004).

Western hemlock soils tend to be dominated by NH_4^+ over NO_3^-, as the pH of these soils is usually lower than the soils under other conifer species (Turner and Franz, 1985; Prescott and Vesterdel, 2005). Many studies have found NH_4^+ to be the preferred N form by western hemlock (Krajina, 1969; Krajina *et al*., 1973; Turner and Franz, 1985; Turner *et al*., 1993; Bennett and Prescott, 2004).

3.1.2 Foliar N and tree growth

Plant foliar analyses can be used to infer nutrient availability in the soil, intra- and interspecies responses to nutrient gradients and if nutrients are at adequate levels for growth

(Kranabetter *et al*., 2003; Kranabetter *et al*., 2009). For example, in conifer species, N concentrations less than 10 to 12 mg/g of leaf mass may indicate N deficiency, while critical P concentrations are around 10% of N concentrations (Binkley and Fisher, 2013). Foliar N concentration is also positively linked to the photosynthetic capacity and growth potential of many tree species (Reich *et al*., 1995; Wright *et al*., 2004) and can be an effective index of site differences in soil quality and N supply (Wang and Klinka, 1997; Kranabetter *et al*., 2003; Duursma *et al*., 2005)

3.1.3 δ^{15}N of Soil, Foliage and Mycorrhizae

The ratio between the two stable isotopes of N, ^{15}N and ^{14}N, varies in the biosphere due to isotopic fractionation. Fractionation occurs when chemical, physical and/or biological processes result in the incorporation of the lighter ^{14}N isotope into products, leaving the substrate enriched in the heavier ^{15}N isotope (Högberg, 1997; Hobbie and Högberg, 2012). Variability in the ratio of ^{15}N:^{14}N in tissues can provide useful information about the sources of N used by the organism and the fluxes of N in the ecosystem (Högberg, 1997). Atmospheric N_2, which has a ^{15}N abundance of 0.3663 atom % and ^{14}N abundance of 99.6337 atom %, is the standard used to compare variation in the ^{15}N:^{14}N ratio of other N pools. These N pools all vary within the narrow range of -0.0040 to + 0.0060 atom % from atmospheric N_2 (Högberg, 1997). For this reason, ^{15}N:^{14}N ratio is expressed as $\delta^{15}N$, which signifies the deviation in parts per thousand (‰) from the ^{15}N:^{14}N ratio of atmospheric N_2 (Högberg, 1997).

Throughout the N cycle, there are many opportunities for isotopic N fractionation (Högberg, 1997; Craine *et al*., 2009). Greater fractionation occurs in denitrification and nitrification processes, causing more discrimination against the heavier ^{15}N, compared to N_2 fixation and N mineralization (ammonification) processes (Högberg, 1997; Craine *et al*., 2009). This causes NH_4^+ to become more enriched in ^{15}N than the organic N from which it was derived, and than the NO_3^- being produced by nitrification (Högberg, 1997; Craine *et al*., 2009). In N limited systems, NO_3^- is usually 1-6‰ depleted in ^{15}N relative to NH_4^+ (Hobbie and Högberg, 2012). Many factors, such as different rates of N transformation, size of N form pools, precipitation and temperature, affect the bulk soil $\delta^{15}N$ of an ecosystem (Handley *et al*., 1999).

For example, high rainfall may cause all NO_3^- produced to be denitrified, leading to no net ^{15}N enrichment of the soil (Hobbie and Högberg, 2012). In most cases, the bulk soil $\delta^{15}N$ averages between 2-6‰, with $\delta^{15}N$ usually increasing with depth in the soil profile (Hobbie and Högberg, 2012). The dominant mycorrhizal type also affects soil $\delta^{15}N$, as ^{15}N enrichment in soil profiles was found to be 4.5‰ higher in ectomycorrhizal systems than arbuscular dominated systems (Hobbie and Högberg, 2012).

Along with soil $\delta^{15}N$, foliar $\delta^{15}N$ values of plants can also be used to study ecosystem N dynamics, and soil N form availability and uptake (Kahmen *et al*., 2008; Craine *et al*., 2009). When N availability is high in the soil, the N lost from the system is more likely to be depleted in ^{15}N, which will increase foliar ^{15}N and thus $\delta^{15}N$ (Craine *et al*., 2009). On the other hand, when N availability is low in the soil, N is lost primarily as organic N, which results in lower foliar ^{15}N (Craine *et al*., 2009). However, foliar $\delta^{15}N$ values may be affected by many factors such as discrimination during N uptake, intra-plant isotope partitioning and mycorrhizal status and type (Kahmen *et al*., 2008). For example, when soil N availability is low, plants may be more dependent on mycorrhizae for N acquisition, and the N received from the fungi is depleted in ^{15}N (Craine *et al*., 2009).

Plants receive much of their N from their mycorrhizal fungal partners, therefore, further ^{15}N fractionation occurs, resulting in distinct $\delta^{15}N$ patterns in host plants and fungi (Kranabetter and MacKenzie, 2010; Mayor *et al*., 2015). The $\delta^{15}N$ of mycorrhizal plants is generally less than that of their mycorrhizal fungi, due to the retention of ^{15}N-enriched N by the fungi and transfer of ^{15}N-depleted NH_4^+ and amino acids to the plant (Mayor *et al*., 2015; Hobbie and Högberg, 2012; Hobbie and Agerer, 2010). In general, ectomycorrhizal plants are more ^{15}N-depleted relative to arbuscular mycorrhizal plants and non-mycorrhizal plants. (Mayor *et al*., 2015; Hobbie and Högberg, 2012; Hobbie and Agerer, 2010). However, even though ectomycorrhizal fungi have higher $\delta^{15}N$ values than their plant hosts, much variability in $\delta^{15}N$ is found among the sporocarps of different ectomycorrhizal species (Hobbie and Agerer, 2010). Some studies link the relationship of sporocarp $\delta^{15}N$ to climate (Mayor *et al*., 2009), forms of N used by the ectomycorrhizal fungi (Gebauer and Taylor, 1999) and proteolytic fungal species (Lilleskov *et al*. 2002). Other studies suggest that ectomycorrhizal sporocarp $\delta^{15}N$ patterns are related to

mycelium morphology, with fungal species with extensive extraradical mycelium having higher sporocarp δ^{15}N values and those species with spatially limited extraradical mycelium having lower sporocarp δ^{15}N values (Hobbie and Agerer, 2010; Trudell *et al*., 2004).

3.1.4 Study Objective

The objective of this study was to investigate the relationships between soil N forms and concentrations, as described in Chapter 2, to the growth of four conifer species. I hypothesized that the trees growing at the N-rich San Juan site would have greater growth, foliar %N and ^{15}N concentrations than trees at the Fairy Lake site, where N was less available. I also hypothesized that growth would vary among the different tree species due to species-specific characteristics and possibly due to differences in N form preferences. I expected all tree species in my study, Douglas-fir, Sitka spruce, western redcedar and western hemlock, to have greater growth at San Juan, but hypothesized that those species with a preference for NO_3^-, such as Douglas-fir and western redcedar, might grow proportionately better at that site, due to the higher NO_3^- :NH_4^+ ratio. This study also investigated differences in foliar %N and δ^{15}N among the different tree species on the two sites, as indicators of soil quality and N supply.

This study examined N and ^{15}N concentrations in ECM species' sporocarps throughout the two sites. I hypothesized that sporocarp N concentrations would be higher in San Juan, owing to higher N availability and thus greater N uptake by the ECM fungi. Due to isotopic fractionation processes, I expected that ECM sporocarps would have higher δ^{15}N values than their host plants and overall higher δ^{15}N values at San Juan. I also expected differences in sporocarp N and ^{15}N concentrations among the different ECM genera and species sampled, indicating differences in fungal N form niches.

3.2 Materials and Methods

3.2.1 Site Description

The study area was located near Port Renfrew, B.C. (latitude 48° 33-36' N; longitude 124° 19-21' W; elevation 90-250m) and is part of the B.C Ministry of Forests, Lands and Natural Resource Operations' (FLNRO) Experimental Project (EP) 571 espacement trial planted in 1960

(Omule, 1988). Two sites from the EP571 trial were used for the study: Fairy Lake (48° 35'N, 124° 19'W, elevation 200-280m) and San Juan (48° 35'N, 124° 12'W, elevation 65-85m). Each site contained 24 700m^2 plots with trees of four species planted at three spacings, in a randomized complete block design. The eight 2.7m spacing plots at each site were used for this study, thus at each site there were two plots each of Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW) (16 plots in total).

3.2.2 Data Collection and Processing

3.2.2.1 Height and DBH

The B.C. Ministry of FLNRO has measured tree height and diameter at breast height (DBH) periodically since 1967 for all sites in EP571. Years measured were: 1967, 1970, 1975, 1979, 1982, 1987, 1992, 1998, 2005 and 2014. This thesis focused on the 2014 height and DBH measurements for Fairy Lake and San Juan sites in the Port Renfrew study area, the most recent sampling year. Using height and DBH data, bole volume was calculated based on the volume of a cone. The heights and volumes of the 15 tallest trees in each plot were also used to represent the codominant trees on each plot, considered to be key indicators of productivity.

3.2.2.2 Foliage collection

Foliar samples from branches blown down during a windstorm were collected in November 2015 from all Fairy Lake and San Juan plots. Five samples were taken from each plot (80 samples in total). The samples were brought back to the lab and 2015 needles were dried and processed for nutrient analysis. The samples were oven dried at 55°C for three days. Once dried, they were ground using a Wig-L-Bug and oven dried again at 55°C overnight. Then 4mg ± 0.1mg of sample was packaged into a 10mg tin capsule. During the packaging process, all surfaces and instruments/tools were cleaned with 90% ethanol between each sample. The packaged foliar samples were analyzed for %N, %C and δ^{15}N on a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) at the Pacific Forestry Centre.

3.2.2.3 Sporocarp survey

In October 2015, all observed sporocarps of ectomycorrhizal fungi were collected from each plot in Fairy Lake and San Juan. Up to five sporocarps of each fungal species found at each plot were collected, placed into wax paper bags and brought back to the lab. In the lab, the sporocarps were cleaned lightly to remove excess soil and identified and grouped according to identification keys, then oven dried at ~50°C for two days. A subsample (~1mg) was taken from each oven dried sporocarp sample and sent to the Pacific Forestry Centre for CTAB DNA extraction and PCR amplification of the fungal ITS region of nuclear rDNA (Kranabetter *et al.*, 2015). The primer pairs used for PCR amplification were the basidiomycete-specific ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4B (5'-CAGGAGACTTGTACACGGTCCAG-3') or universal ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990; Gardes and Bruns, 1993). Methodology for DNA extraction and PCR amplification was as described in Kranabetter *et al.* (2013). The resulting PCR products were sent for sequencing (Centre de Recherche du Centre hospitalier de l'Université Laval). The sequences were aligned and manually corrected in Sequencher (Sequencher 5.4, Ann Arbor, Michigan, USA) and BLAST searched against the UNITE database for molecular identification (Kranabetter *et al*., 2015). Only molecular identifications with a >98.5% match were used for analysis (Table 3.1). The remaining samples were ground into a fine powder using a Retsch Oscillating Mill. All sporocarps of the same species from the same plot were ground together to give one bulk sample. The ground samples were then oven dried at 70°C for one hour and 8mg ± 0.1mg of sample was packaged and analyzed for %C and %N on a Costech Elemental Combustion Analyzer. Another 0.866 – 2.367mg ± 0.01mg of sample, depending on the %N levels found in the combustion analyzer, was packaged for δ^{15}N analysis on the Delta V Advantage IRMS. During the packaging process, all surfaces and instruments/tools were cleaned with 90% ethanol between each sample.

3.2.3 Statistical Analysis

For all data, assumptions of normality and homogeneity of variance were determined using the Kolmogorov-Smirnov test and Levene's test. A nested mixed effects Analysis of

Variance (ANOVA) model and Least Significant Difference (LSD) post hoc analysis for significant means differences were used to analyze height, DBH, bole volume, Top 15 tree heights, Top 15 bole volumes and foliar and sporocarp %N and δ^{15}N data. All ANOVA models were balanced except for foliar %N and δ^{15}N data which were unbalanced (and therefore analyzed using the general linear model procedure (PROC GLM) in SAS). The model contained three factors: site, tree species and plot. There was an interaction between site and tree species and plot was nested in site (McClave and Sincich, 2009).

A one-way ANOVA and LSD post hoc were used to analyze sporocarp %N and δ^{15}N by genus and species. Sporocarp δ^{15}N was also analyzed by genus and species containing more than 3 samples, using a one-way ANOVA (McClave and Sincich, 2009).

A one-way ANOVA and LSD post hoc were used to find the relationship between BEC Site Series: 03, 01, 05/01, 06, 07 and 09 (from Chapter 2), and all tree characteristics (height, DBH, bole volume, Top 15 tree heights and volumes and foliar %N and δ^{15}N) and sporocarp data (%N and δ^{15}N) (McClave and Sincich, 2009).

Spearman Correlation Coefficients were used to find potential correlations between mean tree characteristic and mean soil N form data (NH_4^+-N, NO_3^--N, amino acid-N, inorganic N [NH_4^+-N + NO_3^--N], available N [NH_4^+-N + NO_3^--N+ amino acid-N], NO_3^-/NH_4^+ ratio, mineralizable N, Total N, δ^{15}N forest floor and δ^{15}N mineral soil) by plot from Chapter 2. Soil N form data was log transformed to meet the assumptions of normality (lognormal). Due to multiple comparisons, Bonferroni correction was used to adjust the p-values. Any significant correlations ($R \geq 0.40$ and $p \geq 0.05$) were further analyzed with a simple linear regression to find significant linear relationships (McClave and Sincich, 2009). All ANOVA models used a significance of $\alpha \leq 0.05$ and replicate as the Error term. Statistical analyses were conducted in SAS® (Statistical Analysis System, Cary, NC, USA).

Table 3.1 – Ectomycorrhizal sporocarp species sampled during the Fall of 2015 in Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS) plots in Fairy Lake and San Juan. Sporocarp species are organized by family and genus, with number of samples (n) and tree species with which they were found indicated.

Tree Species	n	Family	Genus	Species
HW, SS	3	Amanitaceae	*Amanita*	*vaginata*
FD, HW, SS	7	Cantharellaceae	*Cantharellus*	*formosus*
FD, HW	3	Cantharellaceae	*Craterellus*	*tubaeformis*
FD, SS	2	Clavulinaceae	*Clavulina*	*cinerea*
FD, HW, SS	3	Clavulinaceae	*Clavulina*	*cristata*
FD	1	Cortinariaceae	*Cortinarius*	*cinnamomeus*
HW	1	Cortinariaceae	*Cortinarius*	*vanduzerensis*
FD	2	Cortinariaceae	*Cortinarius*	*venetus*
FD, HW, SS	8	Cortinariaceae	*Cortinarius*	unknown
SS	1	Hydnangiaceae	*Laccaria*	*bicolor*
FD, SS	3	Hygrophoraceae	*Hygrophorus*	*olivaceoalbus*
SS	1	Hygrophoraceae	*Hygrophorus*	*pudorinus*
SS	1	Hygrophoraceae	*Hygrophorus*	unknown
SS	1	Inocybaceae	*Inocybe*	*sororia*
HW	1	Inocybaceae	*Inocybe*	*rimosa*
FD, SS	2	Inocybaceae	*Inocybe*	unknown
FD, SS	5	Russulaceae	*Russula*	*bicolor*
FD, HW, SS	5	Russulaceae	*Russula*	*dissimulans*
HW, SS	2	Russulaceae	*Russula*	*fragilis*
SS	1	Russulaceae	*Russula*	*pallescens*
SS	1	Russulaceae	*Russula*	*sapinea*
HW	1	Russulaceae	*Lactarius*	*fallax*
FD, HW, SS	9	Russulaceae	*Lactarius*	*hepaticus*
FD, HW, SS	5	Russulaceae	*Lactarius*	*luculentus*
HW	1	Russulaceae	*Lactarius*	*pseudomucidus*
HW	1	Tricholomataceae	*Tricholoma*	*sejunctum*
SS	1	Tricholomataceae	*Tricholoma*	*vaccinum*

3.3 Results

3.4 3.3.1 Comparing Soil N and Tree Growth

3.3.1.1 Height

Overall, trees in San Juan were taller than those in Fairy Lake (28.54 ± 0.39m and 24.67 ± 0.30m, respectively) (Table 3.2). There were also differences in tree height among the four tree species, on average (Table 3.2). The western redcedar trees were the shortest while Douglas-fir and Sitka Spruce trees were the tallest; however, the site*species interaction was also

significant (Table 3.2). In Fairy Lake, Sitka Spruce were the tallest trees, while Douglas-fir were the tallest trees in San Juan (Figure 3.1). At both sites, the western hemlock trees were of intermediate height and the western redcedar trees were the shortest (Figure 3.1). There was also variability in tree height among different plots within each site (Table 3.2).

Table 3.2 – P-values from the nested ANOVAs for height (m), DBH (cm), bole volume (BV) (m^3), Top 15 tree heights (m) and Top 15 bole volumes (m^3).

Source of Variation	Height (m)	DBH (cm)	BV(m^3)	Top15Ht	Top15BV
Site	**< 0.0001**	**0.0161**	**<0.0001**	**< 0.0001**	**0.0056**
Species	**< 0.0001**	**0.0016**	**<0.0001**	**< 0.0001**	**< 0.0001**
Site*Species	**< 0.0001**	0.0787	**0.0044**	**< 0.0001**	0.1987
Plot(Site)	**< 0.0001**	**0.0044**	0.0549	**< 0.0001**	**0.0154**

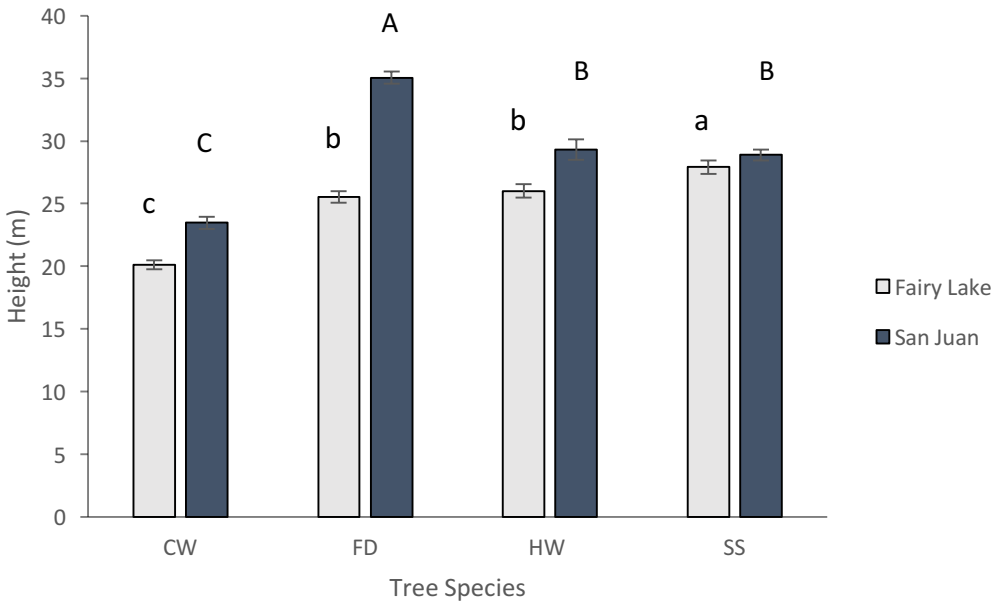


Figure 3.1 – Mean height ± S.E. (m) of each tree species, western redcedar (CW), Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS), from the two sites. Significant mean differences from LSD post hoc tests for site*species interactions are shown by letters; upper and lowercase letters are compared separately.

3.3.1.2 DBH

The DBH of the trees in San Juan was greater than that of trees in Fairy Lake (31.3 ± 0.64cn and 29.34 ± 0.54cm, respectively) (Table 3.2). DBH varied among the different tree species (Table 3.2), averaged across the two sites, the western redcedar trees had the smallest

DBH and the Douglas-fir, hemlock and spruce all had greater DBH (Figure 3.2). The site*species interaction was not significant (Table 3.2). There were significant differences in DBH among the different plots within each site (Table 3.2).

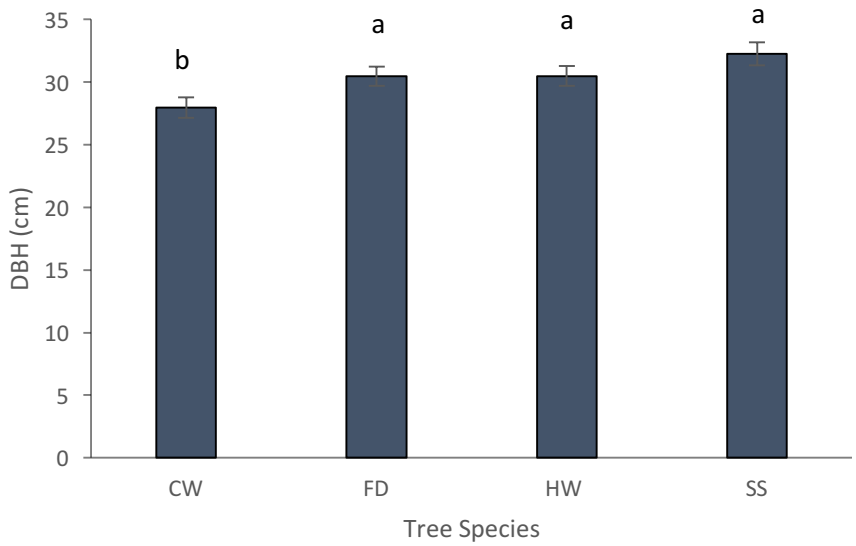


Figure 3.2 – Mean ± S.E. DBH (cm) of each tree species, western redcedar (CW), Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS), averaged over the two sites. Significant mean species differences from LSD post hoc tests are indicated by lowercase letters.

3.3.1.3 Bole Volume

The average bole volume of the trees in San Juan was greater than those in Fairy Lake (0.85 ± 0.04 m^3 and 0.65 ± 0.03 m^3, respectively) (Table 3.2). Bole volume varied among the different tree species (Table 3.2), with western redcedar having the smallest volume on both sites (Figure 3.3). The site*species interaction was also significant (Table 3.2), because in Fairy Lake, Sitka spruce had the largest volume, while in San Juan, Douglas-fir had the larger volume (Figure 3.3).

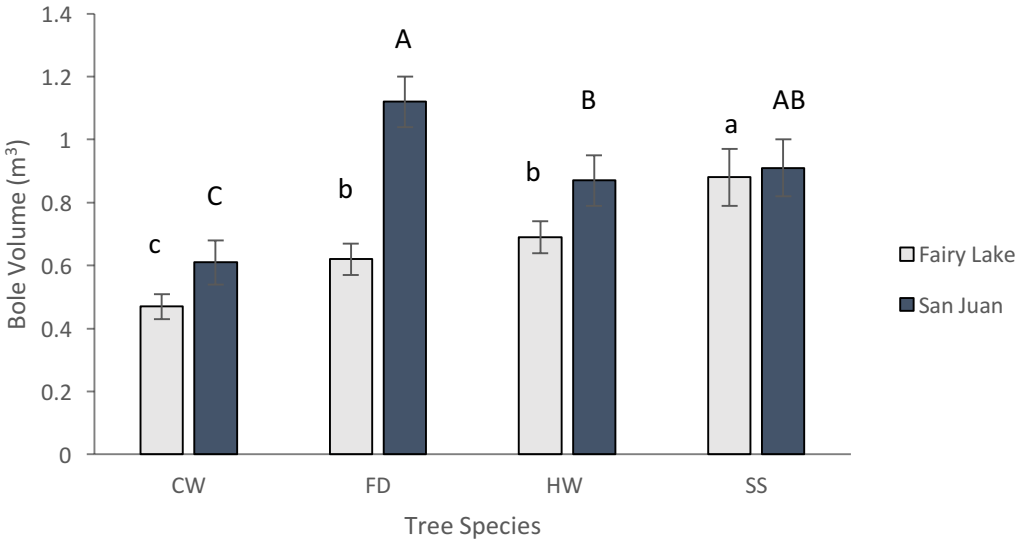


Figure 3.3 – Mean ± S.E. bole volume (m^3) of each tree species, western redcedar (CW), Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS), from the two sites. Significant mean differences from LSD post hoc tests for site*species interactions are shown by letters; upper and lowercase letters are compared separately.

3.3.1.4 Top 15 Height

Of the average top 15 tree heights, San Juan had the tallest trees compared to Fairy Lake (Table 3.2) with means of 31.51 ± 0.38m and 27.75 ± 0.36m, respectively. There were also significant differences among the different tree species (Table 3.2). Douglas-fir had the tallest Top 15 trees and western redcedar had the shortest, with western hemlock and Sitka spruce being in the middle, on average. The site*species interaction was significant (Table 3.2), as Douglas-fir was only tallest in San Juan while Sitka spruce was tallest in Fairy Lake (Figure 3.4). Significant variability was also seen in the Top 15 tree heights among the different plots within each site (Table 3.2).

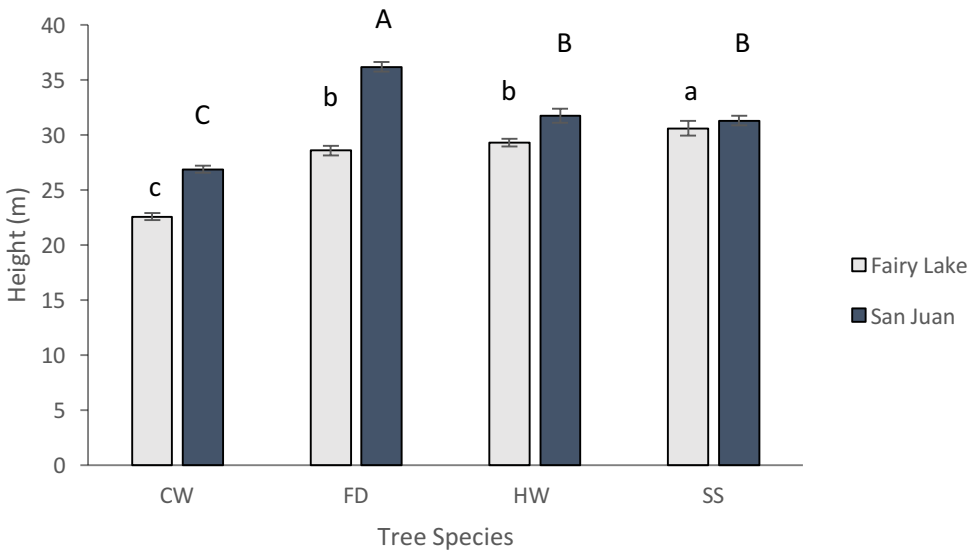


Figure 3.4 - Mean ± S.E. Top 15 tree heights (m) of each species, western redcedar (CW), Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS), from the two sites. Significant mean differences from LSD post hoc tests for site*species interactions are shown by letters; upper and lowercase letters are compared separately.

3.3.1.5 Top 15 Bole Volume

Of the Top 15 tree bole volumes, San Juan had trees with larger volumes, compared to Fairy Lake (Table 3.2), with means of 1.19 ± 0.05 m^3 and 1.02 ± 0.04 m^3, respectively. There were also significant differences among the different tree species (Table 3.2), with the Sitka spruce having the largest volume (Figure 3.5). The site*species interaction term was not significant (Table 3.2). Significant variability in the Top 15 bole volumes was seen among the different plots at each site (Table 3.2).

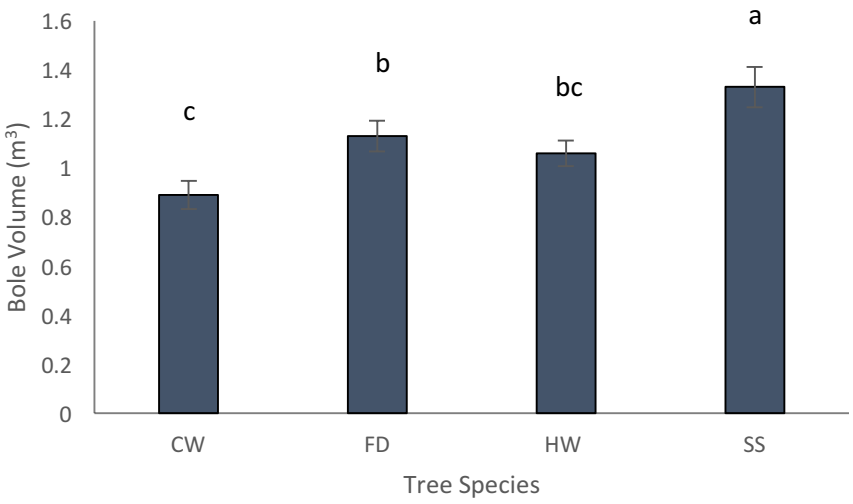


Figure 3.5 – Mean ± S.E. Top 15 tree bole volumes (m^3) of each species, western redcedar (CW), Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS), averaged over the two sites. Significant mean species differences from LSD post hoc tests are indicated by lowercase letters.

3.3.2 Foliar %N and $\delta^{15}N$

3.3.2.1 Foliar %N

There was statistically no difference (Table 3.3) between the %N of the foliage in Fairy Lake and San Juan (1.19 ± 0.03% and 1.21 ± 0.02%, respectively). There were significant differences among the different tree species (Table 3.3), with Douglas-fir having consistently high foliar %N and western redcedar having the lowest foliar N concentration. The site*species interaction was also significant (Table 3.3), because the %N of the spruce foliage was relatively high in San Juan but relatively low at Fairy Lake (Figure 3.6).

Table 3.3 - P-values from the nested ANOVAs for Foliar %N and $\delta^{15}N$.

Source of Variation	%N	$\delta^{15}N$
Site	0.6635	0.0774
Species	**0.0004**	**0.0012**
Site*Species	**0.0502**	**<0.0001**
Plot(Site)	0.4487	**<0.0001**

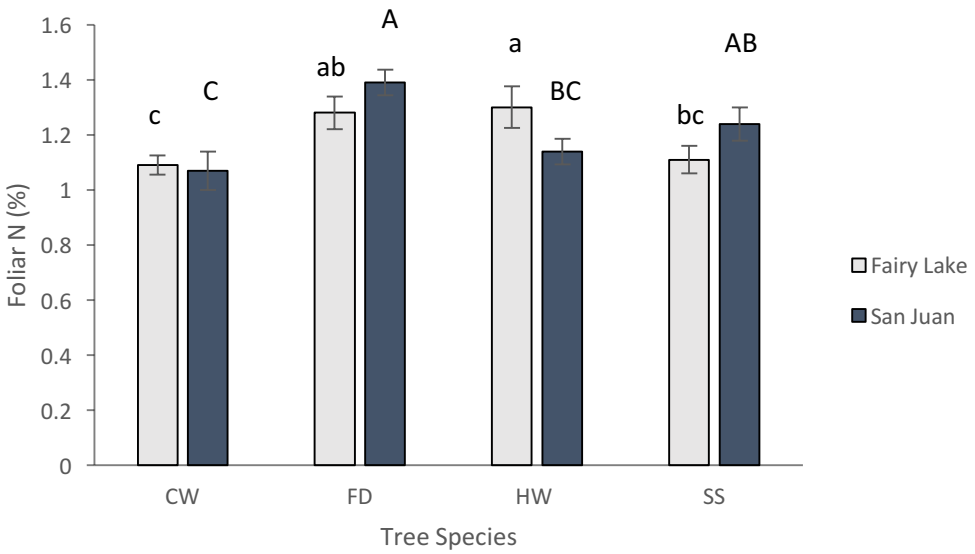


Figure 3.6 – Mean ± S.E. foliar N (%) of each tree species, western redcedar (CW), Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS), from the two sites. Significant mean differences from LSD post hoc tests for site*species interactions are shown by letters; upper and lowercase letters are compared separately.

3.3.2.2 Foliar $\delta^{15}N$

There was statistically no difference (Table 3.3) between the $\delta^{15}N$ of the foliage from Fairy Lake and San Juan (-3.20 ± 0.15‰ and -2.91 ± 0.22‰, respectively). There were significant differences among the different tree species (Table 3.3), but the site*species interaction was also significant because the relative ranking of species changed between sites (Figure 3.7). There was significant variability in foliar $\delta^{15}N$ among the different plots within each site (Table 3.3).

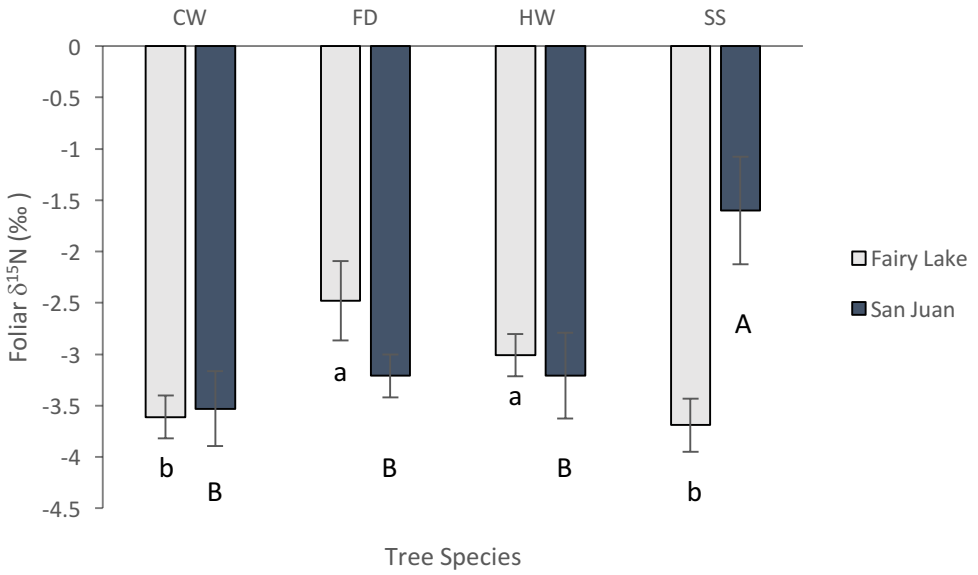


Figure 3.7 - Mean ± S.E. foliar δ^{15}N (‰) of each species, western redcedar (CW), Douglas-fir (FD), western hemlock (HW) and Sitka spruce (SS), from the two sites. Significant mean differences from LSD post hoc tests for site*species interactions are shown by letters; upper and lowercase letters are compared separately.

3.3.3 Correlations and Regression

A significant positive correlation was seen between mean height and T6 soil (forest floor + mineral soil) inorganic N levels (rho=0.50, p=0.05) and available N levels (rho=0.61, p=0.01) (Table A3.1). A significant positive correlation was seen between mean bole volume and T6 soil (forest floor + mineral soil) inorganic N levels (rho=0.49, p=0.05). There was also a significant positive relationship between available N levels and the Top 15 tree heights (rho=0.57, p=0.02) and Top 15 bole volumes (rho=0.50, p=0.05) (Table A3.1). Foliar δ^{15}N was found to have a significant positive correlation with δ^{15}N in the forest floor (rho=0.59, p=0.02) (Table A3.1). Similar significant positive correlations were seen between height, bole volume, Top 15 height and bole volume with net soil available N data (Table A3.2). Using the Bonferroni corrected p-values, no significant correlations were seen for T6 or net soil data (Table A3.1 and Table A3.2).

Linear regressions for relationships with significant correlations (not using the Bonferroni corrected p-values) showed weak to moderate R^2 values (Table A3.3), the highest being the relationships between height and available N (R^2=0.30) (Figure 3.8) and between foliar δ^{15}N and soil δ^{15}N in the forest floor (R^2=0.4273) (Figure 3.9). Similar R^2 values were seen

for regressions of net soil N data based on relationships with significant correlations (Table A3.4).

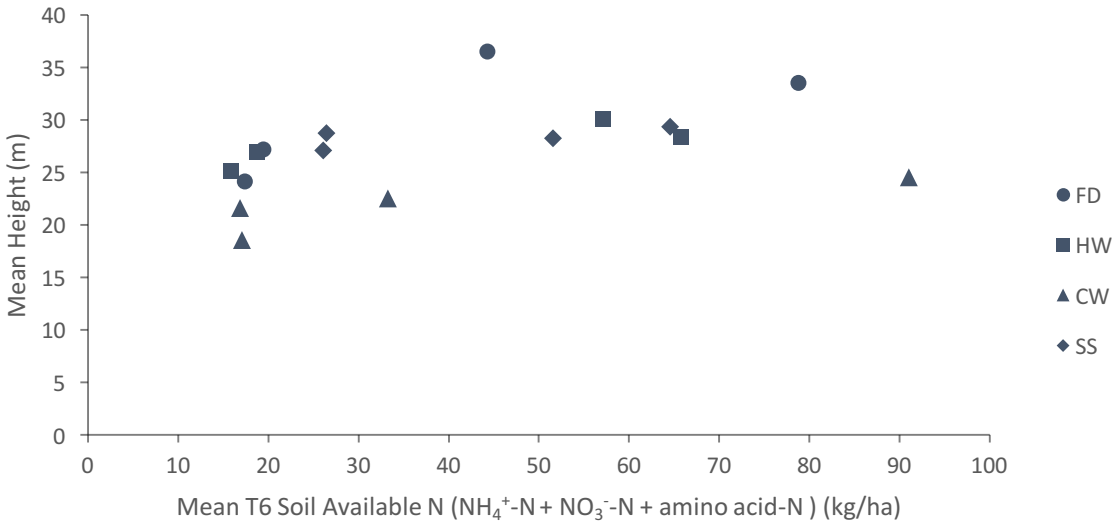


Figure 3.8 – Mean tree height (m) and mean T6 forest floor + mineral soil available N (NH_4^+-N + NO_3^--N + amino acid-N) by species (Douglas-fir (FD), western hemlock (HW), western redcedar (CW) and Sitka spruce (SS)), using non-transformed data. Mean tree height and LOG soil available N data of all species were used in the regression (y=0.0821x + 23.722, R^2 = 0.3030).

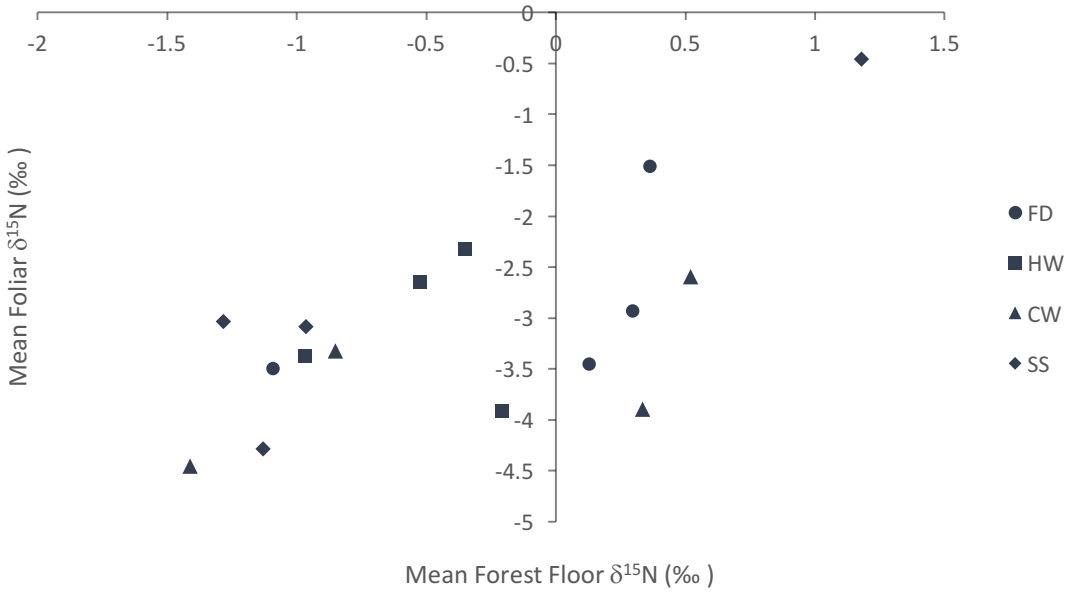


Figure 3.9 - Mean forest floor δ^{15}N (‰) and mean foliar δ^{15}N (‰) by tree species, Douglas-fir (FD), western hemlock (HW), western redcedar (CW) and Sitka spruce (SS). Mean forest floor δ^{15}N and mean foliar δ^{15}N values of all species were used in the regression (y=0.4957x + 1.138, R2=0.4273).

3.3.4 Alignment of Tree Characteristics with BEC Site Series

All measured tree characteristics, height, DBH, bole volume, Top 15 tree height and Top 15 bole volume, showed significant variability among the different BEC Site Series (Table 3.4). No relationship was seen between site richness as indicated by BEC Site Series and height, DBH or bole volume (Figures 3.10-3.12), Top 15 data showed similar trends.

Table 3.4 – P-values from one-way ANOVA between BEC Sites Series with height, DBH, bole volume, Top 15 tree heights and Top 15 bole volumes.

	Height	DBH	Bole Volume	Top15Ht	Top15BV
BEC Site Series	**<0.0001**	**0.0007**	**<0.0001**	**<0.0001**	**0.0040**

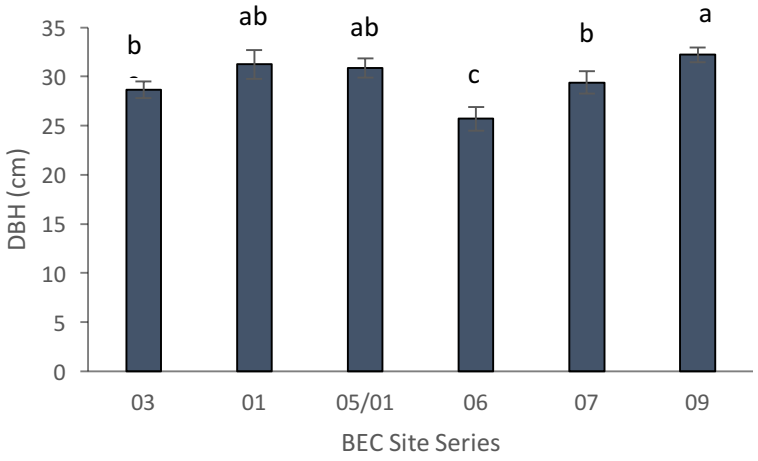


Figure 3.10 – Mean ± S.E. DBH (cm) organized by site richness as indicated by BEC Site Series. Significant mean differences from LSD post hoc tests are indicated by lowercase letters.

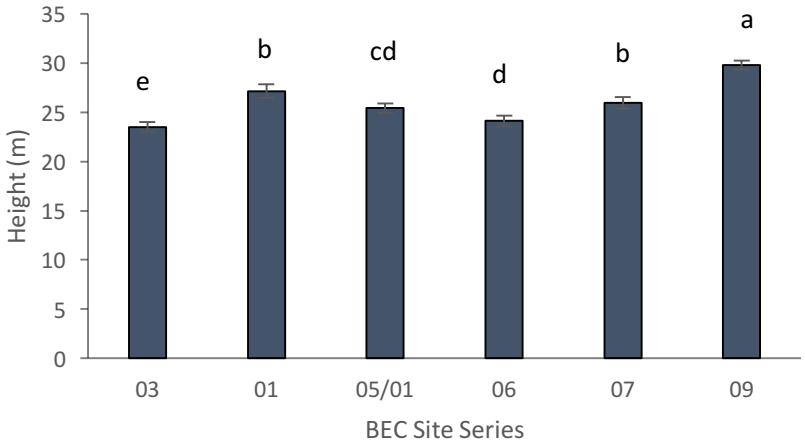


Figure 3.11 – Mean ± S.E. height (m) organized by site richness as indicated by BEC Site Series. Significant mean differences from LSD post hoc tests are indicated by lowercase letters.

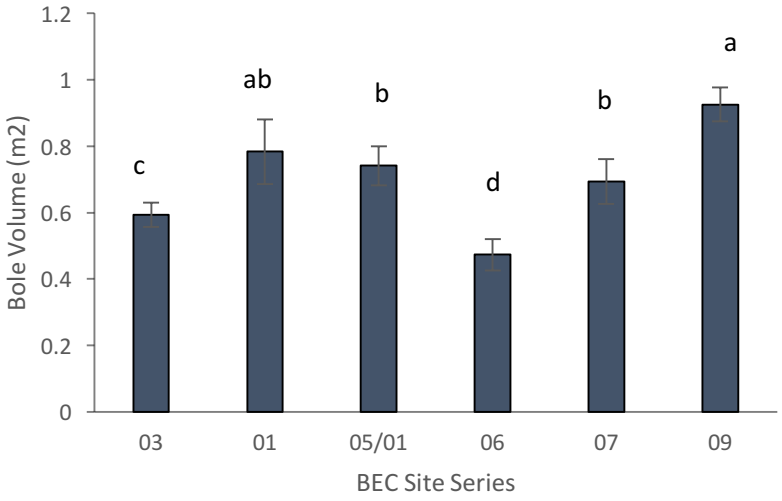


Figure 3.12 – Mean ± S.E. bole volume (m^3) organized by site richness as indicated by BEC Site Series. Significant mean differences from LSD post hoc tests are indicated by lowercase letters.

Significant variability in foliar $\delta^{15}N$ levels was observed among the different BEC Site Series (Table 3.5); however, no relationship was seen between site richness, as indicated by BEC Site Series number, and foliar $\delta^{15}N$ (Figure 3.13). Foliar %N did not differ significantly among the BEC Site Series.

Table 3.5 – P-values from one-way ANOVA of BEC Site Series for foliar %N and $\delta^{15}N$.

	Foliar %N	Foliar $\delta^{15}N$
BEC Site Series	0.1462	**<0.0001**

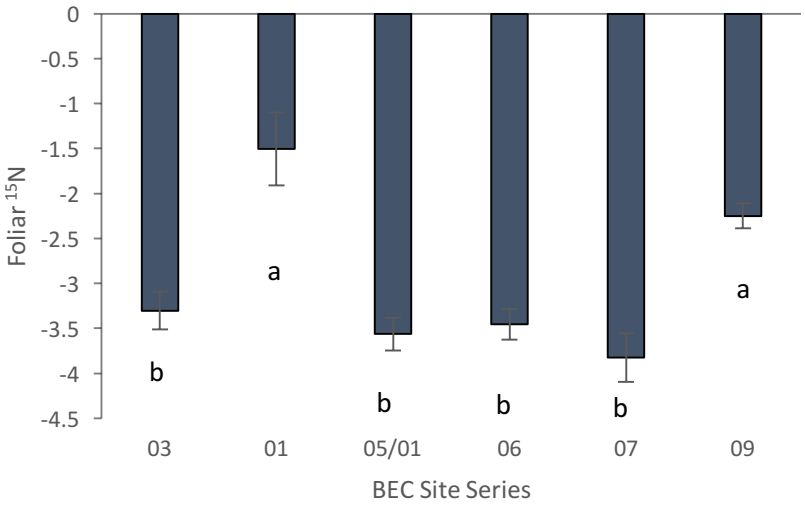


Figure 3.13 – Mean ± S.E. foliar δ^{15}N (‰) ranked by site richness as indicated by BEC Site Series. Significant mean differences from LSD post hoc tests are indicated by lowercase letters.

3.3.5 Sporocarp %N and δ^{15}N

3.3.5.1 Sporocarp %N and δ^{15}N by Community

Overall, only site had a significant effect on sporocarp %N and δ^{15}N levels (Table 3.6). The sporocarps in San Juan had higher %N than those in Fairy Lake (4.39 ± 0.18% and 3.75 ± 0.10%, respectively). The δ^{15}N levels in Fairy Lake were higher than in San Juan (5.49 ± 0.52‰ and 3.55 ± 0.49‰).

Table 3.6 – P-values from nested ANOVA of sporocarp %N and δ^{15}N.

Source of Variation	%N	δ^{15}N
Site	**0.0020**	**0.0077**
Tree Species	0.4760	0.1192
Site*Tree Species	0.3790	0.4434
Plot(Site)	0.2911	0.4578

3.3.5.2 Sporocarp %N and δ^{15}N by Genera and Species

There were significant differences in sporocarp %N and δ^{15}N among the ectomycorrhizal genera sampled across the 12 plots of ECM conifers (Table 3.7). *Amanita* had high %N levels, while *Cortinarius* had low %N (Table 3.7). The genera *Cantharellus*, *Cortinarius*, *Lactarius* and *Tricholoma* all had high δ^{15}N values and *Hygrophorus* had a low δ^{15}N value (Table 3.7). Significant differences in %N and/or δ^{15}N levels between the genera sampled within a family

were seen in Russulaceae (Table 3.7). In Russulaceae, *Lactarius* was found to have significantly higher δ^{15}N levels compared to *Russula* (Table 3.7). Multiple species were sampled within the genera *Russula*, *Lactarius*, *Hygrophorus*, *Cortinarius*, *Clavulina*, *Inocybe* and *Tricholoma* (Table 3.1); however, significant differences in %N and δ^{15}N values among the different species within a genus were only seen in *Russula* and *Lactarius* and. The species within *Russula* and *Lactarius* had significant differences in %N and δ^{15}N values (Table 3.8 and 3.9).

Table 3.7 - P-values from one-way ANOVA and means differences from LSD tests for %N and δ^{15}N (‰) values among the genera sampled in all Douglas-fir, western hemlock and Sitka spruce plots in Fairy Lake and San Juan during the Fall of 2015. Genera are grouped by family and mean ± S.E. for each genus are included.

Family	Genera	%N (**<0.0001**)		δ^{15}N (**<0.0001**)	
		Mean ± SE		Mean ± SE	
Amanitaceae	*Amanita*	a	6.63 ± 0.39	c	1.00 ± 0.53
Cantharellaceae	*Cantharellus*	fg	3.49 ± 0.07	b	5.79 ± 0.61
	Craterellus	g	3.04 ± 0.29	bc	3.72 ± 0.25
Clavulinaceae	*Clavulina*	def	3.85 ± 0.13	c	2.25 ± 0.46
Cortinariaceae	*Cortinarius*	fg	3.28 ± 0.20	a	7.96 ± 0.41
Hygrophoraceae	*Hygrophorus*	fg	3.65 ± 0.12	c	1.26 ± 1.67
Inocybaceae	*Inocybe*	b	4.99 ± 0.19	c	1.26 ± 0.57
Hydnangiaceae	*Laccaria*	bcd	4.96 ± 0	c	0.02 ± 0
Russulaceae	*Lactarius*	bc	4.44 ± 0.10	b	6.12 ± 0.60
	Russula	cde	4.22 ± 0.20	c	2.81 ± 0.69
Tricholomataceae	*Tricholoma*	efg	3.62 ± 0.50	ab	7.29 ± 1.42

Table 3.8 - P-values from one-way ANOVA and means differences from LSD tests for %N and δ^{15}N (‰) values among the species in the genus *Russula* sampled in all Douglas-fir, western hemlock and Sitka spruce plots in Fairy Lake and San Juan during the Fall of 2015. Species are grouped by genus and family and mean ± S.E. values for each variable are included.

Family	Genus	Species	%N (p=**0.0007**)		δ^{15}N (p=**0.0006**)	
Russulaceae	*Russula*	*bicolor*	bc	3.78 ± 0.10	c	0.19 ± 0.46
		dissimulans	a	5.04 ± 0.20	a	5.47 ± 0.60
		fragilis	bc	3.72 ± 0.17	bc	1.58 ± 0.44
		pallescens	c	2.96 ± 0	ab	3.82 ± 0
		sapinea	ab	4.56 ± 0	ab	4.16 ± 0

Table 3.9 - P-values from one-way ANOVA and means differences from LSD tests for %N and δ^{15}N (‰) values among the species in the genus *Lactarius* sampled in all Douglas-fir, western hemlock and Sitka spruce plots in Fairy Lake and San Juan during the Fall of 2015. Species are grouped by genus and family and mean ± S.E. values for each variable are included.

Family	Genus	Species	%N (p=**0.0084**)		δ^{15}N (p=**0.0290**)	
Russulaceae	*Lactarius*	*hepaticus*	a	4.53 ± 0.09	b	5.11 ± 0.51
		luculentus	a	4.46 ± 0.14	a	8.49 ± 1.10
		pseudomucidus	b	3.19 ± 0	b	3.46 ± 0
		fallax	a	4.80 ± 0	b	3.70 ± 0

Using all ectomycorrhizal genera and species with three or more samples, a wide range of mean sporocarp δ^{15}N levels was found (Figure 3.14). *Hygrophorus olivaceoalbus* had the lowest δ^{15}N value (-1.25 ± 0.85‰) with *Cortinarius* species and *Lactarius luculentus* having the highest δ^{15}N values (7.96 ± 0.41‰ and 8.49 ± 1.10‰, respectively).

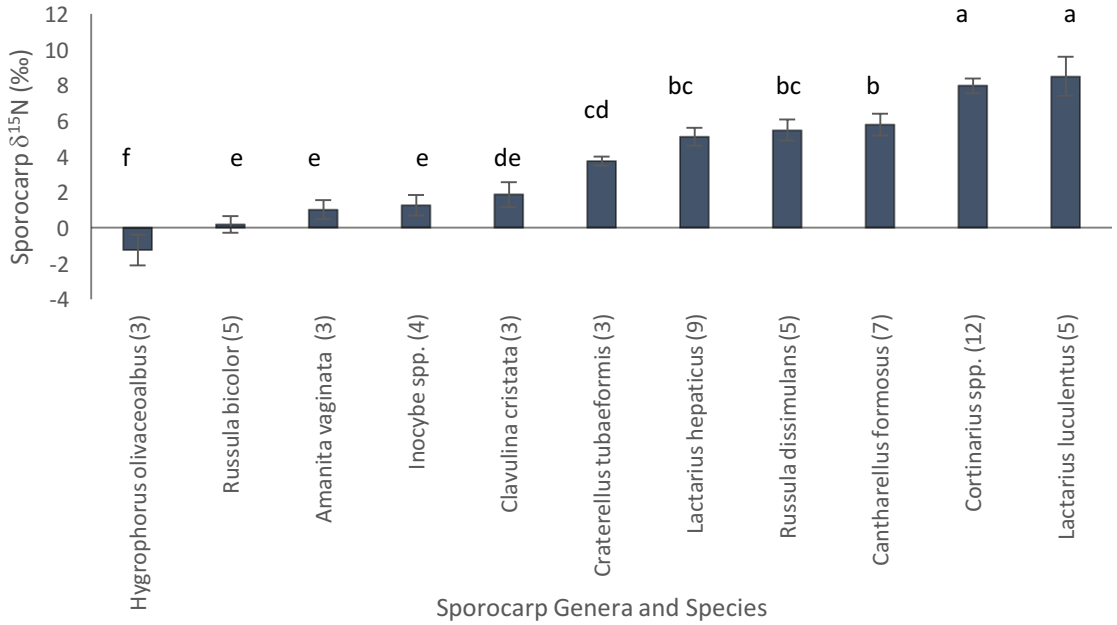


Figure 3.14 – Mean ± S.E. sporocarp δ^{15}N (‰) levels of all ectomycorrhizal genera and species with three or more samples, indicated by numbers next to species name. Significant mean species differences from LSD post hoc tests are indicated by lowercase letters.

3.3.5.3 Sporocarp %N and δ^{15}N by BEC Site Series

Significant variability was found among the different BEC Site Series in sporocarp %N and δ^{15}N levels (p=0.0171 and p=0.0393, respectively). No relationship was seen with site fertility as indicated by BEC Site Series and sporocarp %N and δ^{15}N levels (Figure 3.15 and Figure 3.16).

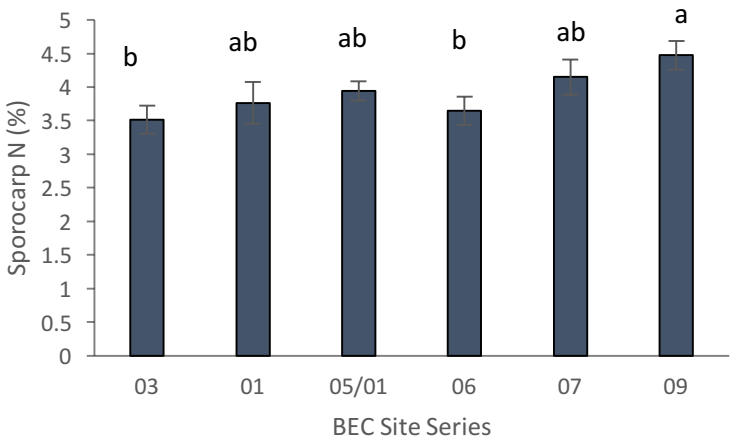


Figure 3.15 – Mean ± S.E. ECM sporocarp N (%) concentrations organized by site richness as indicated by BEC Site Series. Significant mean differences from LSD post hoc tests are indicated by lowercase letters.

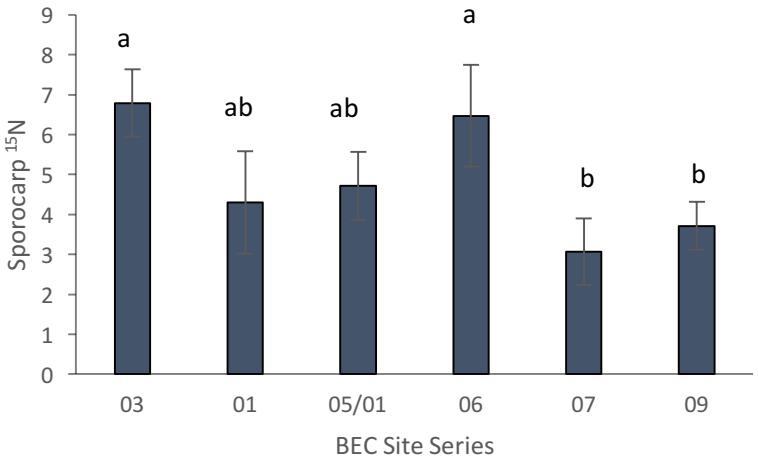


Figure 3.16 - Mean ± S.E. ECM sporocarp δ^{15}N (‰) values organized by site richness as indicated by BEC Site Series. Significant mean differences from LSD post hoc tests are indicated by lowercase letters.

3.4 Discussion

Overall, the trees at San Juan had consistently greater height, DBH and bole volume compared to the trees at Fairy Lake. The soil in San Juan was found to have a higher production of NH_4^+-N, NO_3^--N and amino acid-N compared to Fairy Lake (from Chapter 2), which likely contributed to greater tree growth on that site. When plots were organized by BEC site series, indicating a gradient of increasing N richness, however, no noticeable trends in tree growth were observed. This is likely due to the confounding of tree species with BEC site series.

Mean height of all the trees in San Juan and Fairy Lake had a positive, linear relationship (r^2= 0.3030) with total available N (NH_4^+-N + NO_3^--N+ amino acid-N concentrations). Many studies have found an increase in tree height when N is added to the system (Binkley *et al*., 1995; Footen *et al*., 2009; Littke *et al*., 2014; da Ros *et al*., 2016), as improved N nutrition increases C assimilation, and ultimately, growth. However, more N does not always equate to greater tree height or growth, as forests are often limited by low supplies of several resources, in combination (Chapin *et al*., 1987; Seastedt and Knapp, 1993; Binkley *et al*., 2004; Binkley and Fisher, 2013). Knight and Nicholas (1996) found that adding N alone did not increase *Eucalyptus saligna* growth but in combination with P, growth increased almost 10-fold. The addition of P to low-productivity cedar-salal soils was also seen to increase extractable inorganic N

concentrations (Kranabetter *et al*., 2005). A limited supply of other resources, such as P, at my sites may have masked any response to N, and weakened the correlation between growth and N supply. Negrave *et al.* (2007) found that competition for nutrients is a significant growth limiting factor, as increased stand density reduced height growth in plantations established on salal-dominated western redcedar-western hemlock stands. My study used the plots in the EP571 trial with the closest tree spacing. These factors, in combination, may have hindered N uptake by the trees in this study, causing a limited growth response to N supply. This may also suggest that the conifers in these sites may not be fully utilizing the N, especially NO_3^- (Chapter 2), available here, or the relationship between N supply and growth is weaker than what was hypothesized.

Among the four tree species planted on both sites, Douglas-fir and Sitka spruce were the tallest trees and had the greatest DBH and bole volumes. Western hemlock was intermediate and western redcedar trees were the smallest. Douglas-fir is known to respond well to high N availability, and numerous studies have found a substantial positive response of Douglas-fir to N fertilization (Knight 1963; Crossin *et al.* 1966; Chappell *et al.* 1992; Omule 1990; Weetman *et al.* 1997; Footen *et al*., 2009). Douglas-fir is able to thrive in a variety of climates and habitats (Hermann and Lavender, 1990), corresponding to its ability to take up both NH_4^+ (van den Driessche, 1978; Kamminga-Van Wijk and Prins, 1993; Turner *et al.*, 1993) and NO_3^- (Krajina *et al*., 1973; van den Driessche, 1978). This can be seen in my study, where Douglas-fir had the greatest growth at the more N-rich San Juan site. Similar to Douglas-fir, Sitka spruce also has greater biomass when supplied with higher levels of N, but a preference for NH_4^+ over NO_3^- is seen (Metcalfe *et al*., 2011; Boczulak *et al*., 2014). In my study, Sitka spruce had the greatest growth in Fairy Lake compared to San Juan. While higher NH_4^+ production was seen in San Juan soils than Fairy Lake soils, more than double the amino acid production at San Juan may have inhibited NH_4^+ uptake by spruce. Stoelken *et al.* (2010) found that the presence of glutamine or arginine in a solution with NH_4^+ and NO_3^- inhibited the uptake of NH_4^+ in European beech seedlings. Sitka spruce grows best on deep, moist, well-aerated and drained soils, and grows poorly on swampy sites (Harris, 1990). Since San Juan is characterized as a floodplain, water

accumulation in this site may have hindered spruce growth, versus the well-drained sites at Fairy Lake.

Western hemlock and western redcedar had consistently lower growth than the other two species on both sites. Compared to other conifer species, western hemlock has been shown to have an unpredictable growth response to N fertilization, which may be due to P deficiencies (Brown, 2003 review). Field studies have shown greater growth responses of western hemlock to N and P addition than just N alone (Weetman *et al.*, 1989; Bradley *et al*., 2000; Bennett *et al*., 2003). Blevins *et al*. (2006) found that 10 years after the first fertilization, western hemlock stand volume in plots with the highest rate of N and P fertilization was 10 times larger than the untreated plots. Higher P concentrations can improve tree growth by increasing photosynthetic efficiency, root growth, N uptake and mycorrhizal growth (Cole and Heil, 1981; Jentschke *et al*., 2001; Blevins *et al*., 2006), therefore, low P concentrations on my sites (1.51 ± 0.24 μg/10cm^2/6 months in Fairy Lake and 4.81 ± 1.23 μg/10cm^2/6 months in San Juan, from PRS probe data) may have limited hemlock growth. In contrast, Douglas-fir has been shown to have little response to fertilization with elements other than N (Weetman *et al.*, 1997; Carter *et al.*, 1998).

Western redcedar had the lowest height, DBH and bole volume of the four tree species on both sites. Western redcedar can be considered a stress tolerator, as it can persist and maintain slow growth when resources are limited (Antos *et al*., 2016). Western redcedar can regenerate and survive under low N and low light conditions, contributing to its wide distribution and slow growth (Kranabetter and Simard, 2008). When fertilized with both N and P, western hemlock generally had greater growth than western redcedar (Bennett *et al*., 2003; Negrave *et al.,* 2007), supporting cedar's relatively slow innate growth, compared to other conifers (Antos *et al*., 2016).

No significant difference in foliar %N was observed between the two sites. This contrasts with other studies (Radwan and Harrington, 1986; Kayahara *et al.* 1995; Wang and Klinka, 1997; Kranabetter *et al*. 2003) which found foliar nutrients, such as N, reflected site quality. Foliar δ^{15}N values have also been shown to indicate soil N availability to plants, where

foliar δ^{15}N increased with increasing N availability (Garten and Van Miegroet, 1994; McLauchlan *et al*., 2007; Craine *et al*., 2009). However, similar to foliar N concentrations, no difference in foliar δ^{15}N was observed between the two sites or among plots arranged by BEC site series, possibly due to inherent differences in substrate ^{15}N concentrations (Figure 3.9). The lack of sensitivity in these parameters may have also been due to the collection method; since I collected foliar samples from branchlets blown down by a wind storm. Discrepancies among the age of fallen foliage and where the foliage originated on the tree may have led to high variability in foliar N concentrations.

Other environmental, climatic and geographic factors, in addition to site quality, can influence foliar N concentrations. Balzotti *et al.* (2016) found elevation and precipitation were good predictors of foliar N concentrations, followed by soil and site exposure, but phylogeny explained ~75% of the variation in foliar N data. In my study, differences in foliar nutrients among tree species were seen. On average, Douglas-fir had the greatest foliar %N concentrations, followed by western hemlock and Sitka spruce, with western redcedar having the lowest foliar %N and δ^{15}N. Western redcedar reabsorbs a high percentage of N before its foliage is abscised, which results in lower N concentrations in its foliage, compared to other conifer species (Keenan *et al*., 1995). Kranabetter *et al*. (2009) examined foliar attributes of four tree species across gradients of light availability and found similar results, where western hemlock and white spruce had consistent foliar N concentrations while foliar %N of western redcedar and paper birch decreased with light.

Multiple studies across varying geographic scales have shown positive relationships between foliar N concentrations and foliar δ^{15}N values (Martinelli *et al*., 1999; Hobbie *et al*., 2000; Craine *et al*., 2005; Pardo *et al*., 2006). I also found this trend in my study, where the tree species with higher foliar N concentrations also had higher foliar δ^{15}N values. Douglas-fir, Sitka spruce and western hemlock had similar and high foliar δ^{15}N values compared to western redcerdar. Studies have found Sitka spruce (Nelson and Shelby, 1974; Boczulak *et al.,* 2014) and western hemlock (Krajina *et al*., 1973; Bennett and Prescott, 2004) to preferentially take up NH_4^+ over NO_3^-, while western redcedar seems to prefer NO_3^- (Krajina *et al*., 1973; Turner *et al*., 1993; D'Amore *et al*., 2009). Higher uptake of ^{15}N-enriched NH_4^+ may have contributed to

higher foliar ^{15}N levels for Sitka spruce and western hemlock. While Douglas-fir is found to readily take up both $NH_4^+$and NO_3^-, higher uptake of NH_4^+ may have occurred on my sites, since it is easier to assimilate compared to NO_3^-, leading to higher foliar ^{15}N.

Mycorrhizal associations affect the ^{15}N levels of plants. In general, ectomycorrhizal plants are found to be more ^{15}N-depleted relative to arbuscular mycorrhizal plants and non-mycorrhizal plants, suggesting greater transfer of ^{15}N-depleted N by ectomycorrhizal fungi to plant hosts. (Mayor *et al*., 2015; Hobbie and Högberg, 2012; Hobbie and Agerer, 2009). Craine *et al*. (2009) examined foliar $\delta^{15}N$ and mycorrhizal type for over 11,000 plants worldwide and found that arbuscular mycorrhizal, ectomycorrhizal and ericoid mycorrhizal plants were depleted in foliar $\delta^{15}N$ by 2‰, 3.2‰ and 5.9‰, respectively, relative to nonmycorrhizal plants. This trend was not seen in my study, as the arbuscular mycorrhizal western redcedar had lower foliar $\delta^{15}N$ values than the other three ectomycorrhizal conifer species, indicating greater ^{15}N depletion. The uptake of different N forms, with differences in ^{15}N content, by the tree species in my sites may have had a greater effect on foliar $\delta^{15}N$ than the effect of mycorrhizal type. Studies on ECM plant species in the tropics have suggested that the $\delta^{15}N$ of ECM and AM trees are different from those in high latitude forests (Mayor *et al*., 2015). Research from the Afro-tropics has found $\delta^{15}N$ values in ECM trees to be equivalent to or even higher than co-occurring AM trees (Högberg 1990; Högberg and Alexander 1995; Cerling *et al*. 2004; Tedersoo *et al*. 2012). Other studies have examined temperate forests with high N deposition and found ^{15}N-enrichment of ECM plants relative to co-occurring AM plants, indicating that N saturation may overtake the ECM signal (Schulze *et al*., 1994; Pardo *et al*., 2006). Also, culture studies examining effects of arbuscular mycorrhizal colonization on plant $\delta^{15}N$ have been inconclusive (Hobbie and Hobbie, 2008), therefore, more research needs to be done on the topic before a generalization can be made.

While I did not find any differences in foliar $\delta^{15}N$ between the two sites, the mean foliar $\delta^{15}N$ values of all the plots in San Juan and Fairy Lake were found to have a moderate linear relationship ($r^2 = 0.4273$) with the $\delta^{15}N$ of the forest floor. As $\delta^{15}N$ of the forest floor increased, foliar $\delta^{15}N$ also increased. Greater ^{15}N enrichment of the soil when N availability increases, could lead to increased foliar ^{15}N levels (Craine *et al*., 2009). When N availability increases, NH_4^+

and NO_3^- production also increases. Greater N concentrations lead to greater gaseous N loss during nitrification (Firestone and Davidson, 1989) and greater loss of ^{15}N-depleted NO_3^- to leaching (Högberg, 1997; Koba *et al*., 2003) and denitrification (Hall and Matson, 2003). This all leads to the production of soil available N with high ^{15}N levels, which could lead to higher foliar ^{15}N concentrations.

Epigeous sporocarps of ECM fungi may be important as biotic indicators of forest soil quality and productivity (Kranabetter *et al*., 2009) and in identification of ECM species response to environmental changes over wide spatial and temporal ranges (Tóth and Barta, 2010). In my study, I found the ECM sporocarps in San Juan had the greatest N concentrations, while the sporocarps in Fairy Lake had the greatest $\delta^{15}N$ values. Trudell and Edmonds (2004) characterized ECM sporocarp communities in two old-growth conifer forests with similar dominant tree species but different soil N and moisture contents. Similar to my study, Trudell and Edmonds (2004) saw higher ECM sporocarp N concentrations on a wet, N rich site compared to a dry, N poor site, suggesting greater N uptake and N translocation to sporocarps with increasing N availability. Mayor *et al.* (2015) examined soil, plant and fungal $\delta^{15}N$ values from 40 high- and 9 low-latitude ecosystems and found that ECM sporocarp $\delta^{15}N$ was negatively correlated with soil N concentration. This indicates that high soil N availability leads to lower ^{15}N concentration in ECM fungi. I found similar trends, with my lower N site having higher sporocarp $\delta^{15}N$ values than my richer N site. This trend can be explained if higher inorganic N availability on N rich sites results in greater uptake of ^{15}N-depleted NO_3^- because assimilation of NO_3^- is energetically cheaper than assimilation of ^{15}N-enriched organic N (Bödeker *et al*., 2014). Also, when N availability is high, host plants may have lower N demand on their mycorrhizal symbiont (Näsholm *et al.*, 2013), decreasing the transfer of ^{15}N-depleted NH_4^+ and amino acids from the fungus to the plant.

The transfer of ^{15}N-depleted N from mycorrhizal fungi to host plant produces lower ^{15}N concentrations in the plant, compared to the fungus. Many studies have observed this pattern (Handley *et al*., 1996; Taylor *et al*., 1997; Hobbie *et al*., 1999; Hobbie and Colpaert, 2003), including my study, where sporocarp ^{15}N levels were much higher than foliar ^{15}N levels. This

supports the concept of a fractionation process in the mycorrhizal symbiosis. It has been suggested that mycorrhizal fungi produce N transfer compounds, such as glutamine, that are depleted in ^{15}N relative to the N source, through discriminatory processes such as transaminase reactions (Macko *et al*., 1986; Stoker *et al*., 1996). As an example, the transamination of glutamic acid to aspartic acid results in 9‰ fractionation (Macko *et al*., 1986).

In this study, many ECM fungal species with high sporocarp N concentrations had low $\delta^{15}N$ values and vice versa. *Amanita*, *Inocybe* and *Laccaria* all had high sporocarp N concentrations and low $\delta^{15}N$ values, while *Cortinarius* and *Tricholoma* had high $\delta^{15}N$ values and low N concentrations. Trudell and Edmonds (2004) found many of these similar genera on their N contrasting sites. Genera such as *Cortinarius*, *Tricholoma* and *Hydnellum* dominated the dry, N poor site while genera such as *Inocybe*, *Russula* and *Amanitia* dominated the wetter, higher N site (Trudell and Edmonds, 2004). Lilleskov *et al.* (2001) examined ECM sporocarp communities on sites with N deposition and found that with increasing mineral N concentrations in the organic horizon, taxa such as *Cortinarius* and *Tricholoma* decreased in species richness or abundance. Avis *et al*. (2003) also looked at the effect of increased N supply on ECM community composition and found that the abundance of sporocarps in the genera *Cortinarius* and *Hydnellum* was much lower in fertilized plots, while taxa such as *Boletus*, *Inocybe*, *Lactarius*, and *Russul*a were more abundant. Kranabetter *et al*. (2009) also found *Inocybe*, *Lactariu*s and *Russula* species favoured sites with higher levels of inorganic N, while *Cortinarius*, *Tricholoma* and *Hygrophorus* species favoured mesotrophic sites with mainly organic N cycles. These studies suggest a pattern in ECM species distribution based on site factors, such as nutrient concentrations, and this pattern may be reflected in sporocarp N and ^{15}N concentrations.

Based on field, laboratory and growth culture studies, ECM fungi have been classified by exploration type based on mycelial morphology and hyphal growth (Agerer 2001, 2006, 2007; Agerer and Raidl 2004). There are four exploration types: contact, short-distance, medium-distance and long-distance, with further subdivisions in the medium-distance group (Hobbie and Agerer, 2010). From contact to long-distance types, ECM fungi range from having smooth

mantles, limited hyphae, lack of rhizomorphs and a hydrophilic nature to greater hyphal growth and distance travelled from root, presence of and highly differentiated rhizomorphs and hydrophobic nature (Hobbie and Agerer, 2010). Exploration types are not known for all ECM species and there is interspecific variability within genera, such as in *Russula*, *Lactarius*, and *Craterellus* (Trudell and Edmonds, 2004; Hobbie and Agerer, 2010). To further characterize ECM species based on exploration type, δ^{15}N values have been used and distinct patterns have been found. Hobbie and Agerer (2010) confirmed that N isotopes in ECM fungi correlate with exploration type and hydrophobicity. Hydrophobic ECM fungi had on average 3-4‰ higher ^{15}N concentrations than hydrophilic ECM fungi (Hobbie and Agerer, 2010; Hobbie and Högberg, 2012). ECM fungi with rhizomorphs, cords and mat-forming hyphae are hydrophobic and their development in culture is severely reduced in high moisture conditions (Unestam, 1991; Unestam and Sun, 1995). ECM fungi with these characteristics may be adapted to habitats with seasonal drought and lower moisture conditions (Unestam, 1991; Unestam and Sun, 1995). On average, hydrophobic ECM genera with higher δ^{15}N values are characterized as medium- to long-distance exploration types (Hobbie and Högberg, 2012). These genera include *Cortinarius*, *Tricholoma*, *Hydnellum* and *Suillus* (Hobbie and Högberg, 2012). Conversely, ECM genera with lower δ^{15}N values are mainly hydrophilic and grouped as contact, short- and medium-distance exploration types (Hobbie and Högberg, 2012). ECM fungi with hyphae that form diffuse mycelia are hydrophilic, inhibited by low soil moisture and may be more adapted to mesic habitats (Trudell and Edmonds, 2004). Some of these genera included *Laccaria*, *Inocybe*, *Amanita* and *Russula* (Hobbie and Högberg, 2012). My study supports these findings. *Cortinarius* and *Tricholoma* had high sporocarp δ^{15}N values and were most abundant on the drier, lower N site. The lack of water in drier habitats can inhibit ammonifying and nitrifying microbes (see Chapter 2 Discussion), decreasing available inorganic N. Sites with low levels of available inorganic N may result in higher N demand by host plants from mycorrhizal symbionts, increasing the transfer of ^{15}N-depleted NH_4^+ and amino acids to the plant and increasing ECM sporocarp δ^{15}N. Many genera with high δ^{15}N values, like *Cortinarius*, also have strong proteolytic abilities (Lilleskov *et al*., 2002), reflected in their increased abundance on sites with high levels of organic N and higher δ^{15}N values. Higher δ^{15}N values can also be due to their

specific exploration type. Many of these genera have hyphae that travel medium to long distances from the mycorrhizal root compared to other ECM genera, and since soil $\delta^{15}N$ increases with increasing depth, they are able to access deeper N pools with higher ^{15}N concentrations (Hobbie and Högberg, 2012). In my study, *Lactarius* had higher average $\delta^{15}N$ values compared to many of the other genera; however, there was a large range in $\delta^{15}N$ among the species within *Lactarius*, ranging from 3.46 – 8.49‰. This is not surprising, as *Lactarius* is among the genera with large interspecific variability (Trudell and Edmonds, 2004; Hobbie and Agerer, 2010). Many genera with low $\delta^{15}N$ values in the literature (Hobbie and Högberg, 2012) were seen in my study. I found *Laccaria*, *Amanita* and *Inocybe* to all have lower $\delta^{15}N$ values compared to other genera. These ECM fungi are characterized as being hydrophilic with hyphae travelling short to medium distances from the root, and many of these genera dominate on sites with high levels of inorganic N (Trudell and Edmonds, 2004; Kranabetter *et al*., 2009). Lower $\delta^{15}N$ values may be due to less access to $\delta^{15}N$-enriched, deeper N sources because of shorter hyphal extension. Also, since these genera dominate on inorganic N-rich sites, there may be less demand for N from the fungi by host plants, and thus less isotopic fractionation and retention of ^{15}N-enriched N sources by the fungi.

3.5 Conclusion

The aim of this study was to determine the relationships among soil N availability, tree growth and foliar and sporocarp N. On average, the trees in San Juan had greater growth, compared to the trees in Fairy Lake. From Chapter 2, San Juan was found to be the more N-rich site with greater inorganic and organic N production, likely contributing to greater tree growth on that site. Differences in growth among the four tree species were found, with Douglas-fir and Sitka spruce having the greatest growth and western redcedar having the least growth. This may reflect species-specific characteristics such as N form preferences and soil moisture availability, and inherent differences in growth rates. Soil total available N (NH_4^+-N + NO_3^--N+ amino acid-N concentrations) was found to linearly increase with mean height of the trees, suggesting that improved N nutrition increases growth. However, low supply of other nutrients, such as P, and competition may have limited the growth response. While foliar %N and $\delta^{15}N$

were not related to site quality, differences were observed among tree species. Western redcedar had the lowest foliar %N and δ^{15}N values, supporting western redcedar's ability to reabsorb a higher percentage of foliar N compared to other conifers. Mean foliar δ^{15}N was also found to linearly increase with δ^{15}N of the forest floor, due to greater uptake of ^{15}N-enriched N.

ECM sporocarps reflected site quality, with greater N concentrations in the sporocarps from San Juan, suggesting greater N uptake and N translocation to sporocarps by ECM fungi as N availability increases. ECM sporocarps from Fairy Lake had higher mean δ^{15}N values than those from San Juan, potentially due to the higher ^{15}N concentrations of the specific N forms taken up, or due to greater transfer of ^{14}N to the host tree. The fractionation processes in the mycorrhizal symbiosis were evident from my results, with sporocarp ^{15}N levels being much higher than foliar ^{15}N levels. Differences seen in %N and δ^{15}N values among the sporocarps of different ECM genera and species supports the concept of niches based on functional traits such as N form uptake, drought tolerance and mycelial morphology or exploration type.

Chapter 4 – Nitrogen form uptake by Arbuscular mycorrhizae and Ectomycorrhizae

4.1 Introduction

4.1.1 Mycorrhizal fungal community assembly

Mycorrhizal fungi form mutualistic associations with over 90% of all plant species, from liverworts to gymnosperms (Lanfranco *et al*., 2016). Many different types of mycorrhizal symbiosis occur, including relationships among a diverse range of fungal and plant species. Two of the most common associations are arbuscular mycorrhizae (AM) and ectomycorrhizae (ECM). AM symbiosis occurs between fungi in the phylum Glomeromycota and approximately 80% of all known plant species (Lanfranco *et al*., 2016). While only 250 species of AM fungi have been identified, meta-sequencing of soil samples suggest this number is much higher (Bijl *et al*., 2011; Lanfranco *et al*., 2016). ECM symbiosis, on the other hand, occurs primarily between fungi in the phyla Basidiomycota and Ascomycota, and approximately 3% of plant species (Finlay, 2008). Although the number of host plant species is relatively small, 7750 ECM fungal species have been identified, with estimates of up to 25,000 species in total (Rinaldi *et al*., 2008). With this diverse range of AM and ECM fungal species and host plants, the parameters that organize mycorrhizal communities are of interest, as they may allow for better ecosystem characterization and monitoring. However, due to the difficulties of identifying fungal species, sampling of the hyphal phase, and culturing and manipulation of the fungi, little is known of the factors that affect mycorrhizal community structure (Crowther *et al*., 2014, Kranabetter *et al*., 2015).

One way to characterize mycorrhizal community assembly is by determining the functional traits of the mycorrhizal fungi (Crowther *et al*., 2014). Functional traits influence community structure because the function of organisms determines their ability to tolerate a range of climatic conditions, acquire resources and interact with other individuals, which, together, result in a specific community (Maherali and Klironomos, 2012). ECM fungal communities are influenced by tree species composition, forest structure (Ishida *et al*. 2007) and stand age (Twieg *et al.* 2007; Twieg *et al.* 2009). Many studies have examined different soil parameters affecting ECM community assembly, such as soil nutrients (Avis *et al*., 2003), N

availability, pH and moisture (Scattolin *et al*. 2008; Kranabetter *et al*. 2009; Zhang *et al*. 2013; Moeller *et al*. 2014; Walker *et al.* 2014; Corrales *et al*. 2015). While AM fungi are often considered generalists, with only ~250 species identified, Kivlin *et al*. (2011) found, by examining 14,961 DNA sequences from 111 published studies, that geographic distance, soil temperature and moisture, and plant community type were all significantly related to AM fungal community composition. Competition was also found to be a dominant force structuring AM comminutes, as it prevented functionally similar traits co-existing at small spatial scales (Maherali and Klironomos, 2012).

4.1.2 Mycorrhizal type and Nitrogen form uptake

N-limited forest ecosystems on marginal soils are generally characterized as having low external N input and loss from the atmosphere and hydrosphere, as well as high internal N cycling from the depolymerization and mineralization of leaf and root litter and decaying microbial biomass (Rennenberg *et al*., 2009). For this reason, the N cycle in N-limited soils is dominated by reduced inorganic and organic N compounds like ammonium, amino acids and peptides (Rennenberg *et al*., 2009). This is different from riparian forest ecosystems that receive significant N inputs, as NO_3^-, from flood and groundwater sources (Rennenberg *et al*., 2009). In temperate forest ecosystems, where N is most often the limiting nutrient for growth and there are spatial and temporal differences in N form availability, N concentration can play a role in mycorrhizal niche differentiation, through N form preferences of NH_4^+, NO_3^- and/or amino acids, in AM and ECM fungal communities (Rennenberg *et al*., 2009).

NH_4^+ has often been described as the preferred N form by mycorrhizae, as it is more energetically efficient to assimilate than NO_3^- and amino acids (Bücking *et al*., 2012). However, numerous experiments using radioactive labeled N, measurements of plant enzyme activity in N assimilation, and transcriptional studies of nitrate reductase in the mycobiont have found that AM fungal hyphae have the ability to take up NO_3^- and amino acids, along with NH_4^+, from the soil and translocate these compounds to the host plant (Kaldorf *et al*., 1994; Johansen *et al*., 1996; Cliquet *et al*., 1997; Faure *et al*., 1998; Subramanian and Charest, 1998; Hawkins *et al*., 2000; Leigh *et al*., 2009). Also, as discussed in the Literature Review (Section 1.4.1), AM fungi

and ECM fungi have both been found to have NH_4^+, NO_3^- and amino acid transporters that allow them to assimilate these different N forms and pass N along to their host plant. While the ability of AM fungi to take up different N forms has been determined, numerous studies continue to find NH_4^+ as the preferred N form by AM fungi (Johansen *et al*., 1996; Toussaint *et al*., 2004; Gachomo *et al.*, 2009; Bücking *et al*., 2012; Lanfranco *et al*., 2016). As different plant species have been found to have N form preferences, AM fungal species, too, may have N form preferences, since it has been shown that even the genetic diversity in one AM fungal spore can be enough to develop phenotypically different variants of one fungal species (Ehinger *et al*., 2012; Hogde and Storer, 2015).

The preference of ECM fungi for certain N forms, on the other hand, is debated. Early studies on excised and intact ectomycorrhizal root systems found enhanced NH_4^+ uptake capacities of mycorrhizal plants compared to their non-mycorrhizal counterparts (France and Reid, 1979; Genetet *et al*., 1984; Rygiewicz *et al*., 1984). ECM fungi are found to have a preference for NH_4^+ over NO_3^- *in vitro* (Finlay *et al*., 1992; Rangel-Castro *et al*., 2002; Guidot *et al.,* 2005) and in the field (Grenon *et al*., 2005; Clemmensen *et al*., 2008). Studies using the ECM fungal species *Hebeloma cylindrosporum* found that supplying the fungus with NH_4^+ led to the down-regulation of a NO_3^- transporter and a nitrate reductase (Jargeat *et al*., 2003; Smith and Read, 2008; Rékangalt *et al*., 2009). The majority of the 68 ECM fungal species grown in culture by Nygren *et al.* (2008) were reported to prefer NH_4^+ as an inorganic N source, with the ability to grow on NO_3^- being widely distributed among the different species. Other studies found certain ECM fungal species preferred and even had greater biomass with NO_3^- over NH_4^+ (Plassard *et al*., 1994; Scheromm *et al.*, 1990; Aouadj *et al*., 2000; Montanini *et al*., 2002). ECM fungal species such as *Laccaria laccata* (Ahmad *et al*., 1990), *Pisolithus tinctorius* (France & Reid, 1984; Plassard *et al*., 1991) and *Hebeloma cylindrosporum* (Scheromm *et al*., 1990) are found to easily take up and assimilate NO_3^-. Molecular, growth culture and field studies examining ECM fungal species diversity with inorganic N uptake abilities have found evidence to support niche differentiation (Nygren *et al.* 2008; Jones *et al.* 2009; Pena and Polle 2014), indicating that ECM fungi may be adapted to the available N forms in the soil from which they were isolated, which

could allow the fungi to compete with other soil microbes (Chalot and Plassard, 2011). There is a strong competition in the soil to use N as it becomes available, where soil microbes other than ECM fungi are usually the first sinks for added N (Chalot and Plassard, 2011).

4.1.3 Study Objective

This study searched for evidence of N form preferences of ECM and AM communities associated with two conifer species on the west coast of Vancouver Island. ECM roots were extracted from two pure stands of 50+ year old Douglas-fir trees. The two Douglas-fir plots were chosen from sites with contrasting soil N availability (see Chapter 2), where one site had significantly more NH_4^+ and NO_3^- production than the other. Roots were extracted from a pure stand of 50+ year old western redcedar trees on a N-rich site. While AM associations were not confirmed on the cedar roots, these roots were assumed to be mycorrhizal and referred to as AM roots throughout the rest of the study.

The objective of this study was to quantify NH_4^+ and NO_3^- uptake and H^+ net flux in ECM and AM roots supplied with NH_4^+ and high NO_3^- solution. Based on the type of mycorrhizal symbiosis and N quality of the site from which the mycorrhizal roots were isolated, I hypothesized that there would be differences in N form uptake between sites and among fungal species, contributing to niche differentiation. Since AM and ECM both have the capacity to take up NH_4^+ and NO_3^- from the surrounding soil, due to the presence of NH_4^+ and NO_3^- transporters, I hypothesized that the mycorrhizal roots sampled from the site with higher N availability would be more adapted to these conditions and would, therefore, have higher NH_4^+ and NO_3^- uptake. Also, since the N-rich site had greater production of NO_3^- compared to NH_4^+, I hypothesized that some mycorrhizal roots from this site would have greater uptake of NO_3^- relative to NH_4^+ when supplied with a high NO_3^- solution.

4.2 Materials and Methods

4.2.1 Site Description

The study area was located near Port Renfrew, B.C. (latitude 48° 33-36' N; longitude 124° 19-21' W; elevation 90-250m) and is part of the B.C Ministry of Forests, Lands and Natural Resource Operations' Experimental Project (EP) 571 espacement trial planted in 1960 (Omule, 1988). Two sites from the EP571 trial were used for the study: Fairy Lake (48° 35'N, 124° 19'W, elevation 200-280m) and San Juan (48° 35'N, 124° 12'W, elevation 65-85m). Each site contained 24, 700m^2 plots with trees of four species planted at three spacings, in a randomized complete block design. The 2.7m spacing plots were used for this study. Both sites consisted of two plots each of Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW) (16 plots in total).

4.2.2 Mycorrhizal root Collection

During the month of April 2016 (Spring), 10 root samples were collected every Monday for three weeks from each of three plots, Douglas-fir plot 44 (SJ44FD) and western redcedar plot 48 (SJ48CW) from San Juan and Douglas-fir plot 18 from Fairy Lake (FL18FD), thus 30 samples in total were collected each week. Locations for root samples were chosen at random across the entire plot, with the condition that samples must be 0.5-1m away from a tree. At each location, the forest floor and mineral soil were removed by hand until mycorrhizal tree roots were found, then roots were gently separated and a 15-20cm section was cut with a soil knife. The root sections were covered in moss to keep them moist, brought back to the lab and stored in a 10°C fridge overnight. The following Tuesday to Thursday, at least two root samples were analyzed per plot each day (6-7 roots per day in total). Each root sample was cleaned by soaking in a buffer solution of 200μM $CaSO_4 \bullet 2H_2O$ at pH5 and removing clumps of soil and debris with forceps under a dissecting microscope.

4.2.3 Ion Flux Analysis

The net fluxes of NH_4^+, NO_3^- and H^+ ions in the root samples were measured over 72 hours a week (Tuesday to Thursday) for three weeks using a microelectrode ion flux measurement system (MIFE™, Unitas Consulting, Hobart, Australia). Prior to flux measurements, electrode blanks were pulled from 1.5mm borosilicate glass capillaries, dried in

an oven at 220°C for 4 h and silinized with tributylchlorosilane (catalog no. 90796, Fluka). Cooled microelectrodes were backfilled with 200mM NH_4Cl for NH_4^+, 500mM KNO_3 + 100mM KCl for NO_3^- and 15mM NaCl + 40mM KH_2PO_4 (adjusted to pH 6.0 using 0.1 NaOH) for H^+. Every day for three days following the retrieval of the root samples, the tips of the microelectrodes were filled with ion-selective H^+ or NH_4^+ resin cocktails (Fluka catalog no. 95297 and 09882, respectively) or a NO_3^- selective cocktail containing 0.5% methyltridodecylammoniumnitrate (MTDDA NO3-), 0.084% methyltriphenylphosphonium bromide (MTPPB) and 99.4% n-phenyloctylether (NPOE) (Plassard *et al*. 2002). The H^+ microelectrode was calibrated with pH 4.89, 5.01 and 6.88 solutions, the NH_4^+ electrode was calibrated with 25, 50 and 100μM NH_4^+ solutions (25, 50 and 100μM NH_4NO_3 solutions) at pH 5 and the NO_3^- electrode was calibrated with 225, 500 and 1000μM NO_3^- solutions (25μM NH_4NO_3 + 100μM $Ca(NO_3)_2 \bullet 4H_2O$, 50μM NH_4NO_3 + 225μM $Ca(NO_3)_2 \bullet 4H_2O$ and 100μM NH_4NO_3 + 450μM $Ca(NO_3)_2 \bullet 4H_2O$ solutions) at pH 5. The calibrated electrodes were mounted onto an electrode holder (NMT-5, Narishige, Tokyo, Japan), which provided three-dimensional positioning. The electrodes were positioned in a line, tips spaced 3-4μm apart and 20μm above the root surface. The holder was attached to a computer controlled micromanipulator (PatchMan NP2, Eppendorf AG, Hamburg, Germany). For flux measurements, the MIFE computer gently moved the electrode holder up and down between two positions, 20μm and 60μm, above the root surface, in a 10s square-wave cycle. The ion concentrations were determined by their electrochemical potential at each position and ion flux measurements were determined by the difference in electrochemical potential between the two positions (Shabala *et al*., 1997; Alber *et al*., 2012). The flux measurements for each sample were taken for 10 minutes and averaged, with the first 2 minutes removed. Descriptions of MIFE™ set up and measurements are based on those discussed in Shabala *et al*., (1997) and Shabala and Newman (1997).

The cleaned root samples were tied and submerged into a 50μM NH_4^+ + 500μM NO_3^- aerated measuring solution (50μM NH_4NO_3 + 225μM $Ca(NO_3)_2 \bullet 4H_20$) at pH 5, for at least 30 minutes prior to analysis. After acclimating for 30 minutes, one sample was removed at a time and inserted into the MIFE™ chamber. Fresh measuring solution was added and three to five measurements of net fluxes on each root (colony) were made. This procedure was repeated in

the Fall of 2016 (end of September to the beginning of October) with similar calibration solutions, but with an NH_4^+-free 500μM NO_3^- measuring solution (225μM $Ca(NO_3)_2 \bullet 4H_2O$) at pH 5, in order to examine the effects of season and presence of NH_4^+ (Table 4.2).

4.2.4 Molecular Genetic Analysis

Molecular techniques were used to confirm taxonomic identifications for ectomycorrhizal Douglas-fir roots. Approximately 1cm of mycorrhizal tip from each sample was cut and frozen at -80°C and sent to the Pacific Forestry Centre for CTAB DNA extraction and PCR amplification of the fungal internal transcribed spacer (ITS) region of nuclear rDNA (Kranabetter *et al.*, 2015). The primer pairs used for PCR amplification were the basidiomycetes-specific ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4B (5'-CAGGAGACTTGTACACGGTCCAG-3') or universal ITS5
(5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990; Gardes and Bruns, 1993). Methodology for DNA extraction and PCR amplification was as described in Kranabetter *et al.* (2013). The resulting PCR products were sent for sequencing (Centre de Recherche du Centre hospitalier de l'Université Laval). The sequences were aligned and manually corrected in Sequencher (Sequencher 5.4, Ann Arbor, Michigan, USA) and BLAST searched against the UNITE database for molecular identification (Kranabetter *et al*., 2015). Only molecular identifications with a >98.5% match were used for analysis (Table 4.1 and 4.2).

4.2.5 Statistical Analysis

For all data, assumptions of normality and homogeneity of variance were determined using the Kolmogorov-Smirnov test and Levene's test. A nested two-way mixed effects Analysis of Variance (ANOVA) model and Least Significant Difference (LSD) post hoc analysis for significant means differences were used to analyze the NH_4^+, NO_3^- and/or H^+ flux by the mycorrhizal roots sampled from the three plots in the Spring and Fall. The model contained two factors, plot and root, with root nested in plot, and individuals from each root were pseudoreplicates. For the two Douglas-fir plots, the NH_4^+, NO_3^- and/or H^+ flux by the mycorrhizal roots in the Spring and Fall were analyzed by fungal genera and species using a one-way ANOVA and LSD test. Spring and Fall 2016 mean H^+, NH_4^+ and/or NO_3^- flux data were

compared, using a scatterplot, with Fall 2015 mean sporocarp δ^{15}N data of the same genera (Chapter 3) to find potential relationships. All ANOVA models used replicate as the Error term and a significance of α = 0.05 (McClave and Sincich, 2009). Statistical analyses were conducted in SAS® (Statistical Analysis System, Cary, NC, USA).

Table 4.1 – Ectomycorrhizal species sampled in the Spring of 2016 from Douglas-fir plot 18 in Fairy Lake (FL18FD) and Douglas-fir plot 44 in San Juan (SJ44FD). Ectomycorrhizal species are organized by family and genus, with number of colonies tested (n) and plot on which they were found.

Plot	n	Family	Genus	Species
SJ44DF	2	Atheliaceae	*Amphinema*	*byssoides*
FL18DF	2	Atheliaceae	*Amphinema*	unknown
FL18DF	2	Atheliaceae	*Piloderma*	unknown
SJ44DF	1	Boletaceae	*Xerocomellus*	*zelleri*
FL18DF	2	Corticiaceae	*Tylospora*	unknown
FL18DF	1	Cortinariaceae	*Cortinarius*	*obtusus*
SJ44DF	1	Discinaceae	*Hydnotrya*	*cubispora*
FL18DF	1	Gloniaceae	*Cenococcum*	*geophilum*
SJ44DF	1	Inocybaceae	*Inocybe*	*agglutinata*
SJ44DF	1	Inocybaceae	*Inocybe*	*lacera*
FL18DF & SJ44DF	5	Russulaceae	*Lactarius*	*hepaticus*
SJ44DF	1	Russulaceae	*Russula*	*chloroides*
SJ44DF	1	Russulaceae	*Russula*	*nigricans*
FL18DF	1	Sebacinaceae	*Sebacina*	unknown
FL18DF	1	Thelephoraceae	unknown	unknown
SJ44DF	1	Thelephoraceae	*Tomentella*	*galzinii*
FL18DF & SJ44DF	2	Thelephoraceae	*Tomentella*	*stuposa*
FL18DF & SJ44DF	5	Thelephoraceae	*Tomentella*	*sublilacina*

Table 4.2 – Ectomycorrhizal species sampled in the Fall of 2016 from Douglas-fir plot 18 in Fairy Lake (FL18FD) and Douglas-fir plot 44 in San Juan (SJ44FD). Ectomycorrhizal species are organized by family and genus, with number of colonies tested (n) and plot on which they were found.

Plot	n	Family	Genus	Species
SJ44DF	2	Atheliaceae	*Amphinema*	*byssoides*
FL18DF & SJ44DF	2	Atheliaceae	*Amphinema*	unknown
FL18DF & SJ44DF	3	Atheliaceae	unknown	unknown
FL18DF	2	Corticiaceae	*Tylospora*	*fibrillosa*
FL18DF	1	Corticiaceae	*Tylospora*	unknown
FL18DF	1	Cortinariaceae	*Cortinarius*	unknown
SJ44DF	1	Clavulinaceae	*Clavulina*	*coralloides*
SJ44DF	2	Clavulinaceae	*Clavulina*	unknown
SJ44DF	1	Inocybaceae	*Inocybe*	*whitei*
FL18DF & SJ44DF	2	Russulaceae	*Lactarius*	*hepaticus*
FL18DF & SJ44DF	4	Russulaceae	*Russula*	*emetica*
SJ44DF	1	Russulaceae	*Russula*	*pallescens*
SJ44DF	1	Thelephoraceae	*Thelephora*	unknown
SJ44DF	1	Thelephoraceae	*Tomentella*	*stuposa*
FL18DF & SJ44DF	5	Thelephoraceae	*Tomentella*	*sublilacina*

4.3 Results

4.3.1 Spring and Fall 2016 Ion Flux Measurements by Plot

There were significant differences in average H^+, NH_4^+ and NO_3^- flux among the mycorrhizal roots sampled from the three plots: FL18DF, SJ44DF and SJ48CW in the Spring of 2016 (Table 4.3). On average, there was substantial H^+ efflux, but NH_4^+ and NO_3^- uptake, and NH_4^+ uptake on average was approximately 10 times NO_3^- uptake. The roots sampled from the Douglas-fir plot in Fairy Lake had significantly greater efflux of H^+ ions than the roots sampled from the two plots in San Juan (Figure 4.1). There were no significant differences in the efflux of H^+ ions between the Douglas-fir plot and western redcedar plot in San Juan (Figure 4.1). There were significant differences in the NH_4^+ and NO_3^- ion fluxes among the three plots (Table 4.3). Both Douglas-fir plots had no significant differences in NH_4^+ nor NO_3^- uptake, however, both Douglas-fir plots had greater NH_4^+ uptake than the western redcedar plot (Figure 4.2 and 4.3). The Douglas-fir plot in Fairy Lake had a greater uptake of NO_3^- compared to the NO_3^- efflux in the San Juan western redcedar plot, and the San Juan Douglas-fir plot was intermediate (Figure

4.3). There were significant differences in H^+, NH_4^+ and NO_3^- flux among the different roots sampled within each plot (Table 4.3).

Table 4.3 – P-values from the nested two-way ANOVA comparing H^+, NH_4^+ and NO_3^- flux among the mycorrhizal roots sampled in Spring 2016 from three plots: Fairy Lake Douglas-fir plot 18 (FL18FD), San Juan Douglas-fir plot 44 (SJ44FD) and San Juan western redcedar plot 48 (SJ48CW).

	H^+ Flux	NH_4^+ Flux	NO_3^- Flux
Plot	**<0.0001**	**<0.0001**	**0.0289**
Root(plot)	**<0.0001**	**0.0034**	**0.0021**

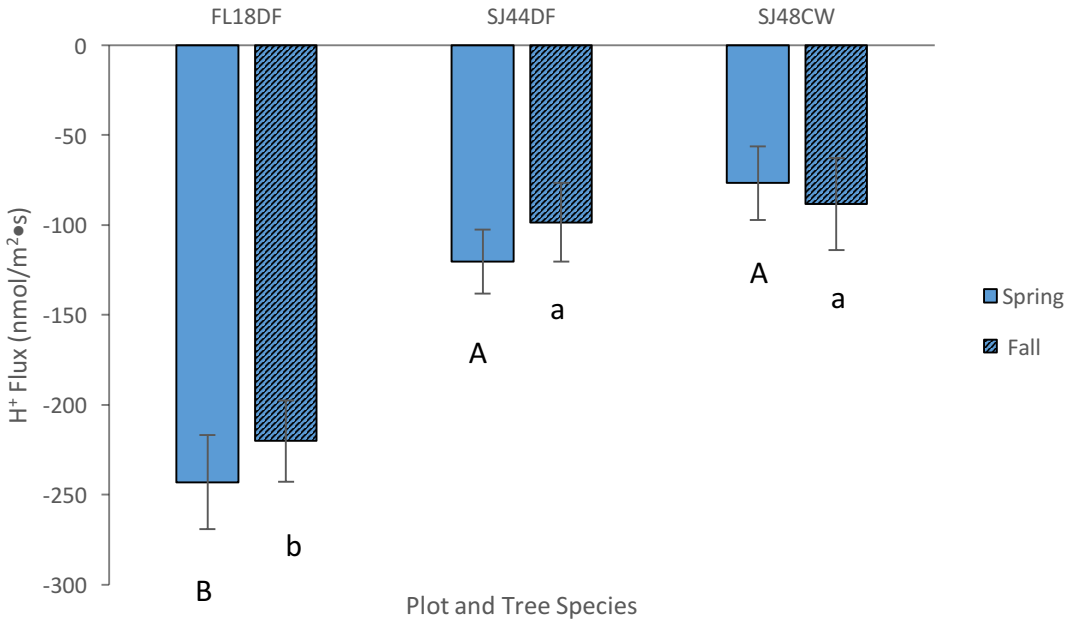


Figure 4.1 – Mean net H^+ efflux (nmol/m^2•s) from the mycorrhizal roots sampled in the Spring and Fall of 2016 from three plots: Fairy Lake Douglas-fir plot 18 (FL18FD), San Juan Douglas-fir plot 44 (SJ44FD) and San Juan western redcedar plot 48 (SJ48CW). Significant mean plot differences from LSD post hoc tests are shown by letters; upper and lowercase letters are compared separately.

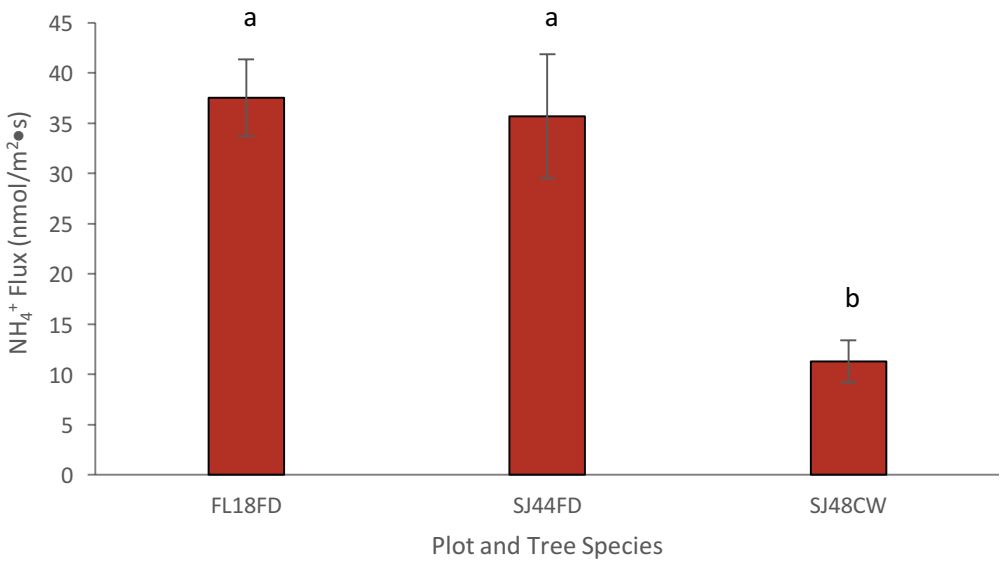


Figure 4.2 – Mean net NH_4^+ flux (nmol/m^2•s) from the mycorrhizal roots sampled in the Spring of 2016 from three plots: Fairy Lake Douglas-fir plot 18 (FL18FD), San Juan Douglas-fir plot 44 (SJ44FD) and San Juan western redcedar plot 48 (SJ48CW). Significant mean plot differences from LSD post hoc tests are indicated by lowercase letters.

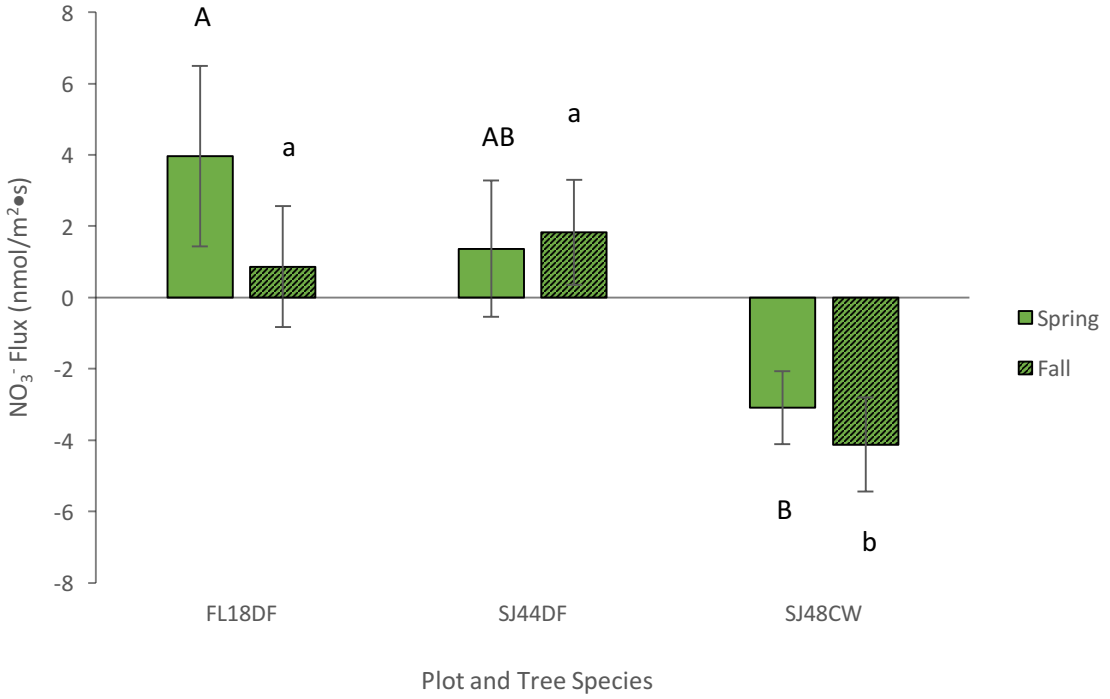


Figure 4.3 – Mean net NO_3^- flux (nmol/m^2•s) from the mycorrhizal roots sampled in the Spring and Fall of 2016 from three plots: Fairy Lake Douglas-fir plot 18 (FL18FD), San Juan Douglas-fir plot 44 (SJ44FD) and San Juan western redcedar plot 48 (SJ48CW). Significant mean plot differences from LSD post hoc tests are shown by letters; upper and lowercase letters are compared separately.

There were significant differences in average H^+ and NO_3^- net flux among the mycorrhizal roots sampled from the three plots: FL18DF, SJ44DF and SJ48CW in the Fall of 2016 (Table 4.4). On average, there was substantial H^+ efflux, but NO_3^- uptake. Roots from the Douglas-fir plot in Fairy Lake had the largest efflux of H^+ ions, while the two plots in San Juan had no significant differences in H^+ efflux (Figure 4.1). Significant differences were also found in NO_3^- flux among the three plots (Table 4.4). Both Douglas-fir plots had NO_3^- uptake with no significant differences in uptake rate, while the western redcedar plot in San Juan was found to have an efflux of NO_3^- ions (Figure 4.3). There were significant differences in H^+ and NO_3^- flux among the different roots sampled within each plot (Table 4.4).

Table 4.4 - P-values from the nested two-way ANOVA comparing H^+ and NO_3^- flux among the mycorrhizal roots sampled in the Fall of 2016 from three plots: Fairy Lake Douglas-fir plot 18 (FL18FD), San Juan Douglas-fir plot 44 (SJ44FD) and San Juan western redcedar plot 48 (SJ48CW).

	H^+ Flux	NO_3^- Flux
Plot	**<0.0001**	**0.0040**
Root(plot)	**<0.0001**	**0.0001**

4.3.2 Spring and Fall 2016 Ion Flux Measurement by Ectomycorrhizal Genera

There were significant differences in H^+ and NO_3^- fluxes among the ectomycorrhizal genera sampled in the two Douglas-fir plots in the Spring of 2016 (Table 4.5). On average, *Inocybe* and *Sebacina* had uptake of H^+ while all other genera had H^+ efflux, with *Lactarius* showing a very high H^+ efflux (Table 4.5). *Inocybe* was found to have relatively high NO_3^- uptake, while *Hydnotrya* had high NO_3^- efflux (Table 4.5). Russulaceae was the only family that had significant differences in H^+, NH_4^+ and/or NO_3^- fluxes between the different genera sampled within that family (Table 4.5). On average, *Lactarius* had greater H^+ efflux compared to *Russula*, while *Russula* had uptake of NO_3^- and *Lactarius* had NO_3^- efflux (Table 4.5). While, overall, there were no significant differences in NH_4^+ flux among the sampled ECM genera, *Lactarius* had relatively high NH_4^+ uptake and *Cortinarius* and relatively low NH_4^+ uptake (Table 4.5)

Table 4.5 – P-values from one-way ANOVA and means differences from LSD test, indicated by lowercase letters, for net H^+, NH_4^+ and NO_3^- fluxes (nmol/m^2•s) among the genera sampled in the Douglas-fir plot 18 in Fairy Lake and the Douglas-fir plot 44 in San Juan during the Spring of 2016. Genera are grouped by family and mean ± S.E. for each genus are included.

Family	Genus	H^+ Flux (nmol/m^2•s) (p=0.0019)		NH_4^+ Flux (nmol/m^2•s) (p=0.4994)		NO_3^- Flux (nmol/m^2•s) (p=0.0027)	
Atheliaceae	*Amphinema*	ab	-165.98 ± 80.97	ab	35.61 ± 8.76	bc	6.74 ± 3.52
	Piloderma	ab	-102.25 ± 31.93	ab	42.75 ± 15.01	abc	10.44 ± 11.23
Boletaceae	*Xerocomellus*	ab	-95.91 ± 43.42	ab	20.82 ± 16.68	bcd	-1.32 ± 2.28
Corticiaceae	*Tylospora*	bc	-197.61 ± 44.53	ab	54.53 ± 10.29	bc	8.00 ± 7.22
Cortinariaceae	*Cortinarius*	abc	-202.45 ± 83.01	b	12.53 ± 4.47	bcd	-3.77 ± 1.73
Discinaceae	*Hydnotrya*	abc	-218.74 ± 62.87	ab	19.05 ± 7.72	d	-19.07 ± 9.84
Gloniaceae	*Cenococcum*	bc	-330.27 ± 68.98	ab	40.39 ± 4.66	abc	5.28 ± 5.30
Inocybaceae	*Inocybe*	a	1.27 ± 9.55	ab	47.03 ± 18.43	a	21.12 ± 6.06
Russulaceae	*Lactarius*	c	-384.97 ± 69.33	a	53.85 ± 12.76	c	-1.17 ± 2.94
	Russula	ab	-130.12 ± 74.33	ab	32.35 ± 6.42	ab	11.23 ± 5.48
Sebacinaceae	*Sebacina*	a	56.59 ± 98.64	ab	10.36 ± 7.06	abc	6.33 ± 10.31
Thelephoraceae	*Tomentella*	b	-170.02 ± 24.19	ab	40.51 ± 7.88	bc	0.50 ± 1.33
	Unknown	ab	-67.48 ± 138.03	ab	12.09 ± 7.53	abc	12.12 ± 7.03

In the Fall of 2016, significant differences were found only in H^+ flux among the ectomycorrhizal genera sampled in the two Douglas-fir plots (Table 4.6). On average, *Inocybe* had high H^+ uptake while *Cortinarius* had very high H^+ efflux (Table 4.6). While, overall, there were no significant differences in NO_3^- flux among the sampled ECM genera, *Clavulina* was found to have high NO_3^- uptake compared to the NO_3^- efflux seen by *Cortinarius*, *Inocybe* and *Tomentella* (Table 4.6).

Table 4.6 - P-values from one-way ANOVA and means differences from LSD test, indicated by lowercase letters, for net H^+ and NO_3^- flux (nmol/m^2•s) among the genera sampled in the Douglas-fir plot 18 in Fairy Lake and the Douglas-fir plot 44 in San Juan during the Fall of 2016. Genera are grouped by family and mean ± S.E. for each genus are included.

Family	Genus	H^+ Flux (nmol/m^2•s) (p=0.0005)		NO_3^- Flux (nmol/m^2•s) (p=0.1137)	
Atheliaceae	*Amphinema*	ab	-47.30 ± 42.88	ab	4.32 ± 3.09
	Unknown	ab	-86.53 ± 33.12	ab	3.50 ± 2.93
Corticiaceae	*Tylospora*	abc	-96.16 ± 38.69	ab	4.96 ± 3.20
Cortinariaceae	*Cortinarius*	d	-399.11 ± 81.08	b	-9.04 ± 1.93
Clavulinaceae	*Clavulina*	bc	-158.95 ± 48.35	a	11.58 ± 2.52
Inocybaceae	*Inocybe*	a	94.74 ± 65.13	b	-8.04 ± 22.33
Russulaceae	*Lactarius*	abcd	-149.96 ± 60.43	ab	1.72 ± 5.97
	Russula	cd	-245.80 ± 45.04	b	0.11 ± 3.47
Thelephoraceae	*Thelephora*	ab	30.25 ± 21.16	ab	6.75 ± 0.70
	Tomentella	cd	-247.67 ± 54.78	b	-1.55 ± 1.66

4.3.3 Spring and Fall 2016 Ion Flux and Sporocarp $\delta^{15}N$ Comparison

Of the mycorrhizal roots sampled in the Spring and Fall of 2016 and ectomycorrhizal sporocarps sampled in the Fall of 2015 (see Chapter 3), four genera were found in common: *Lactarius*, *Russula*, *Cortinarius* and *Inocybe*. The mean Spring net NH_4^+ and mean Spring + Fall net NO_3^+ flux of each genus was compared with mean sporocarp $\delta^{15}N$ values of the same genus (Figure 4.4 and 4.5). A relationship may be seen between mycorrhizal NO_3^- flux and sporocarp $\delta^{15}N$, where, as NO_3^- uptake increases, sporocarp $\delta^{15}N$ decreases (Figure 4.5). More NO_3^- flux measurements and sporocarp $\delta^{15}N$ values of other mycorrhizal genera are needed to statistically support this relationship.

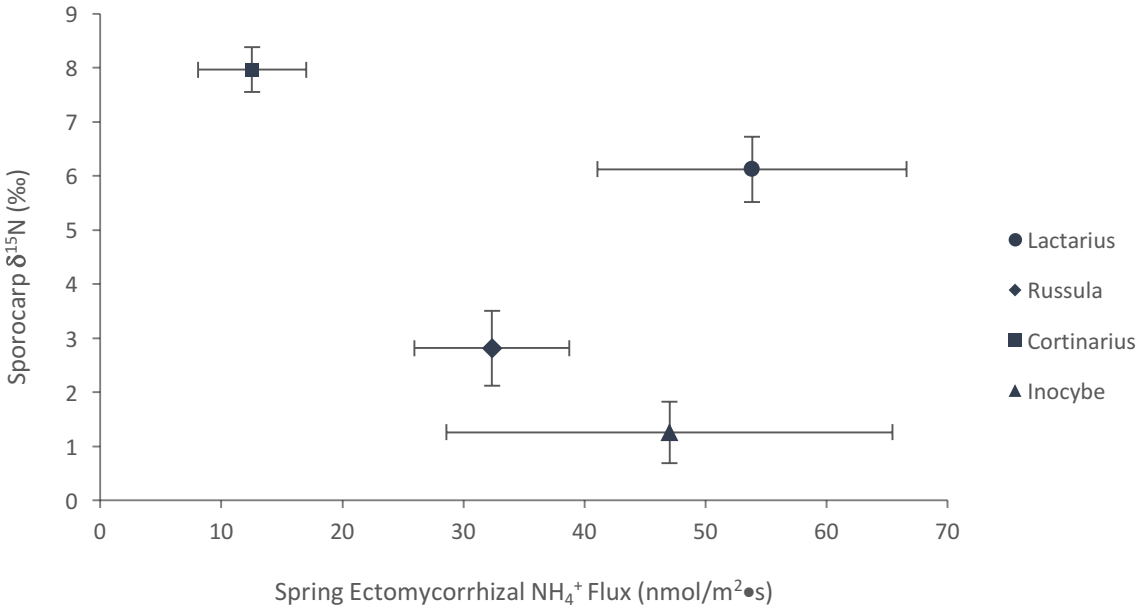


Figure 4.4 – Mean ± S.E. NH_4^+ flux (nmol/m^2•s) of ectomycorrhizal genera sampled in the Spring of 2016 with mean $\delta^{15}N$ of sporocarps of the same genera sampled in the fall of 2015. Genera include: *Cortinarius, Russula, Inocybe* and *Lactarius*.

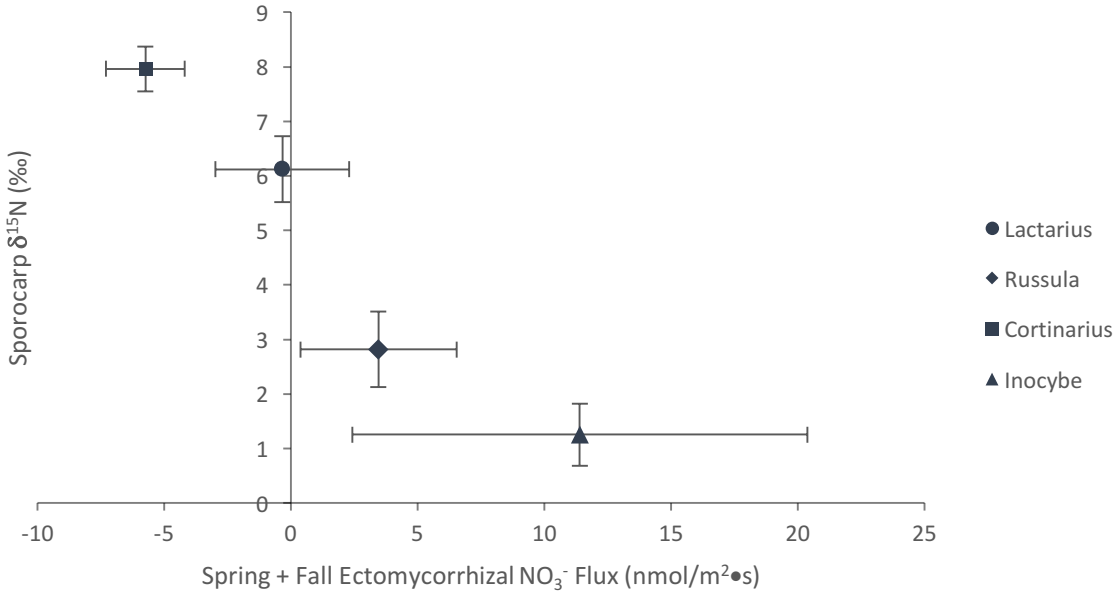


Figure 4.5 – Mean ± S.E NO_3^- flux (nmol/m^2•s) of ectomycorrhizal genera sampled in the Spring and Fall of 2016 with mean $\delta^{15}N$ of sporocarps of the same genera sampled in the fall of 2015. Genera from left to right are: *Cortinarius*, *Lactarius*, *Russula* and *Inocybe*.

4.4 Discussion

Higher NH_4^+ uptake than NO_3^- uptake was observed in the ECM roots sampled from Douglas-fir in both the lower N Fairy Lake and higher N San Juan plots. Kranabetter *et al.* (2015) conducted a similar microelectrode ion flux experiment, comparing NH_4^+ and NO_3^- uptake by non-mycorrhizal Douglas-fir seedlings and excised mycorrhizal Douglas-fir roots from sites with a gradient of N availability, and found comparable results. Kranabetter *et al.* (2015) incubated ECM roots and measured H^+, $NH_4^+$and NO_3^- fluxes in a solution with equal parts NH_4^+ and NO_3^-, while my experiment used measuring solutions of 1:10 NH_4^+ to NO_3^- in the spring and 100% NO_3^- in the fall. On average, Kranabetter *et al.* (2015) found NO_3^- uptake was only 3% of NH_4^+ uptake and I found that NH_4^+ uptake was ~10 times higher than NO_3^- uptake in the spring. Overall, there was no substantial uptake of NO_3^- by any of the mycorrhizal roots sampled in the Spring and Fall in my sites compared to the high NH_4^+ uptake rates (ranging from 52.0 - 361.5 nmol/m^2•s) seen by Kranabetter *et al.* (2015) using a higher NH_4^+ measuring solution, suggesting a preference for NH_4^+.

Many studies have found negative effects of NH_4^+ on net NO_3^- uptake in non-mycorrhizal woody plants. When NH_4^+ and NO_3^- were supplied at a 1:1 ratio, NO_3^- uptake was not affected in Scots pine (*Pinus sylvestris*) (Boxman & Roelofs 1988), but was significantly reduced in Douglas-fir (Kamminga-Van Wijk and Prins, 1993) and Norway spruce (*Picea abies*) (Marschner *et al*., 1991). However, when NH_4^+ concentrations exceeded NO_3^- concentrations in all three studies, NO_3^- uptake was significantly reduced. This suggests that the negative effects of NH_4^+ are dependent on the NH_4^+/ NO_3^- ratio (Kreuzwieser *et al*. 2000), as well as tree species. Species such as Douglas-fir and Norway spruce may be more sensitive to NH_4^+, thus low NH_4^+ concentrations will reduce net NO_3^- uptake in these species (Gobert and Plassard, 2007).

Fewer studies have examined the effects of NH_4^+ on NO_3^- uptake in ECM fungi. Prima Putra *et al.* (1999) grew the ECM fungus *Scleroderma verrucosum* in pure culture and found that in a 1:1 NH_4^+/NO_3^- solution, NH_4^+ uptake was significantly higher than NO_3^- uptake, and NO_3^- uptake only occurred when NH_4^+ concentrations were considerably lower. They found that the fungal nitrate reductase (NR) enzyme was induced by NO_3^- and repressed by NH_4^+ (Prima Putra *et al.,* 1999). Gobert and Plassard (2007) also used ion-selective microelectrodes to

analyze NO_3^- uptake in pine seedlings associated with the ECM fungus *Rhizopogon roseolus* and found that an NH_4^+ pretreatment led to a threefold reduction in NO_3^- uptake, compared to a pretreatment of N-free or NO_3^- only solution. On the other hand, Kreuzwieser *et al*. (2000) found no difference in NO_3^- uptake of excised beech (*Fagus sylvatica* L.) roots inoculated with the ECM fungus *Laccaria laccata* in the presence of NH_4^+ before and during ^{15}N measurements. Net uptake of NO_3^- reflects a balance between NO_3^- influx into cells and NO_3^- efflux out of cells, therefore, compounds that reduce net NO_3^- uptake may act as inhibitors of NO_3^- influx or stimulators of NO_3^- efflux (Kreuzwieser *et al*., 2000). Before ECM fungi can assimilate NO_3^-, via NR and nitrite reductase (NIR), NO_3^- is taken up through NO_3^- transporters (NTs) in the plasma membrane (Chalot and Plassard, 2011; Montanini *et al*., 2006). Fungal NT genes are usually clustered with NR and NIR genes and are generally induced by NO_3^- and repressed by reduced N forms like NH_4^+ and glutamine (Montanini *et al*., 2006). Only one NT in ECM fungi has so far been identified, NRT2 in *H. cylindrosporum* (*HcNRT2*, Jargeat *et al*. 2003) and in *Tuber borchii* (*TbNRT2*, Montanini *et al*. 2006). Montanini *et al*. (2006) found that NRT2 in *T. borchii* was upregulated by N-starvation in NO_3^--grown mycelia and by NO_3^- in NH_4^+-grown mycelia, but, downregulated by NH_4^+ and glutamine in NO_3^--grown and N-starved mycelia. These findings support the general trends found in my research, where ECM communities on the Douglas-fir plots had significantly higher NH_4^+ uptake compared to NO_3^- uptake when incubated in solutions containing both NH_4^+ and NO_3^-. ECM fungal species with similar NT genes may have had those genes downregulated by the presence of NH_4^+, even when NH_4^+ concentration was a fraction of NO_3^- concentration. NH_4^+ is also the more energetically favourable N form for uptake, as it does not require the further energy-consuming assimilation steps mediated by NR and NIR. A combination of NH_4^+ and NO_3^- uptake by the ECM communities was seen, but NH_4^+ inhibition of NT and easier assimilation of NH_4^+ may have contributed to lower net NO_3^- uptake.

The AM roots sampled from the western redcedar plot in San Juan also had higher net NH_4^+ uptake compared to NO_3^-, which showed net efflux in the Spring and Fall. While studies have found NTs and NO_3^- uptake capabilities in AM fungi (Tobar *et al*., 1994; Hawkins *et al*., 2000), more studies still point to NH_4^+ being the preferred N source, as it is energetically easier

to assimilate (Hawkins *et al*., 2000; Toussaint *et al*., 2004; Jin *et al*., 2005). A number of studies have documented the preferential utilization of NH_4^+ over NO_3^- by the AM fungus *Glomus intraradices (*Johansen *et al.* 1996, Toussaint *et al*. 2004, Gachomo *et al.* 2009), and that NH_4^+ present in the medium repressed the incorporation of $^{15}NO_3^-$ into free amino acids (Gachomo *et al*., 2009). NH_4^+ is taken up from the soil by NH_4^+ transporters (AMT) in AM fungi and only one gene, GintAMT, identified in the AM fungus *Rhizophagus irregularis*, has so far been characterized (Smith and Smith, 2011; Lanfranco *et al*., 2011; Bücking and Kalfe, 2015). High expression levels of GintAMT1 in the extraradical mycelium (ERM) indicate that this transporter is mainly responsible for NH_4^+ uptake by the fungal hyphae from the soil, while higher expression of GintAMT2 in the intraradical mycelium (IRM) indicates the transporter helps with re-uptake of NH_4^+ by the fungus from the symbiotic interface (Lanfranco *et al*., 2011; Bücking and Kalfe, 2015). The expression of GintAMT1 was found to be induced by low additions of NH_4^+ in the medium, in the presence of relatively high concentrations of NO_3^- (Bücking and Kalfe, 2015). GintAMT2 expression was found to be unaffected by N concentrations in culture media but was found to be upregulated in the presence of NO_3^- (Perez-Tienda *et al*., 2011). The higher expression of GintAMT2 in the IRM may lead to the role of GintAMT2 in retrieving NH_4^+ leaked during fungal metabolism (Perez-Tienda *et al*., 2011), making AM fungi more efficient in assimilating NH_4^+.

Similar to ECM fungi, in AM roots, an external supply of NO_3^- was found to increase NT expression in the ERM of *Rhizophagus irregularis* (Tian *et al*., 2010), but this gene was repressed by an increase in internal NH_4^+ or downstream metabolites such as glutamine (Fellbaum *et al*., 2012). The suppression of NTs by the availability of a more preferred N source, like NH_4^+, occurs in many organisms and is known as N catabolite repression (Cooper and Sumrada, 1983; Bücking and Kalfe, 2015). This regulatory process also controls the expression of AMTs in AM, ECM and other fungi (Cooper and Sumrada, 1983; Bücking and Kalfe, 2015). High affinity AMTs in the ECM fungus *Hebeloma cylindrosporum* were supressed by high concentrations of intracellular glutamine (Javelle *et al*., 2003).

My study found almost three times lower net NH_4^+ uptake by the AM roots compared to the ECM roots in the spring, even compared to the ECM roots sampled on the lower N site. Both phosphorus (P) and N are needed for AM symbiosis, where AM fungal colonization of plants is controlled by feedback mechanisms between the two nutrients (Fellbaum *et al*, 2014). Under P and N stress, plant defence genes are down-regulated and genes involved in strigolactone biosynthesis, signals for AM fungal colonization that stimulate hyphal branching, are up-regulated (Bonneau *et al*., 2013). Low P concentrations in my sites (4.81 ± 1.23 μg/10cm^2/6 months) in San Juan (from PRS probe data in Appendix), may have contributed to lower AM colonization, and therefore, lower NH_4^+ uptake. My soils were also found to be more acidic (mineral soil pH of ~4.4 at San Juan) which may have reduced the availability of P, since maximum availability occurs at ~pH 6.5 (Kimmins, 2004). Lastly, ECM fungal associations form distinct hyphal sheaths/mantles around host plant roots, which do not occur in AM fungal associations. ECM fungal mantles are structurally very diverse and involved in nutrient storage and transfer (Bücking *et al*., 2012), which may contribute to greater N uptake because they are able to store N in these structures and assimilate when conditions are optimal.

Similar to Kranabetter *et al.* (2015), H^+ efflux occurred from mycorrhizal roots from all three plots in the spring and fall. H^+ movement is an indicator of plasma membrane H^+-ATPase activity and ion transport. The plasma membrane H^+-ATPase in plants and fungi are proteins that couple ATP hydrolysis to H^+ transport, creating pH and electrochemical gradient across the plasma membrane (Morsomme and Boutry, 2000). The energy created by the electrochemical gradient allows for active secondary transport of ions and minerals into the cell against concentration gradients (Morsomme and Boutry, 2000). Many transporters work in symport or antiport with protons, depending on the charge of the ion (Morsomme and Boutry, 2000). This process causes root-induced pH changes in the rhizosphere by releasing H^+ to compensate for unbalanced uptake of cations and anions (Hinsinger *et al*., 2003). N has a major role in the cation-anion balance of the rhizosphere because it is the nutrient that is taken up at the highest rate by most plant species (Hinsinger *et al*., 2003). NO_3^- uptake in roots and hyphae occurs through a H^+-symport system that produces a net H^+ influx and/or corresponding OH^- efflux

along with NO_3^-, which causes alkalinization through higher pH of the rhizosphere (Hinsinger *et al*., 2003; Smith and Smith, 2011; Bücking and Kalfe, 2015). On the other hand, NH_4^+ is taken up with net H^+ efflux by an antiport mechanisms, causing acidification and a decrease in rhizosphere pH (Hinsinger *et al*., 2003; Smith and Smith, 2011; Bücking and Kalfe, 2015). This process supports the H^+ efflux seen in my experiment, as higher net NH_4^+ uptake compared to net NO_3^- uptake, and even net NO_3^- release in some cases, was found. The Douglas-fir plot in Fairy Lake had significantly higher H^+ efflux compared to the other two plots in San Juan. Since NH_4^+ can be chemically held to the cation exchange sites of soil particles and negative charges of clay particles (Sylvia *et al*., 1999; Morot-Gaudry and Touraine, 2001), more H^+ may be released by roots in N-poor sites to mobilize NH_4^+ and other cations.

Kranabetter *et al.* (2015) found greater NH_4^+ uptake from ECM roots sampled from medium and N-rich sites compared to N-poor sites, possibly indicating ECM fungal species specializing in NH_4^+ uptake on those sites. In contrast, I found no difference in NH_4^+ and NO_3^- uptake between the ECM roots sampled in the lower versus higher N sites in the spring and no differences in NO_3^- uptake in the fall. This is most likely due to low NH_4^+ concentrations in the measuring solution in my study which may have diminished any potential site effects. Also, the sites used by Kranabetter *et al.* (2015) may have had a greater range of N concentrations compared to my sites. Kranabetter *et al.* (2015) had almost a 3.5-fold increase in total N from the low to high N sites, and while my sites contrasted in total N, the contrast was not as large (Chapter 2 Figure 2.7).

While I was unable to statistically compare ECM fungal species on contrasting sites due to the low number of replicates, I found trends in NO_3^- and NH_4^+ uptake among ECM genera, overall. Coastal Douglas-fir hosts a diverse range of ECM fungal species, such as those from the genera *Amphinema*, *Clavulina*, *Inocybe*, *Lactarius*, *Russula*, *Sebacina*, *Thelephora*, *Tomentella* and *Tylospora*, which were represented on my sites and those of Kranabetter *et al.* (2015). Kranabetter *et al.* (2015) found the genus *Cortinarius*, which is associated with low-fertility soils (Lilleskov *et al.* 2011), had very low NH_4^+ uptake compared to other genera, such as *Tomentella*,

which had 3 times greater NH_4^+ uptake. I also found *Cortinarius* to have low NH_4^+ uptake and all *Cortinarius* samples were found on the low N site. I found the genus *Lactarius* and species *L. hepaticus*, which is normally associated with high N available sites (Lilleskov *et al.* 2011) but was found on both of my sites, had high NH_4^+ uptake. Kranabetter *et al.* (2015) also found *Lactarius hepaticus* to have high NH_4^+ uptake, along with *Tomentella sublilacina* and the genus *Tomentella* overall. I also found *Tomentella* to have high NH_4^+ uptake compared to many other genera.

Of the 68 ECM fungal species examined by Nygren *et al.* (2008), all were able to grow on NO_3^-, indicating that majority of ECM fungal species likely contain the gene necessary for the initial stages of NO_3^- metabolism. However, there was a large gradient in the growth of those 68 species on NO_3^-, with species of *Amanita*, *Russula* and *Lactarius* having the lowest rates of mycelial growth, and even growing better on NH_4^+, compared to other species (Nygren *et al.*, 2008). This supports my findings, where *Lactarius* had high NH_4^+ uptake rates compared to other genera but low NO_3^- uptake rates in both the spring and fall, even showing a net efflux of NO_3^- in the spring. *Russula*, by contrast, was found to have more moderate NH_4^+ and NO_3^- uptake in my study, which is not surprising, as this genus is found to have a mixed response to N addition (Lilleskov *et al.* 2011).

Niche differentiation in ECM fungal species and N forms may be due to mantle morphologies. Most *Lactarius* and *Russula* species form hydrophilic, smooth mantles with few emanating hyphae, and the ECM root tips are generally in close contact with the surrounding substrate (Agerer, 2001). Due to the hydrophilic nature of these mantles, NO_3^- may be able diffuse straight through the mantle, into the Hartig net, and pass directly into the host plant without the fungus actually taking up and metabolizing the NO_3^- (Nygren *et al.*, 2008), supporting the lower NO_3^- uptake seen in my study. Also, due to the close proximity of these ECM root tips to substrates, NH_4^+ may be more quickly taken up, by a higher production of AMTs, before it gets attracted and locked onto soil particles. On the other hand, *Cortinarius*, and other ECM, like *Piloderma*, *Suillus* and *Tricholoma,* have hydrophobic mantles and extensive mycelium, with nutrients being taken up further away from the mycorrhizal root tips

(Agerer, 2001). These genera would need to metabolize NO_3^-, NO_2^- and NH_4^+, in order to avoid toxicity effects, before translocating the N to the plant (Nygren *et al.*, 2008). This would create a large carbon drain for the ECM fungus (Nygren *et al.*, 2008), which would make it in the fungus' interest to take up less NO_3^- and NH_4^+. This was seen in the *Cortinarius* species in my study, as not only was NH_4^+ taken up at lower rates, but there was an efflux of NO_3^- in both seasons. In my study, *Inocybe* was found to have high NO_3^+ uptake in the spring compared to many other genera. *Inocybe* has a similar mantle morphology to *Lactarius*, however, its hyphae grow further from the ECM tip (characterized as short-distance mantle type), but not as far as *Cortinarius* (Agerer, 2001). Lower carbon use for less extensive, shorter-distance mycelium with no rhizomorphs may contribute to higher NO_3^- uptake by *Inocybe* species compared to *Cortinarius*. Also, having hyphae that extend further away from the ECM root tip may allow for greater soil exploration and greater access to mobile NO_3^-, compared to *Lactarius* species. *Inocybe*, having a medium hyphae length compared to *Lactarius* and *Cortinarius,* may create a 'Goldilocks effect', where just enough carbon is expended to create these structures, but not enough to hinder N uptake. However, similar to *Russula*, *Inocybe* has also been found to have a mixed response to N addition (Lilleskov *et al.* 2011). This was seen in my study, as *Inocybe* had net NO_3^- efflux in the fall.

When mean ECM sporocarp $\delta^{15}N$ values and NO_3^- flux of similar genera were compared in my study, a potential linear relationship was seen. I found sporocarp $\delta^{15}N$ was lower in ECM species with higher NO_3^- uptake. Since NO_3^- is more depleted in ^{15}N compared to NH_4^+, greater uptake of NO_3^- and translocation to sporocarps would cause the sporocarps to become progressively more depleted in ^{15}N. Also, on higher NO_3^- sites with greater NO_3^-- uptake, hosts plants may require less N from mycorrhizal symbionts, decreasing the ^{15}N-depleted N being transferred to the plant and increasing retention by the ECM fungi (Chapter 3). The reverse relationship would be expected for sporocarp $\delta^{15}N$ and NH_4^+ uptake, since NH_4^+ is more ^{15}N-enriched. This trend was not clearly shown in my study, potentially due to a small sample size. More sporocarp and N flux sampling of the same ECM genera will need to occur to further examine this relationship.

4.5 Conclusion

Mycorrhizal symbiosis is one of the most important relationships on Earth, with the majority of land plants forming these associations with fungi. While research on the importance of mycorrhizal symbioses for N uptake by plants, especially in N limited systems, continues to grow, understanding of the mechanisms of N uptake and transfer by mycorrhizae is lacking. I set out to investigate the N form preferences of ECM roots from Douglas-fir trees and AM roots from western redcedar trees, from sites of contrasting N availability, when supplied with NH_4^+ and high NO_3^- solution. I also aimed to determine potential niche formation by ECM fungal species based on N form and supply as a functional trait.

Overall, there was no substantial uptake of NO_3^- by the ECM roots and AM roots, despite a greater supply of NO_3^-. The greater uptake of NH_4^+ compared to NO_3^- seen in both the ECM and AM roots suggests a preference for NH_4^+. The presence of NH_4^+ may have hindered NO_3^- uptake, since NO_3^- transporters in ECM and AM fungi are found to be suppressed by NH_4^+ and NH_4^+ is the energetically easier N form to metabolize. In the spring, there was no difference in NH_4^+ and NO_3^- uptake between the ECM roots from the Douglas-fir plots in the lower N site versus the N-richer site, and no difference in NO_3^-uptake in the fall. However, differences in NH_4^+ and NO_3^- uptake were seen among the different ECM genera, potentially due to differences in mantle morphologies. This provides evidence for niche formation by ECM species and for adaptation of ECM species to the dominant N forms in their native habitat.

Chapter 5 – Future Research and Conclusion

Mycorrhizal symbiosis plays a major role in nutrient acquisition by most plants in terrestrial ecosystems, especially in ecosystems with low nitrogen (N) availability, such as temperate and boreal forests. Since N is often the limiting nutrient in forests, understanding N uptake patterns by mycorrhizae is critical to fully understanding N nutrition of trees. Not only is N usually limiting for plant growth, the forms it takes and its availability can vary considerably across and within different ecosystems. Organisms may respond accordingly to the N profile in their habitat, and all aspects of growth and development may be affected. This thesis sought to determine the effects of differences in soil N availability on conifer growth and association with mycorrhizal fungi. The response of conifers to N availability was assessed through growth and foliar N concentrations. The response of mycorrhizae was assessed through N form uptake capacities using microelectrode ion flux measurements and sporocarp N and ^{15}N concentrations.

5.1 Summary of Results

Soil N availability differed between the two sites in this study, with the forest floor and mineral soil in San Juan having a higher production of inorganic and organic N compared to Fairy Lake. On the other hand, N production in the soil under the four conifer species did not differ, in general. There was no significant difference in mineral soil NH_4^+-N production and forest floor and mineral soil NO_3^--N and amino acid-N production among the species. Different mycorrhizal types were represented in our conifer species, with western redcedar forming AM associations and Douglas-fir, Sitka spruce and western hemlock forming ECM associations. However, differences in mycorrhizal type were not reflected in soil N production.

The contrasting N availability between the sites was reflected in tree growth, with greater growth on the higher N site. Growth also differed among the four conifer species, with Douglas-fir and Sitka spruce having the greatest growth and western redcedar having the least growth. While site quality was not reflected in foliar %N, foliar $\delta^{15}N$ was found to increase with increasing $\delta^{15}N$ of the forest floor. Foliar %N and $\delta^{15}N$ also differed among the conifer species

and western redcedar was found to have the lowest foliar N concentrations. Similarly, ECM sporocarps reflected site quality, with greater %N concentrations in sporocarps from San Juan but greater enrichment of ^{15}N in sporocarps from Fairy Lake, indicating greater relative uptake of NH_4^+ and organic N. Differences in sporocarp N concentrations were also seen among the different ECM genera and species. Sporocarp ^{15}N concentrations were higher than foliar ^{15}N concentrations, supporting the concept of N isotope fractionation by mycorrhizae and enrichment of the heavier N isotope in fungal tissue.

Soil N form availability was not related to N form uptake by the ECM roots in the spring or the fall. Overall, there was no substantial uptake of NO_3^- by the ECM roots and AM roots, despite a greater supply of NO_3^-. However, there was greater uptake of NH_4^+ compared to NO_3^-, suggesting a preference for NH_4^+. Differences in NH_4^+ and NO_3^- uptake were observed among the different ECM genera that, in some cases, related to the N availability of soils in which the species are commonly found.

5.2 Future Research

The focus of this thesis was on N, since it is one of the key nutrients in temperate ecosystems, but forests are often limited by low supplies of more than one resource (Binkley and Fisher, 2013). Nutrient dynamics in the natural environment are often interrelated. For example, the addition of P to low-productivity soil can increase extractable inorganic N concentrations (Kranabetter *et al*., 2005). P is also a key nutrient for mycorrhizal associations, owing to a feedback mechanism between P and N (Fellbaum, 2014). Although I did use PRS probes (Table A2.24) to examine a variety of nutrients in the soil, low rainfall during the burial period resulted in low uptake of free cations and anions, leading to poor results. Future work should examine other nutrients, such as P, in combination with N, in order to give a clearer picture of N dynamics and nutrient production in forest ecosystems. Since my study analyzed mycorrhizal roots from sites contrasting in N availability, future studies should consider sites contrasting in other nutrients such as P and/or using incubation solutions with other nutrients in addition to N.

Although a thorough assessment of the N productivity of my sites was made, I did not determine the N loss from the system. Forest ecosystems are not closed systems and substantial N loss can occur through numerous paths, such as the leaching and denitrification of NO_3^-. Both sites may have had N loss, especially of NO_3^-. Fairy Lake is located on a slope and is topographically much steeper than San Juan, which could lead to greater leaching and runoff of NO_3^-. Conversely, since San Juan is a floodplain, greater denitrification may occur here, especially during periods of high rainfall. I determined the N produced in my sites, without measuring the N lost from the system, thus I do not know the actual amount of N available in the system. Future studies examining tree growth in natural forest ecosystems could consider estimating the loss of N, or other nutrients, as losses could be vastly different on sites contrasting in topography and moisture.

In my ECM sporocarp study, I collected sporocarps during one sampling period from the end of September to the beginning of October. While September has been found to be the most species-rich month for ECM sporocarps and below-ground mycorrhizae in European temperate forests (Hering 1966; Courty *et al.*, 2008), sampling throughout the fall and during the sporulation season of ECM fungi would give a clearer picture of species diversity, since there are temporal variations in ECM sporocarp fruiting (O'Hanlon, 2012). Future studies should sample ECM sporocarps throughout the fall and even sample below-ground ECM mycorrhizae throughout the year to examine species diversity and change overtime.

The microelectrode study focused only on the inorganic forms of N, but, organic N forms are also available to mycorrhizae and plants. Since some ECM fungi have been found to have strong proteolytic abilities compared to other ECM species (Lilleskov *et al*., 2002), future studies should focus on the role of organic N uptake in niche differentiation by mycorrhizal species.

5.3 Conclusion

As hypothesized, differences in N availability between the two sites were seen, and were likely due to differences in topography and moisture. Tree growth and sporocarp N values were found to be the best indicators of N availability, with differences seen in growth among the four conifer species reflecting known species characteristics.

This study expanded on past work using an uncommon approach for investigating the inorganic N uptake capacities of the mycorrhizal symbiont, via microelectrode measurements. Based on the mycorrhizal roots sampled, it can be inferred that ECM and AM prefer NH_4^+ over NO_3^-, as seen in the literature, regardless of the levels of inorganic N in the soil from which they are removed and the proportions of inorganic N forms with which they are supplied. This may be due to NH_4^+ being energetically cheaper to metabolize compared to NO_3^- and being able to suppress NO_3^- transporters in ECM and AM fungi. Nevertheless, differences in N form uptake and sporocarp N concentration were observed among ECM genera, indicating potential niche adaptation based on functional traits such as inorganic N uptake capacity and mycelial morphology.

Appendices

Table A2.1 - Mean ± S.E. pH values of the forest floor and mineral soil in all plots and averaged for Fairy Lake and San Juan.

Plot	Forest Floor	Mineral Soil
18	3.69 ± 0.03	3.99 ± 0.13
22	3.35 ± 0.02	3.71 ± 0.18
23	3.54 ± 0.12	3.71 ± 0.11
24	3.53 ±- 0.05	3.57 ± 0.03
25	3.83 ± 0.11	3.97 ± 0.15
32	4.59 ± 0.09	4.49 ± 0.15
36	3.75 ± 0.12	4.02 ± 0.33
38	4.17 ± 0.18	4.45 ± 0.17
41	3.90 ± 0.15	4.11 ± 0.22
42	3.67 ± 0.14	4.05 ± 0.24
43	3.53 ± 0.08	4.14 ± 0.16
44	4.37 ± 0.36	4.69 ± 0.29
45	4.13 ± 0.23	4.37 ± 0.24
46	4.92 ± 0.07	5.12 ± 0.07
47	4.20 ± 0.36	4.47 ± 0.38
48	4.06 ± 0.24	4.33 ± 0.24
Fairy Lake	3.81 ± 0.07	3.99 ± 0.08
San Juan	4.10 ± 0.10	4.41 ± 0.09

Table A2.2 – P-values from one-way ANOVAs for forest floor and mineral soil pH values.

Source of Variation	Forest Floor	Mineral Soil
Site	**0.0006**	**<0.0001**
Species	0.0613	**0.0142**

Table A2.3 - Mean ± S.E. pH values and significant means differences for the mineral soil by species (Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW)).

Tree Species	pH	
CW	b	4.16 ± 0.11
FD	b	4.18 ± 0.12
HW	b	4.03 ± 0.14
SS	a	4.43 ± 0.14

Table A2.4 - The mean ± S.E. bulk density (kg/m^3) of the forest floor and mineral soil in each plot and averaged for Fairy Lake and San Juan sites.

Plot	Forest Floor	Mineral Soil
18	205.16 ± 44.45	463.45 ± 74.25
22	99.56 ± 7.75	340.75 ± 101.63
23	120.41 ± 23.83	379.75 ± 46.55
24	105.89 ± 27.10	155.43 ± 28.95
25	202.33 ± 46.14	431.29 ± 90.14
32	409.50 ± 97.56	528.53 ± 117.50
36	124.76 ± 47.97	209.26 ± 53.84
38	229.29 ± 96.70	392.74 ± 53.69
41	475.61 ±174.62	541.94 ± 110.50
42	207.54 ± 28.72	511.87 ± 103.96
43	157.14 ± 17.49	574.69 ± 85.47
44	321.45 ± 91.81	614.34 ± 88.83
45	324.86 ± 59.16	708.52 ± 98.33
46	394.43 ± 61.96	553.42 ± 52.13
47	226.47 ± 52.49	582.67 ± 131.83
48	436.39 ± 144.71	626.48 ± 94.00
Fairy Lake	187.11 ± 24.09	362.65 ± 30.84
San Juan	317.99 ± 34.70	589.24 ± 32.68

Table A2.5 – P-values from one-way ANOVAs for forest floor and mineral soil bulk density.

Source of Variation	Forest Floor	Mineral Soil
Site	**0.0002**	**<0.0001**
Species	**0.0029**	0.1896

Table A2.6 – Mean ± S.E. NH_4^+-N (kg/ha) concentrations and significant means differences for T0 forest floor, mineral soil and forest floor + mineral soil by site (Fairy Lake (FL) and San Juan (SJ)).

Site	T0 Forest Floor		T0 Mineral Soil		T0 FF+MS	
FL	b	0.20 ± 0.01	b	1.84 ± 0.10	b	2.03 ± 0.11
SJ	a	0.40 ± 0.04	a	3.79 ± 0.21	a	4.19 ± 0.21

Table A2.7 – Mean ± S.E. NH_4^+-N (kg/ha) concentrations and significant means differences for T0 forest floor, mineral soil and forest floor + mineral soil by species (Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW)).

Tree Species	T0 Forest Floor		T0 Mineral Soil		T0 FF+MS	
CW	a	0.35 ± 0.09	c	2.57 ± 0.36	b	2.92 ± 0.42
FD	a	0.32 ± 0.05	ab	3.12 ± 0.61	a	3.44 ± 0.66
HW	b	0.24 ± 0.02	b	2.79 ± 0.44	b	3.03 ± 0.46
SS	a	0.28 ± 0.03	a	2.78 ± 0.23	a	3.05 ± 0.26

Table A2.8 - Mean ± S.E. T0 forest floor, mineral soil and forest floor + mineral soil NH_4^+-N (kg/ha) concentrations, p-values from nested ANOVAs and significant means differences for site*species interactions.

Fairy Lake	T0 Forest Floor (p<0.0001)		T0 Mineral Soil (p<0.0001)		T0 FF+MS (p<0.0001)	
CW	c	0.18 ± 0.01	b	1.70 ± 0.06	b	1.87 ± 0.06
FD	bc	0.18 ± 0.01	c	1.51 ± 0.10	c	1.70 ± 0.09
HW	b	0.19 ± 0.02	b	1.67 ± 0.07	b	1.86 ± 0.07
SS	a	0.23 ± 0.01	a	2.46 ± 0.07	a	2.69 ± 0.07
San Juan	T0 Forest Floor (p=0.0067)		T0 Mineral Soil (p<0.0001)		T0 FF+MS (p<0.0001)	
CW	a	0.53 ± 0.12	c	3.44 ± 0.33	b	3.97 ± 0.27
FD	a	0.45 ± 0.05	a	4.73 ± 0.14	a	5.18 ± 0.13
HW	b	0.29 ± 0.005	b	3.90 ± 0.24	b	4.20 ± 0.24
SS	b	0.33 ± 0.04	d	3.09 ± 0.42	c	3.42 ± 0.47

Table A2.9 – Mean ± S.E. NO_3^--N (kg/ha) concentrations and significant means differences for T0 forest floor, mineral soil and forest floor + mineral soil by site (Fairy Lake (FL) and San Juan (SJ)).

Site	T0 Forest Floor		T0 Mineral Soil		T0 FF+MS	
FL	b	0.06 ± 0.01	b	0.49 ± 0.02	b	0.55 ± 0.03
SJ	a	0.20 ± 0.04	a	2.43 ± 0.62	a	2.63 ± 0.64

Table A2.10 – Mean ± S.E. NO_3^--N (kg/ha) concentrations and significant means differences for T0 forest floor, mineral soil and forest floor + mineral soil by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW).

Tree Species	T0 Forest Floor		T0 Mineral Soil		T0 FF+MS	
CW	bc	0.09 ± 0.01	c	0.75 ± 0.13	c	0.83 ± 0.14
FD	c	0.07 ± 0.01	c	0.74 ± 0.17	d	0.81 ± 0.18
HW	a	0.21± 0.06	a	3.00 ± 1.21	a	3.21 ± 1.26
SS	b	0.14 ± 0.05	b	1.37 ± 0.42	b	1.51 ± 0.47

Table A2.11- Mean ± S.E. T0 forest floor, mineral soil and forest floor + mineral soil NO_3^--N (kg/ha) concentrations, p-values from nested ANOVA and significant means differences for site*species interactions.

Fairy Lake	T0 Forest Floor (p=0.0423)		T0 Mineral Soil (p=0.0004)		T0 FF+MS (p=0.0006)	
CW	a	0.08 ± 0.02	b	0.48 ± 0.02	b	0.56 ± 0.02
FD	b	0.05 ± 0.01	bc	0.46 ± 0.01	bc	0.51 ± 0.01
HW	b	0.05 ± 0.01	c	0.42 ± 0.04	c	0.47 ± 0.06
SS	ab	0.06 ± 0.01	a	0.62 ± 0.04	a	0.68 ± 0.03
San Juan	T0 Forest Floor (p=0.0001)		T0 Mineral Soil (p<0.0001)		T0 FF+MS (p<0.0001)	
CW	c	0.10 ± 0.01	c	1.02 ± 0.18	c	1.11 ± 0.19
FD	c	0.10 ± 0.01	c	1.02 ± 0.30	c	1.11 ± 0.31
HW	a	0.37 ± 0.05	a	5.58 ± 1.54	a	5.95 ± 1.54
SS	b	0.22 ± 0.08	b	2.11 ± 0.69	b	2.33 ± 0.77

Table A2.12 - Mean ± S.E. amino acid-N (kg/ha) concentrations and significant means differences for T0 forest floor, mineral soil and forest floor + mineral soil by site (Fairy Lake (FL) and San Juan (SJ)).

Site	T0 Forest Floor		T0 Mineral Soil		T0 FF+MS	
FL	b	0.24 ± 0.01	b	1.23 ± 0.08	b	1.47 ± 0.09
SJ	a	0.47 ± 0.05	a	2.18 ± 0.13	a	2.65 ± 0.17

Table A2.13 – Mean ± S.E. amino acid-N (kg/ha) concentrations and significant means differences for T0 forest floor by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW).

Tree Species	T0 Forest Floor	
CW	b	0.40 ± 0.09
FD	a	0.41 ± 0.06
HW	d	0.26 ± 0.02
SS	c	0.34 ± 0.07

Table A2.14 - Mean ± S.E. T0 forest floor, mineral soil and forest floor + mineral soil amino acid-N (kg/ha) concentrations, p-values from nested ANOVA and significant means differences for site*species interactions.

Fairy Lake	T0 Forest Floor (p=0.0012)		T0 Mineral Soil (p=0.0005)		T0 FF+MS (p=0.0003)	
CW	b	0.20 ± 0.01	bc	1.11 ± 0.05	b	1.30 ± 0.05
FD	a	0.26 ± 0.04	c	1.03 ± 0.06	b	1.30 ± 0.09
HW	b	0.21 ± 0.01	b	1.20 ± 0.06	b	1.42 ± 0.1
SS	a	0.28 ± 0.01	a	1.58 ± 0.26	a	1.86 ± 0.26
San Juan	T0 Forest Floor (p<0.0001)		T0 Mineral Soil (p=0.0019)		T0 FF+MS (p=0.0002)	
CW	a	0.61 ± 0.08	a	2.45 ± 0.16	a	3.05 ± 0.13
FD	a	0.56 ± 0.05	a	2.26 ± 0.11	a	2.82 ± 0.15
HW	b	0.30 ± 0.004	a	2.18 ± 0.16	b	2.48 ± 0.16
SS	b	0.40 ± 0.15	b	1.84 ± 0.48	c	2.24 ± 0.63

Table A2.15 – Mean ± S.E. forest floor bulk density (kg/m^3) and significant means differences by species (Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW)).

Tree Species	Bulk density (kg/m^3)	
CW	a	360.48 ± 65.41
FD	b	234.12 ± 28.93
HW	b	189.19 ± 28.83
SS	b	226.41 ± 37.44

Table A2.16 – Percent moisture content (as % of oven dried soil weight) of forest floor and mineral soil fresh samples at T0 by plot and averaged for sites (± S.E.) (dried for 2 days in oven @ 55°C). Only one sample per plot was used.

Forest Floor		Mineral soil	
Plot		Plot	
18	65.54	18	6.62
22	133.87	22	31.53
23	191.92	23	62.22
24	143.33	24	35.81
25	151.75	25	15.29
32	120.61	32	41.95
36	150.86	36	71.43
38	69.01	38	14.74
41	70.00	41	2.14
42	102.08	42	3.21
43	97.93	43	7.04
44	117.16	44	14.62
45	68.82	45	23.11
46	25.44	46	4.68
47	74.39	47	31.08
48	86.36	48	21.19
Fairy Lake	128.36 ± 15.16	Fairy Lake	34.95 ± 8.17
San Juan	80.27 ± 9.88	San Juan	13.38 ± 3.82

Table A2.17 - P-values from one-way ANOVAs for forest floor and mineral soil % moisture content.

Source of Variation	Forest Floor	Mineral Soil
Site	**0.0292**	**0.0310**
Species	0.7013	0.3182

Table A2.18 - The exchangeable cations (cmol(+)/kg) and CEC (cmol(+)/kg) in the forest floor samples from each plot and averaged (± S.E.) for each site.

Plot	Al	Ca	Fe	K	Mg	Mn	Na	Total EC	CEC
18	3.86	7.86	1.06	7.17	2.90	0.20	0.69	23.73	22.94
22	2.53	10.33	0.42	1.31	4.19	0.20	0.80	19.79	24.55
23	3.19	14.20	0.73	0.75	3.81	0.23	0.58	23.49	25.25
24	1.12	11.97	0.16	1.11	3.75	0.28	0.47	18.84	25.97
25	4.18	12.20	0.77	0.62	3.25	0.98	0.48	22.49	24.22
32	1.98	18.49	0.25	0.29	2.77	0.91	0.30	25.00	26.19
36	2.79	13.08	0.36	1.12	3.23	0.44	0.53	21.54	24.48
38	3.68	10.03	0.49	0.59	2.06	1.18	0.26	18.29	19.36
41	4.25	8.37	1.12	0.46	1.81	0.38	0.29	16.68	19.29
42	4.36	11.10	0.72	0.44	2.91	0.70	0.33	20.56	21.77
43	1.49	8.67	0.54	0.65	1.82	0.30	0.00	13.47	26.44
44	4.18	11.14	0.25	0.33	2.09	0.83	0.32	19.14	20.06
45	4.32	8.36	0.31	0.28	1.80	0.94	0.23	16.23	18.35
46	1.97	6.05	0.11	0.15	0.83	0.40	0.20	9.72	9.83
47	2.10	16.87	0.06	0.44	2.69	3.84	0.40	26.40	27.35
48	4.14	8.96	0.43	0.26	1.76	0.92	0.20	16.66	18.72
Fairy Lake	2.92 ± 0.36	12.27 ± 1.13	0.53 ± 0.11	1.62 ± 0.80	3.25 ± 0.24	0.55 ± 0.14	0.51 ± 0.06	21.65 ± 0.87	24.12 ± 0.77
San Juan	3.35 ± 0.44	9.94 ± 1.14	0.44 ± 0.12	0.37 ± 0.05	1.97 ± 0.22	1.04 ± 0.41	0.25 ± 0.04	17.36 ± 1.75	20.23 ± 1.92

Table A2.19 - The exchangeable cations (cmol(+)/kg) and CEC (cmol(+)/kg) in the mineral soil samples from each plot and averaged (± S.E.) for each site.

Plot	Al	Ca	Fe	K	Mg	Mn	Na	Total EC	CEC
18	5.12	1.04	0.53	0.08	0.47	0.02	0.05	7.29	14.93
22	6.03	0.93	0.60	0.11	0.55	0.01	0.07	8.29	43.20
23	8.46	1.27	0.63	0.10	1.14	0.02	0.17	11.80	14.69
24	6.73	4.32	0.55	0.20	2.39	0.01	0.23	14.42	15.26
25	4.98	1.39	0.47	0.07	0.42	0.02	0.04	7.38	11.71
32	4.35	2.70	0.25	0.07	0.71	0.06	0.10	8.25	10.82
36	5.11	4.02	0.21	0.18	0.99	0.05	0.15	10.71	20.61
38	4.62	2.69	0.33	0.15	0.69	0.17	0.15	8.80	10.06
41	4.38	0.93	0.39	0.08	0.30	0.05	0.06	6.18	9.22
42	4.93	1.42	0.46	0.07	0.48	0.04	0.08	7.48	11.62
43	3.30	0.70	0.33	0.06	0.20	0.04	0.02	4.66	8.62
44	1.61	1.90	0.04	0.03	0.27	0.21	0.00	4.06	8.79
45	4.36	2.37	0.20	0.11	0.61	0.18	0.15	7.98	9.89
46	1.19	4.33	0.02	0.07	0.54	0.22	0.12	6.48	7.00
47	1.79	1.34	0.02	0.03	0.29	0.44	0.00	3.92	7.44
48	3.85	1.14	0.17	0.06	0.43	0.20	0.07	5.92	9.66
Fairy Lake	5.67 ± 0.48	2.29 ± 0.48	0.45 ± 0.06	0.12 ± 0.02	0.92 ± 0.23	0.04 ± 0.02	0.12 ± 0.02	9.62 ± 0.88	17.66 ± 3.83
San Juan	3.18 ± 0.51	1.77 ± 0.41	0.20 ± 0.06	0.06 ± 0.01	0.39 ± 0.05	0.17 ± 0.05	0.06 ± 0.02	5.84 ± 0.54	9.03 ± 0.51

Table A2.20 – P-values from nested ANOVA for NH_4^+-N and NO_3^--N concentrations (µg/10cm^2/6 months) from PRS probes.

Source of Variation	NH_4^+-N	NO_3^--N
Site	**0.0002**	**<0.0001**
Species	**0.0520**	**0.0023**
Site*Species	0.1862	**0.0037**
Plot(Site)	0.7697	**0.0030**

Table A2.21 – Mean ± S.E. PRS probe NH_4^+-N and NO_3^--N concentrations (µg/10cm^2/6 months) and significant mean differences by site.

Site	NH_4^+-N (µg/10cm^2/6 months)		NO_3^--N (µg/10cm^2/6 months)	
Fairy Lake	a	2.00 ± 0.29	b	0.00 ± 0
San Juan	b	0.69 ± 0.18	a	101.19 ± 26.43
WC 1000	a	2.75 ± 0.41	a	100.06 ± 24.98

Table A2.22 - Mean ± S.E. PRS probe NH_4^+-N and NO_3^--N concentrations (µg/10cm^2/6 months) and significant mean differences by tree species (Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW)).

Tree Species	NH_4^+-N (µg/10cm^2/6 months)		NO_3^--N (µg/10cm^2/6 months)	
CW	b	1.50 ± 0.19	a	110.92 ± 38.15
FD	b	1.42 ± 0.34	ab	83.50 ± 26.74
HW	a	2.67 ± 0.51	bc	52.33 ± 24.02
SS	b	1.67 ± 0.53	c	21.58 ± 8.29

Table A2.23 - Mean ± S.E. PRS probe NO_3^--N concentrations (µg/10cm^2/6 months), p-values for the effect of species within site from ANOVAs and significant mean differences for site*species interactions (Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW)).

Tree Species	Fairy Lake		San Juan (p=0.4594)		WC 1000 (p<0.0001)	
CW	a	0 ± 0	a	114.00 ± 71.39	a	218.75 ± 54.17
FD	a	0 ± 0	a	117.50 ± 62.60	b	133.00 ± 20.45
HW	a	0 ± 0	a	133.25 ± 52.90	c	23.75 ± 13.36
SS	a	0 ± 0	a	40.00 ± 20.60	c	24.75 ± 7.69

Table A2.24 - Mean ± S.E. concentrations (µg/10cm^2/6 months) for all 15 elements analyzed by the PRS Probes, by plot and site. Only NH_4^+-N and NO_3^--N concentrations were analyzed in the plots in WC1000.

Plot	NH_4^+-N	NO_3^--N	Ca	Mg	K	P	Fe	Mn	Cu	Zn	B	S	Pb	Al	Cd
2	4.00 ± 0	1.50 ± 0.50													
3	1.50 ± 0.50	117.00 ± 34.00													
4	2.50 ± 0.50	129.50 ± 12.50													
5	2.00 ± 0	23.50 ± 18.50													
6	1.50 ± 0.50	308.00 ± 39.00													
7	2.00 ± 0	149.00 ± 29.00													
13	4.00 ± 3.00	26.00 ± 3.00													
16	4.50 ± 0.50	46.00 ± 9.00													
18	1.50 ± 0.50	0 ± 0	1239.10 ± 130.31	656.36 ± 104.39	77.12 ± 43.41	2.46 ± 0.66	6.27 ± 1.19	39.71 ± 22.71	0.15 ± 0.03	2.29 ± 1.33	0.20 ± 0.11	45.01 ± 3.59	0.22 ± 0.08	57.79 ± 33.21	0 ± 0
22	3.50 ± 1.5	0 ± 0	820.80 ± 140.10	809.73 ± 14.73	108.67 ± 52.11	1.58 ± 0.39	6.60 ± 2.50	13.22 ± 1.53	0.155 ± 0.02	4.03 ± 1.51	0.06 ± 0.06	45.43 ± 5.86	0.25 ± 0.11	30.79 ± 8.03	0 ± 0
23	1.50 ± 0.50	0 ± 0	713.03 ± 182.25	1113.72 ± 128.46	19.92 ± 7.24	0.74 ± 0.69	5.22 ± 1.54	11.39 ± 1.57	0.08 ± 0	6.115 ± 2.88	0.09 ± 0.09	43.95 ± 3.94	0.14 ± 0.12	43.335 ± 18.86	0.015 ± 0.02
24	2.5 ± 0.50	0 ± 0	1115.88 ± 112.23	580.59 ± 135.67	130.71 ± 25.42	2.245 ± 0.76	6.005 ± 2.01	15.11 ± 6.34	0.14 ± 0.02	3.405 ± 0.91	0.54 ± 0.02	92.93 ± 26.9	0.23 ± 0.08	46.655 ± 25.58	0.03 ± 0.01
25	3.00 ± 1.00	0 ± 0	1476.10 ± 169.99	575.61 ± 83.71	102.12 +/- 19.10	1.32 +/- 0.10	7.975 +/- 1.90	12.685 +/- 6.45	0.17 +/- 0.02	1.34 +/- 0.16	0.49 +/- 0.21	95.95 +/- 7.03	0.07 +/- 0.07	71.545 +/- 25.14	0.015 +/- 0.015
32	1.00 ± 0	0 ± 0	1595.90 ± 117.03	458.78 ± 55.33	12.87 ± 1.67	0.835 ± 0.125	9.57 ± 0.07	14.925 ± 1.92	0.32 ± 0	2.64 ± 0.86	0.21 ± 0.04	37.815 ± 5.28	0.13 ± 0	31.42 ± 0.17	0 ± 0
36	1.50 ± 0.50	0 ± 0	1546.70 ± 242.20	450.16 ± 2.73	85.86 ± 65.63	2.11 ± 1.36	8.64 ± 0.31	12.63 ± 6.07	0.135 ± 0.01	0.805 ± 0.235	0.475 ± 0.07	78.34 ± 11.67	0.11 ± 0.09	60.96 ± 6.88	0.015 ± 0.005

Table A2.24 Continued - Mean ± S.E. concentrations (µg/10cm^2/6 months) for all 15 elements analyzed by the PRS Probes, by plot and site. Only NH_4^+-N and NO_3^--N concentrations were analyzed in the plots in WC1000.

Plot	NH_4^+-N	NO_3^--N	Ca	Mg	K	P	Fe	Mn	Cu	Zn	B	S	Pb	Al	Cd
38	1.50 ± 0.50	0 ± 0	996.105 ± 401.17	314.915 ± 83.32	208.56 ± 88.74	0.755 ± 0.30	11.86 ± 1.31	24.60 ± 12.26	0.17 ± 0.01	1.50 ± 0.45	0.205 ± 0.05	36.91 ± 13.54	0.11 ± 0.11	52.32 ± 8.18	0 ± 0
41	1.50 ± 0.50	3.00 ± 2.00	785.81 ± 293.37	259.01 ± 84.93	67.94 ± 25.84	2.42 ± 0.84	16.73 ± 2.82	31.13 ± 8.41	0.18 ± 0.03	2.43 ± 1.03	0.29 ± 0.02	32.13 ± 7.90	0.12 ± 0.06	122.21 ± 6.68	0.025 ± 0.03
42	0.50 ± 0.50	67.00 ± 67.00	1720.86 ± 142.61	485.25 ± 34.86	65.04 ± 48.52	9.84 ± 4.19	6.60 ± 0.93	34.75 ± 4.54	0.12 ± 0.03	3.72 ± 0.75	0.25 ± 0	33.00 ± 1.29	0.11 ± 0.06	32.46 ± 9.11	0 ± 0
43	1.00 ± 0	4.50 ± 4.50	1329.07 ± 89.71	528.63 ± 76.00	95.80 ± 43.21	14.62 ± 1.41	9.65 ± 1.93	25.81 ± 8.54	0.14 ± 0.02	1.62 ± 0.43	0.24 ± 0.10	27.38 ± 7.71	0.18 ± 0.01	45.72 ± 11.76	0.01 ± 0.01
44	0 ± 0	168.00 ± 118.00	1773.26 ± 443.72	382.12 ± 136.37	24.73 ± 12.95	1.78 ± 0.64	40.85 ± 30.40	8.60 ± 4.00	1.24 ± 1.11	2.49 ± 0.14	0.38 ± 0.23	67.89 ± 26.58	0.62 ± 0.38	82.69 ± 14.96	0 ± 0
45	1.00 ± 1.00	55.50 ± 43.50	1371.31 ± 170.66	344.51 ± 37.57	142.57 ± 2.74	2.82 ± 0.75	11.59 ± 0.94	4.92 ± 2.50	0.26 ± 2.50	18.57 ± 0.09	0.295 ± 17.05	73.26 ± 0.04	0.125 ± 0.07	91.30 ± 15.53	0.01 ± 0.01
46	0 ± 0	75.50 ± 2.50	2299.86 ± 197.32	254.03 ± 30.11	11.49 ± 3.93	2.23 ± 0.88	23.00 ± 6.27	8.29 ± 3.81	4.29 ± 1.38	4.97 ± 1.81	0.29 ± 0	206.86 ± 40.47	0.53 ± 0.22	58.69 ± 0.57	0 ± 0
47	0.50 ± 0.50	211.00 ± 53.00	1707.25 ± 75.92	391.53 ± 78.85	234.76 ± 219.52	2.88 ± 0.70	23.69 ± 7.79	39.94 ± 24.17	1.64 ± 0.64	1.84 ± 0.14	0.45 ± 0.09	170.55 ± 9.62	0.23 ± 0.07	125.30 ± 62.81	0 ± 0
48	1.00 ± 0	225.00 ± 77.00	1516.86 ± 225.25	289.285 ± 0.435	60.165 ± 19.58	1.87 ± 0.06	18.86 ± 3.86	24.68 ± 2.90	0.55 ± 0.03	2.52 ± 0.32	0.20 ± 0.04	103.22 ± 33.86	0.03 ± 0.03	162.56 ± 57.85	0.01 ± 0.01
Fairy Lake	2.00 ± 0.29	0 ± 0	1187.95 ± 96.94	619.98 ± 64.26	93.23 ± 19.37	1.51 ± 0.24	7.77 ± 0.67	18.03 ± 3.48	0.16 ± 0.02	2.77 ± 0.54	0.28 ± 0.05	59.54 ± 6.84	0.16 ± 0.03	49.35 ± 6.01	0.01 ± 0
San Juan	0.69 ± 0.18	101.19 ± 26.43	1563.03 ± 121.33	366.79 ± 30.72	87.81 ± 27.35	4.81 ± 1.23	18.87 ± 3.95	22.26 ± 4.12	1.05 ± 0.38	4.77 ± 2.08	0.30 ± 0.03	89.28 ± 17.16	0.24 ± 0.07	90.11 ± 13.50	0.01 ± 0
WC 1000	2.75 ± 0.41	100.06 ± 24.98													

Table A2.25 - P-values from nested ANOVAs for forest floor, mineral soil and forest floor + mineral soil NH_4^+-N concentrations (kg/ha) from mineralizable N incubations.

Source of Variation	Forest Floor	Mineral Soil	Forest Floor + Mineral Soil
Site	**<0.0001**	**<0.0001**	**<0.0001**
Species	0.9934	**0.0116**	**0.0124**
Site*Species	**0.0091**	**0.0229**	**0.0158**
Plot(Site)	**0.0004**	0.0900	0.0905

Table A2.26 - Mean ± S.E. forest floor, mineral soil and forest floor + mineral soil NH_4^+-N concentrations (kg/ha) from mineralizable N incubations and significant mean differences by site.

Site	Forest Floor		Mineral Soil		FF+MS	
Fairy Lake	b	2.30 ± 0.14	b	29.86 ± 3.42	b	32.16 ± 3.49
San Juan	a	4.17 ± 0.31	a	52.60 ± 3.64	a	56.77 ± 3.72

Table A2.27 - Mean ± S.E. mineral soil and forest floor + mineral soil NH_4^+-N concentrations (kg/ha) from mineralizable N incubations and significant mean differences by species (Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW)).

Tree Species	Mineral Soil		Forest Floor + Mineral Soil	
CW	ab	41.96 ± 6.03	ab	45.10 ± 6.23
FD	ab	38.30 ± 4.60	ab	41.60 ± 4.89
HW	b	33.99 ± 5.46	b	37.24 ± 5.72
SS	a	50.67 ± 5.86	a	53.92 ± 5.95

Table A2.28 - Mean ± S.E. forest floor, mineral soil and forest floor + mineral soil NH_4^+-N concentrations (kg/ha) from mineralizable N incubations, p-values from nested ANOVAs and significant means differences for site*species interactions (Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW)).

Fairy Lake	T0 Forest Floor (p=0.0752)		T0 Mineral Soil (p=0.0006)		T0 FF+MS (p=0.0004)	
CW	ab	2.58 ± 0.37	b	29.71 ± 6.16	b	32.28 ± 6.47
FD	b	2.00 ± 0.17	bc	23.80 ± 2.51	bc	25.80 ± 2.36
HW	b	1.92 ± 0.14	c	16.64 ± 1.04	c	18.56 ± 1.07
SS	a	2.71 ± 0.28	a	49.30 ± 9.61	a	52.02 ± 9.67
San Juan	T0 Forest Floor (p=0.1602)		T0 Mineral Soil (p=0.9732)		T0 FF+MS (p=0.9752)	
CW	a	3.70 ± 0.37	a	54.21 ± 9.06	a	57.91 ± 9.25
FD	a	4.60 ± 0.52	a	52.80 ± 6.04	a	57.41± 6.30
HW	a	4.58 ± 0.62	a	51.35 ± 7.61	a	55.93 ± 7.71
SS	a	3.79 ± 0.88	a	52.04 ± 7.22	a	55.82 ± 7.42

Table A3.1 - Spearman Correlation Coefficient (rho), p-value and Bonferroni corrected p-value of T6 mean NH_4^+-N, NO_3^--N, amino acid-N, inorganic N (NH_4^+-N + NO_3^--N) and available N (NH_4^+-N + NO_3^--N + amino acid-N) in the soil (forest floor + mineral soil), total N of the forest floor + mineral soil, and $\delta^{15}N$ of the forest floor and the mineral soil with height, DBH, bole volume, Top 15 height, Top 15 bole volume and foliar %N and $\delta^{15}N$. Log values were used for all soil characteristics except for $\delta^{15}N$.

	NH_4^+-N	NO_3^--N	amino acid-N	Inorganic N (NH_4^+-N + NO_3^--N)	Available N (NH_4^+-N + NO_3^--N + amino acid-N)	Total N	$\delta^{15}N$ forest floor	$\delta^{15}N$ mineral soil
Ht	0.42647 0.0995 1.0000	0.33235 0.2085 1.0000	0.42647 0.0995 1.0000	**0.50294** **0.0471** 1.0000	**0.60882** **0.0123** 0.6888	0.45000 0.0803 1.0000	-0.13235 0.6251 1.0000	-0.03824 0.8882 1.0000
DBH	0.24706 0.3563 1.0000	0.28529 0.2841 1.0000	0.15294 0.5717 1.0000	0.41176 0.1130 1.0000	0.37941 0.1472 1.0000	0.10000 0.7125 1.0000	-0.06176 0.8202 1.0000	-0.19706 0.4645 1.0000
BV	0.31765 0.2306 1.0000	0.35000 0.1839 1.0000	0.22941 0.3927 1.0000	**0.49412** **0.0517** 1.0000	0.48235 0.0585 1.0000	0.21471 0.4246 1.0000	-0.08529 0.7535 1.0000	-0.17353 0.5204 1.0000
Top15 Ht	0.36765 0.1612 1.0000	0.30000 0.2589 1.0000	0.40882 0.1159 1.0000	0.42353 0.1021 1.0000	**0.56765** **0.0218** 1.0000	0.40882 0.1159 1.0000	-0.11471 0.6723 1.0000	0.04706 0.8626 1.0000
Top15 BV	0.40294 0.1217 1.0000	0.21176 0.4311 1.0000	0.38824 0.1373 1.0000	0.44118 0.0872 1.0000	**0.49706** **0.0501** 1.0000	0.26471 0.3218 1.0000	-0.18529 0.4921 1.0000	-0.17059 0.5276 1.0000
Foliar %N	-0.19118 0.4782 1.0000	0.02647 0.9225 1.0000	-0.22059 0.4117 1.0000	0.00882 0.9741 1.0000	0.04118 0.8797 1.0000	-0.16765 0.5349 1.0000	0.34706 0.1878 1.0000	0.20000 0.4577 1.0000
Foliar $\delta^{15}N$	-0.21176 0.4311 1.0000	0.15588 0.5643 1.0000	-0.05000 0.8541 1.0000	0.20588 0.4443 1.0000	0.23529 0.3804 1.0000	-0.36471 0.1649 1.0000	**0.58529** **0.0172** 0.9632	0.27059 0.3108 1.0000

Table A3.2 - Spearman Correlation Coefficient (rho), p-value and Bonferroni Correction p-value of net mean NH_4^+-N, NO_3^--N, amino acid-N, inorganic N (NH_4^+-N + NO_3^--N) and available N (NH_4^+-N + NO_3^--N+ amino Acid-N) in the soil (forest floor + mineral soil) with height, DBH, bole volume, Top 15 height, Top 15 bole volume and foliar %N and δ^{15}N. Log values were used for all soil characteristics.

	NH_4^+-N	$NO3^-$-N	Amino Acid-N	Inorganic N (NH_4^+-N + NO_3^--N)	Available N (NH_4^+-N + NO_3^--N + Amino Acid-N)
Ht	0.28235 0.2893 1.0000	0.37458 0.1529 1.0000	0.44118 0.0872 1.0000	0.47647 0.0621 1.0000	**0.59412** **0.0152** 0.5320
DBH	0.13529 0.6174 1.0000	0.31010 0.2424 1.0000	0.19706 0.4645 1.0000	0.35000 0.1839 1.0000	0.41176 0.1130 1.0000
BV	0.17647 0.5133 1.0000	0.37765 0.1493 1.0000	0.26471 0.3218 1.0000	0.43235 0.0944 1.0000	**0.50000** **0.0486** 1.0000
Top15 Ht	0.23529 0.3804 1.0000	0.31624 0.2328 1.0000	0.40882 0.1159 1.0000	0.39118 0.1341 1.0000	**0.55882** **0.0244** 0.8540
Top15 BV	0.28529 0.2841 1.0000	0.22413 0.4040 1.0000	0.42059 0.1048 1.0000	0.37059 0.1577 1.0000	**0.53529** **0.0326** 1.0000
Foliar %N	-0.21176 0.4311 1.0000	0.02149 0.9370 1.0000	-0.21471 0.4246 1.0000	0.00294 0.9914 1.0000	0.05294 0.8456 1.0000
Foliar δ^{15}N	-0.22647 0.3990 1.0000	0.13816 0.6099 1.0000	-0.00882 0.9741 1.0000	0.19118 0.4782 1.0000	0.27059 0.3108 1.0000

Table A3.3 – Simple linear regression R^2 value for T6 soil (forest floor + mineral soil) inorganic N (NH_4^+-N + NO_3^--N), available N (NH_4^+-N + NO_3^--N+ amino acid-N), Total N and δ^{15}N of the forest floor with height, bole volume, Top 15 tree heights, Top 15 bole volumes and foliar δ^{15}N, where correlations were significant ($p<0.05$). Log values were used for all soil characteristics.

	Inorganic N	Available N	δ^{15}N Forest Floor
Height	0.2717	**0.3030**	----------
Bole volume	0.2985	----------	----------
Top15Height	----------	0.2885	----------
Top15BV	----------	0.2415	----------
Foliar δ^{15}N	----------	----------	**0.4273**

Table A3.4 – Simple linear regression R^2 values for net soil (forest floor + mineral soil) available N (NH_4^+-N + NO_3^--N + amino acid-N) with height, bole volume Top 15 heights and Top 15 bole volume. Log values were used for all soil characteristics during analysis.

	Available N
Height	0.2910
Bole volume	0.3113
Top 15 height	0.2765
Top 15 bole volume	0.1828

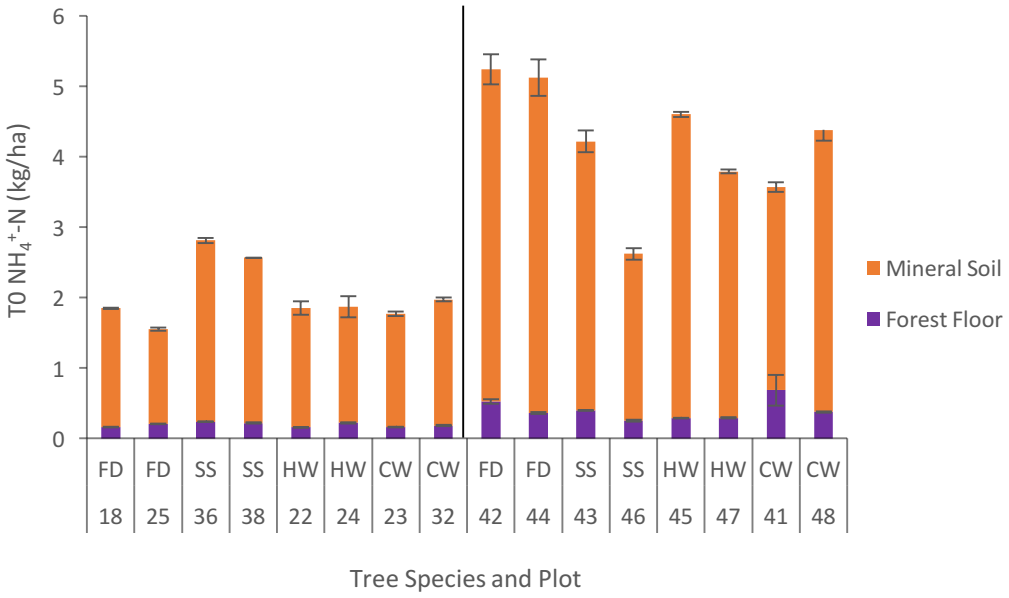


Figure A2.1 – Mean ± S.E. T0 forest floor and mineral soil NH_4^+-N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

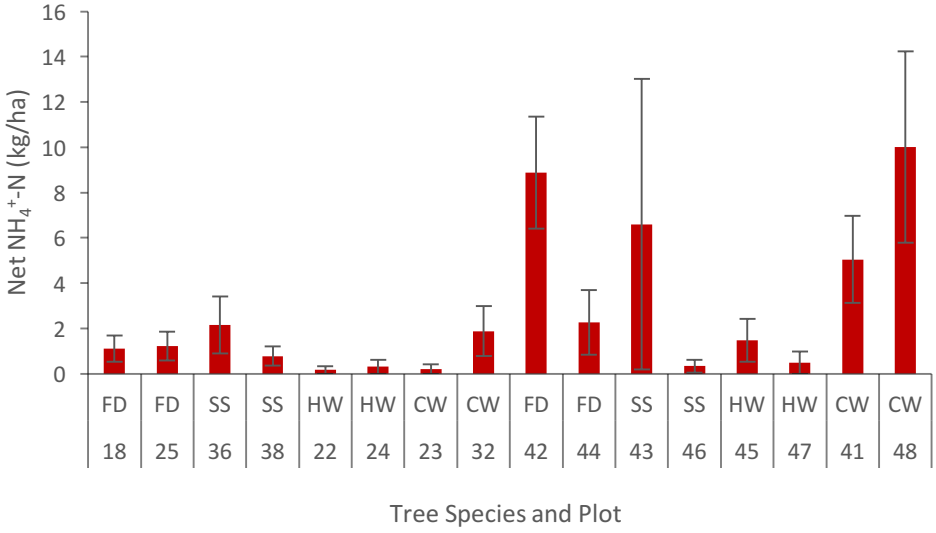


Figure A2.2 – Mean ± S.E. net forest floor + mineral soil NH_4^+-N concentrations(kg/ha) by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

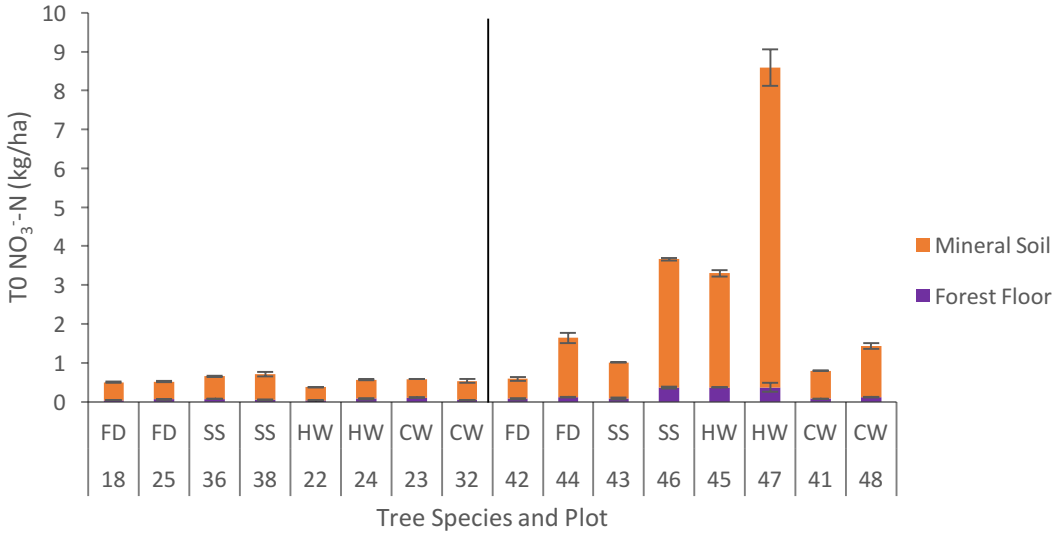


Figure A2.3 – Mean ± S.E. T0 forest floor and mineral soil NO_3^--N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

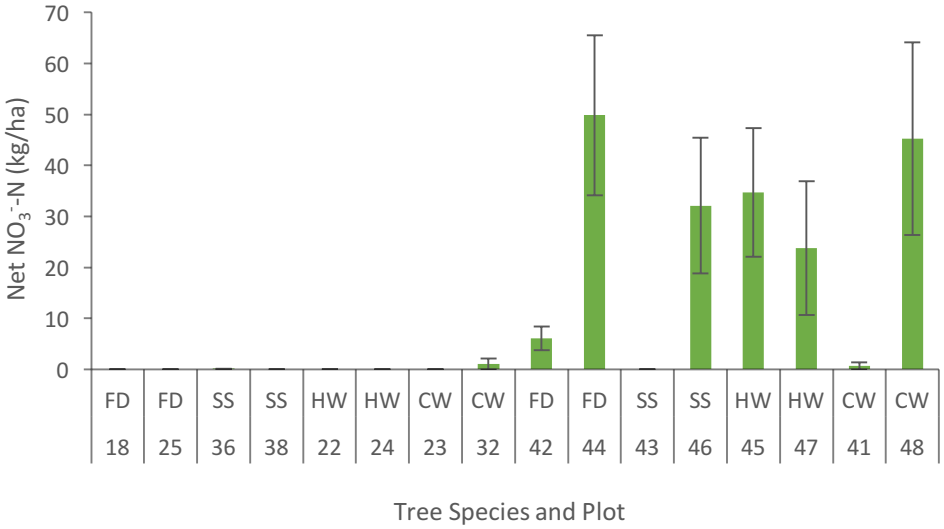


Figure A2.4 – Mean ± S.E. net forest floor + mineral soil NO_3^--N concentrations(kg/ha) by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

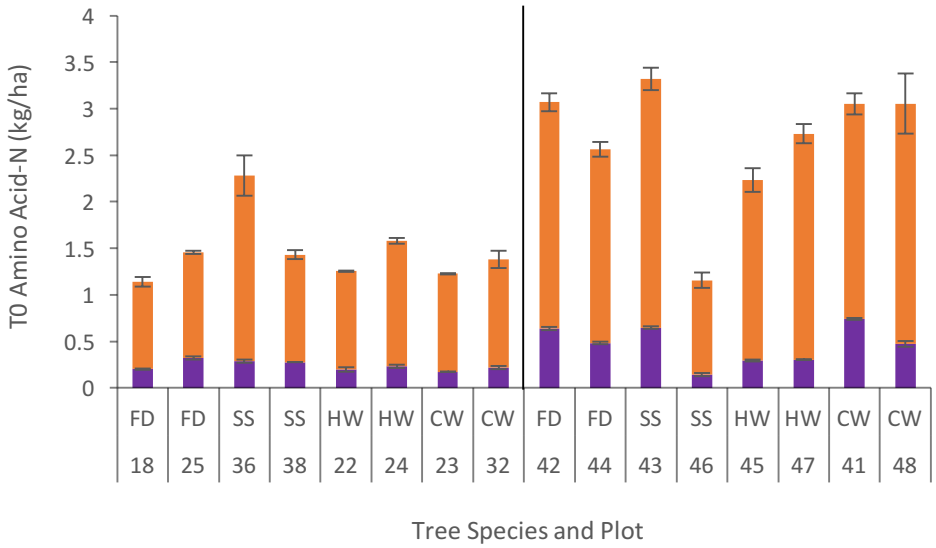


Figure A2.5 - Mean ± S.E. T0 forest floor and mineral soil amino acid-N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan. SE bars included.

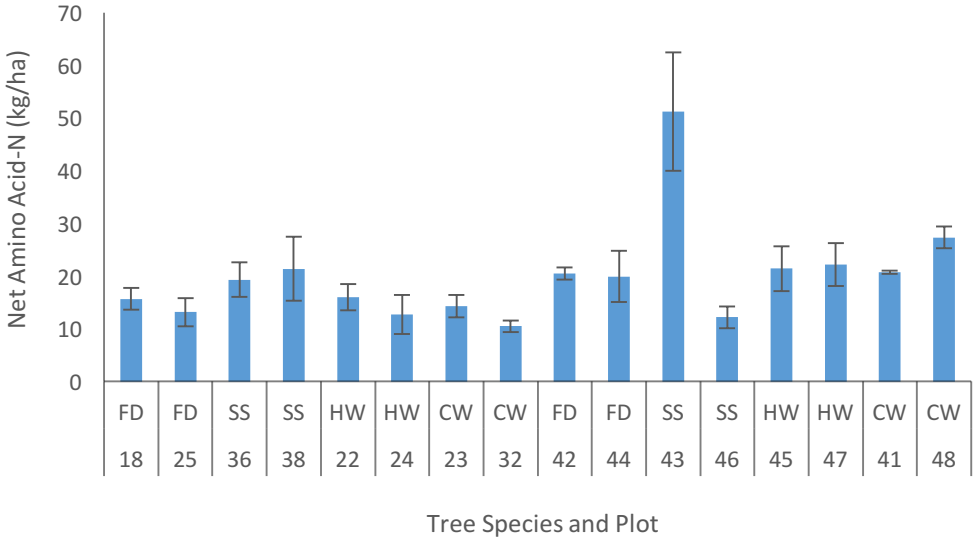


Figure A2.6 - Mean ± S.E. net forest floor + mineral soil amino acid-N (kg/ha) concentrations by tree species, Douglas-fir (FD), Sitka spruce (SS), western redcedar (CW) and western hemlock (HW), and plot. The first eight plots are in Fairy Lake and the last eight plots are in San Juan.

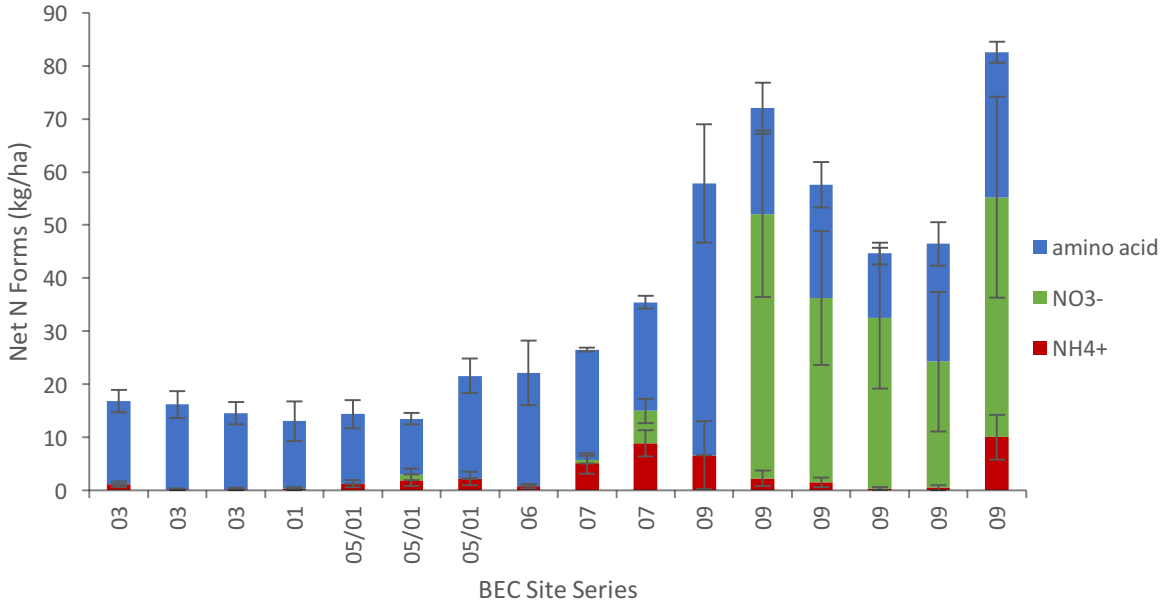


Figure A2.7 – Mean ± S.E. net forest floor + mineral soil NH_4^+-N, NO_3^--N and amino acid-N concentrations (kg/ha) by BEC site series.

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