The effect of soil temperature on soil nitrogen form availability and nitrogen uptake by conifers of British Columbia.

By

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Supervisory Committee

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Dr. Doug Maynard, Departmental Member
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ABSTRACT

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With climate change, forest soils of British Columbia (B.C.) will likely undergo significant increases in temperature. Changes in temperature may differentially alter steps of N cycling, altering the amount of N in various pools of the cycle. Furthermore, plant species may show a preference for certain N forms available in soils, such as ammonium, nitrate or organic N. Changes in soil N forms and plant N preferences can shift competitive interactions among conifer species in B.C. forests. Using a greenhouse incubation of forest soils from two elevations, I aimed to determine how temperature affects N cycling in soils that differ in temperature adaptations. With a conifer growth experiment where ammonium, nitrate and a mix of amino acids were applied to trees, I studied N form preferences and uptake rates of three conifer species from contrasting environments (*Pseudotsuga menziesii*, *Picea sitchensis*, and *Picea engelmannii*), and how N uptake in these species reacted to increases in soil temperature. Results show that the abundance of all N forms increased with temperature, but the response to warming was stronger in soils from a low elevation. Furthermore, ammonium and soluble organic N in soils increased faster with warming than nitrate. Nitrification potential was higher in the low elevation soil. This indicates that rates of soil processes, producing plant available N may increase with warming and the balance of different N forms may change. Differences in the abundance, composition, or activity of soil biota at these two locations likely caused dissimilar reactions to warming in two chemically and physically similar soils. Conifers exhibited preferences towards N forms, and these preferences are likely due to adaptation to the N form most available in native soils. On average, Douglas-fir showed preference for nitrate (a N form commonly found in warmer areas), Sitka spruce preferred ammonium (a N form high in cooler areas), and Engelmann spruce showed equal preference for organic N and ammonium (organic N is usually abundant in very cold areas). Preference as indicated by plant growth changed when species were grown at different temperatures, showing ability for acclimation in these conifers. Understanding that a soil's history greatly affects its response to perturbation is important if we are to make predictions on how N cycling in soils may change with changing climate. Knowing how conifers utilize available soil nutrients at different temperatures will help to predict species' future performance, composition and abundance in B.C. forests as soils warm and tree lines move north or to higher elevations.

Table of Contents

List of Tables

List of Figures

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CHAPTER 1. Literature Review

1.1 Nitrogen in Conifers

Although nitrogen (N) comprises 78% of the Earth's atmosphere, it is usually the most limiting nutrient for plant growth in natural terrestrial ecosystems such as temperate forests. Nitrogen limitation is due to (1) very high nutritional requirements of plants, as it is the most abundant mineral element in many plant tissues, and (2) relatively low availability, as the majority of nitrogen is immobilized in soils or otherwise made unavailable to plants (Miller and Cramer, 2004). It is estimated that only 0.00024% of N on earth is available for plant use (Miller and Cramer, 2004). For example, N_2 gas comprises the majority of the planet's N; however, it is held in a non-reactive form due to a strong triple bond between the two nitrogen atoms (Miller and Cramer, 2004). Furthermore, in temperate or boreal forests, a large amount of N in soils is contained within large organic molecules (often complexed with other elements), which are unavailable for plant uptake (Vitousek and Howarth, 1991; Mengel, 1996).

Nitrogen uptake is usually positively correlated with photosynthesis and plant biomass as N is used in the formation of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) and other enzymes of the Calvin cycle (Lukac *et al.*, 2011; Taiz and Zeiger, 2011). Additionally, N is a building block for proteins, DNA, and chlorophyll, which are essential for plant functioning and growth (Öhlund and Näsholm, 2004). Thus, when nitrogen is available, it is readily taken up and either incorporated immediately or stored by plants (Rennenberg and Schmidt, 2010).

1.1.1 Nitrogen Uptake

Nitrogen in the soil is present in various inorganic and organic forms resulting in a heterogeneous distribution that undergoes seasonal changes (Miller and Cramer, 2004). Most plants (including conifers) can only take up certain forms of nutrients from soils. Uptake of N by plants occurs either by fungal, symbiotic associations (mycorrhizae) or by direct uptake by root tips and root hairs. Roots of many northern temperate and boreal trees only associate with ectomycorrhizal fungi, while most

angiosperms associate with vesicular arbuscular (AM) mycorrhizae (Peterson *et al*., 2004). However, many members of *Cupressaceae* associate with VA mycorrhizae, and some members of *Pinaceae* associate with ectendomycorrhizal fungi, which form a Hartig net while penetrating plant cells (Peterson *et al*., 2004; Hanif *et al.,* 2012). *Populus* is one of the few genera known to associate with both ectomycorrhizae and AM (Vozzo & Hacskaylo 1974; Peterson *et al*., 2004).

In the mycorrhizal symbiosis, the tree supplies the fungi with photosynthates (energy), while the fungi act as an extension of the root hair, increasing the volume of soil accessed for nutrients and water. Ectomycorrhizal fungi are capable of taking up organic and inorganic N forms (Miller and Cramer, 2004). Ectomycorrhizal fungi are also able to excrete hydrolytic enzymes, which break down proteins into amino acids; influencing amino acid uptake by plants (Miller and Cramer, 2004; Frank and Groffman, 2009). Besides fungal associations, the uptake of N also depends on the distribution of roots, rooting depth, and root:shoot ratio (Miller and Cramer, 2004).

Nutrients, such as ammonium (NH_4^+) or nitrate (NO_3^-), must cross the plasma membrane of root cells to become available to plant metabolism. As this membrane is only permeable to gasses and small molecules, nutrients must be transported to the root, then across this membrane. Nutrient transport towards the root can either be driven against a concentration gradient by mass flow, transpiration or gravity, or more commonly, following a concentration gradient between the root surface and soils by diffusion over short distances (Miller and Cramer, 2004). It is important to consider the diffusion rate of each N form along with soil moisture when determining plant utilization (Gjisman, 1991; Miller and Cramer, 2004). For NO_3^-, the diffusion coefficient in soil is $1x10^{-10}$ m^2/s while the diffusion coefficient for NH_4^+ is approximately 10-100 times lower (Miller and Cramer, 2004).

Once N is transported to the root via mass flow or diffusion, N enters a network of pores within the cell wall of the roots (Marschner, 2002). Within these pores are carboxyl groups that can bind to cations such as NH_4^+ or positively charged amino acids, increasing the proximity of these molecules to sites of active uptake in the plasma membrane (Öhlund & Näsholm, 2004). The NO_3^- ion can enter the cell cytoplasm via

active transport after the establishment of a proton gradient, while NH_4^+ uptake is passive through ion channels.

Inorganic nitrogen uptake by root hairs is said to occur via high affinity transport systems (HATS) and low affinity transport systems (LATS) (Miller & Cramer, 2004). HATS follow Michaelis Menton kinetics and can become saturated (Glass *et al*., 2001). This indicates that HATS are controlled by protein carriers- a form of active transport. Several gene families have been identified to control HATS uptake for N (Glass *et al.*, 2001). LATS; however, is thought to act through ion channels, as saturation does not occur even at high N concentrations (Kronzucker *et al.*, 1996).

The influx of NO_3^- and NH_4^+ can occur simultaneously through different uptake systems (Crawford and Glass, 1998). Simultaneous efflux can also occur; however, this is due to passive diffusion of nutrients out of carriers and channels and occurs when N saturation is reached within a plant (Crawford and Glass, 1998). Plant uptake of N is affected by internal nutrient status and external concentrations; thus, plants are able to regulate gene expression of HATS and LATS to match their nutritional requirements (Glass *et al.*, 2001).

Once N is in the root, it is transported to the shoot or leaves via the xylem- or the xylem and phloem for amino acids (Marschner *et al.*, 1991; Miller & Cramer, 2004). Through nutrient cycling between the roots and shoots within plants, plants can detect the nutrient content of their tissues. Internal plant concentrations act as signals and influence how much of a mineral is taken up (Marschner *et al.*, 1991).

It was traditionally accepted that the majority of the N taken up by plants is in the inorganic forms of NH_4^+ or NO_3^- (Tamm, 1991). However, over the last 20 years, an increasing number of studies have challenged this view by showing that plants can also directly take up organic N forms such as amino acids or small peptides, and in some cases, plants may actually take up organic N at rates comparable to inorganic N uptake (Näsholm *et al.*, 2009; Persson, 2003; Öhlund & Näsholm, 2004; Miller & Cramer, 2004). Organic N uptake is beneficial to plants as it bypasses N mineralization and allows direct N assimilation. Amino acids (a.a.) are both transported through the xylem and phloem and between cellular organelles. The transport of a.a. across the plasma membrane is thought to occur by a proton coupled symporter with a broad substrate

specificity (Neelam *et al*., 1999). Although it is known that organic N uptake does occur and that it is important to the nutritional status of a plant, it is still unclear (1) what regulates the uptake of a.a., (2) how essential organic N uptake is for plants, (3) what is the comparative availability of organic and inorganic N in forest soils, and (4) what are the relative uptake rates of organic and inorganic N by forest trees.

1.1.2 Nitrogen Preferences

There is flexibility in which form of N plants take up. Due to the variations in availability with seasonal/diurnal changes and soil type, plants have evolved mechanisms to control the uptake of NO_3^- and NH_4^+. Furthermore, various plant species have shown preferences (in regards to enhanced growth or uptake) towards a certain N form, which may relate to the most abundant N form available in soils (adaption) (Dong *et al.*, 2001; Miller & Cramer, 2004; Nordin *et al*., 2004; Houlton *et al*., 2007). For example, cold, acidic forest soils are associated with higher levels of NH_4^+ (Rengel, 2002) and it is shown that NH_4^+ uptake is preferred to NO_3^- by a number of conifers (Kronzucker *et al.*, 1997; Öhlund & Näsholm, 2001). This is likely due to lower nitrate reductase activity and a low expression of NO_3^- transporters in conifers (Kronzucker *et al.*, 1995; Rengel, 2002). However, preference can also be linked to energy required for uptake, assimilation, and storage. Thus, if available in equal concentrations, some plants will take up NO_3^- rather than NH_4^+, as NO_3^- is a less toxic form that is able to be stored directly in vacuoles as it does not alter cellular pH (Miller & Cramer, 2004; Houlton, 2007); however, NH_4^+ takes less energy to assimilate.

In a study realized in the tundra, the uptake of NH_4^+ by species of *Rubus* and *Tofieldia* was greatest followed by the uptake of amino acids and finally NO_3^- (Nordin *et al*., 2004). This mirrored the relative concentrations of the N forms found in the soil of the area. However, species of *Arctostaphylos* and *Cassiope* took up more NO_3^- than NH_4^+, showing a preference for NO_3^- despite soil concentration (Nordin *et al*., 2004).
In a grassland study, soils were rich in amino acids. The grass *Nardus stricta* showed the expected consequent high uptake of the amino acid serine, while *Lolium perenne* showed a preference for inorganic N forms by taking in 14 times more dissolved inorganic nitrogen (DIN) than dissolved organic nitrogen (DON) (Weigelt et al., 2005).

In agricultural studies, preferences for certain N forms have also been demonstrated. Using hydroponically grown spinach, Elia *et al.* (1998) demonstrated a preference for NO_3^-. Plants supplied with NO_3^- had a larger leaf area as well as a higher overall biomass. In another study, whole tomato plants supplied with NO_3^- or NH_4^+ exhibited no preferential uptake or significant difference in total plant N (Evans *et al*., 1996). There was a difference in growth allocation; however, as plants supplied with NO_3^- possessed a larger leaf area while plants supplied with NH_4^+ allocated growth towards the roots (Evans *et al*., 1996).

Kronzucker *et al.* (1997, 2013) suggested that nutrient preference might affect plant fitness in a certain ecosystem. Thus, information on N preference and uptake can be used to predict productivity or competitive ability. If there is a change in the environment, we can use these predictions to forecast changes in species composition.

1.1.3 Effects of Temperature on Nitrogen Uptake

The preference and uptake of certain N forms can be affected by soil temperature (Bonan, 1991; Scholberg *et al.*, 2002). Warming increases the overall availability of N in soils (Lukac *et al.* 2011) and increases the ability of plants to take up N (Bloom and Chapin, 1981); thus, the uptake of most N forms is positively correlated with temperature (Dong, 2001). For example, in cool climates, NO_3^- influx into roots decreases (Bloom, 1985). It has also been shown that algal utilization of NH_4^+ decreases up to 25% with decreases in temperature of 8 °C (Berg *et al*., 1997). To compensate for the lowered N uptake and utilization, cooling can alter biomass allocation, increasing the root:shoot ratio of plants (Clarkson *et al*., 1986).

Although uptake of N decreases with decreasing temperature, this decrease is not uniform across N forms. In grasses, at cooler temperatures (< 14 °C) it has been shown that the root uptake of NH_4^+ exceeds NO_3^- (Clarkson & Warner, 1979; Clarkson, 1986). The difference in uptake with temperature was thought to be due to differences in transition temperatures of cellular compartments, as assimilation of NO_3^- and NH_4^+ may occur in different parts of a cell differing in lipid concentrations (Clarkson & Warner, 1979; Miller & Cramer, 2004).

It has been demonstrated that root uptake of N is also affected by the N form to which a plant was previously exposed (Bassisirad *et al.*, 1993). This has been demonstrated in a study by Clarkson & Warner (1979) where the ratio of NH_4^+:NO_3^- uptake decreased when ryegrass plants were pre-treated with NO_3^-. Thus, plants may exhibit acclimation to their available N source.

1.2 Nitrogen in Soils

Soil organic N is estimated to contain 40% proteinaceous materials (including proteins, peptides, and amino acids), 19% NH_3, 6% amino sugars, and 35% heterocyclic N compounds throughout terrestrial ecosystems spanning arctic to tropical regions (Schulten & Schnitzer, 1998). Nitrogen in soils is continually transformed from organic to inorganic forms in processes controlled by soil microbes.

The concentration of NH_4^+, NO_3^-, and organic nitrogen forms vary greatly depending on the location of the soil, vegetation, topography, type of soil, extraction method and other chemical, physical and biological factors. For example, temperate coniferous forests of British Columbia (B.C.) have been shown to contain 6-42 μmol NH_4^+/g soil, 21-50 μmol NO_3^-/g, and 0.1-0.5 μmol a.a./g (Tables 1.1 and 1.2; Prescott *et al*., 2000; Hannan & Prescott, 2003). However, tropical regions' overall concentrations of N are considerably lower with 0.84 μmol NH_4^+/g soil, 0.37 μmol NO_3^-/g, and 0.039 μmol a.a./g (Tables 1.1 and 1.2; Schmidt & Stewart, 1999).

Table 1.1. Summary of studies of NH_4^+ and NO_3^- concentrations in soils from various terrestrial habitats. Table is adapted from Metcalfe (2005).

Reference	Site	Extraction method	NH_4^+-N (μmol g^{-1} soil)	NO_3^--N (μmol g^{-1} soil)
Sahrawat et al. (1985)	Variety of deciduous and coniferous sites in Wisconsin	Steam distillation	5 - 36 (deciduous) 11 - 70 (coniferous)	12 - 26 (deciduous) 1 - 21 (coniferous)
Adams et al. (1989 a)	Temperate rainforest of Tasmania	1M KCl	31.1	0.6
Prescott et al. (1992 b)	Coniferous pine forest in Rocky Mountains	KCl	20.5 - 133.2 (forest floor)	0.2 - 2.3 (forest floor)
Prescott et al. (1992 b)	Coniferous spruce forest in Rocky Mountains	KCl	84.6 - 29.2 (forest floor)	0 - 2.8 (forest floor)
Prescott et al. (1992 b)	Coniferous fir forest in Rocky Mountains	KCl	103.8 - 32.7 (forest floor)	0.4 - 4.6 (forest floor)
Schnell & King (1994)	Mixed conifer and deciduous forest in Maine	2M KCl	308	22.4
Stark & Hart (1997)	Douglas-fir forest in Oregon	2M KCl	2.59	0.54
Stark & Hart (1997)	Ponderosa pine forest in Oregon	2M KCl	0.98	0.05
Stark & Hart (1997)	Western hemlock/ Sitka spruce forest in Oregon	2M KCl	4.53	0.08
Stark & Hart (1997)	Red alder/ Douglas-fir forest in Oregon	2M KCl	4.11	1.24
Erskine et al. (1998)	Subantarctic herbs	water	6.776	0.231
Schmidt & Stewart (1999)	Subtropical coral cay	water	0.336	3.29
Schmidt & Stewart (1999)	Subtropical rainforest	water	0.91	0.854
Schmidt & Stewart (1999)	Tropical savanna woodland	water	0.84	0.371

Table 1.1. Continued

Laverman et al. (2000)	Nitrogen saturated Scots pine forest in the Netherlands	1M KCl	60.3 - 384 (forest floor) 9.1 (mineral soil)	5.8 - 23.1 (forest floor) 2.4 (mineral soil)
Prescott et al. (2000)	Temperate coniferous forests of coastal British Columbia	KCl	6 - 42 (forest floor)	21 - 50 (forest floor)
Hope et al. (2003)	British Columbia dry interior Douglas-fir forest	2M KCl	ca. 75- 200 (forest floor) ca. 5 - 20 (mineral soil)	ca. 10 - 250 (forest floor) ca. 1 -20 (mineral soil)
Lavoie & Bradley (2003)	A range of coniferous and deciduous vegetation in southwestern Québec	1M KCl	10 - 20 (forest floor) 2 - 4 (mineral soil)	1 - 5 (forest floor) ~0.5 (mineral soil)
Grenon et al. (2004)	Old growth coniferous forest ecosystems in British Columbia	2M KCl	ca.10 - 50 (forest floor)	ca. 1 (forest floor)
Grenon et al. (2004)	Clearcut coniferous forest ecosystems in British Columbia	2M KCl	ca.10 - 100 (forest floor)	ca. 1 - 50 (forest floor)
Berthrong & Finzi (2006)	Cold-temperate forests in Connecticut	2M KCl	1.71 - 25.8 (Total inorganic N)	

Table 1.2. Summary of studies of amino acid concentrations in soils from various terrestrial habitats. Table is adapted from Metcalfe (2005).

Reference	Site	Extraction method	Amino acid-N ($\mu mol\ g^{-1}$ soil)	Most common amino acids
Nemeth et al. (1988)	Pine and deciduous forests	Electro-ultrafiltration	7.954 (Pine) 5.320 (deciduous)	
Kielland (1995)	Arctic ecosystem dominated by shrubs and lichen	Water	1.5 - 8	Glycine, aspartic acid, glutamic acid, serine, and arginine
Erskine et al. (1998)	Subantarctic herbs	Water	0.077	
Senwo & Tabatabai (1998)	Agricultural soils in Iowa	Acid hydrolysis	573 - 1384	Asparagine, aspartic acid, glutamine and glutamic acid
Schmidt & Stewart (1999)	Subtropical coral cay	Water	0.014	
Schmidt & Stewart (1999)	Subtropical rainforest	Water	0.06	
Schmidt & Stewart (1999)	Tropical savanna woodland	Water	0.039	
Nordin et al. (2001)	Boreal forest	Water	ca. 2 - 5	Asparagine, glutamine, arginine, serine, alanine, and glycine
Persson & Näsholm (2001)	Boreal forest	Water	0.005 - 0.330	
Jones et al. (2002)	Temperate wet conifer forest in Ireland	2M KCl	1.4 - 2.8 (mineral soil)	
Nemeth et al. (1988)	Pine and deciduous forests	Electro-ultrafiltration	7.954 (Pine) 5.320 (deciduous)	
Kielland (1995)	Arctic ecosystem dominated by shrubs and lichen	Water	1.5 - 8	Glycine, aspartic acid, glutamic acid, serine, and arginine
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1.3 Nitrogen Cycling

1.3.1 Nitrogen Fixation

As most organisms cannot use N_2 gas from the atmosphere, the reduction of N_2 by bacteria is essential to biological uptake (Figure 1.1; Reaction 1). Nitrogen-fixing microorganisms are responsible for 90% of natural N fixation, while lightning accounts for the other 10% (Miller & Cramer, 2004, Taiz & Ziegler, 2011). Nitrogen fixation is catalyzed by the nitrogenase enzyme, and requires a high input of energy; thus, it is only done in N limiting conditions. In a study using detached nodules of four non-legumes *(Alnus, Hippophas, Myrica, Casuarina),* the effect of temperature on nitrogenase activity was tested, and the activity was shown to be highest around 25-30 °C, with a relatively high sensitivity to temperature (Waghman, 1976). After NH_3 is produced, it is rapidly converted into NH_4^+ due to acidic conditions within the periplasm of the bacterial cell (Sellstedt, 1999).

Reaction 1. *Reduction of N_2 gas to NH_3*

$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$ (catalyzed by nitrogenase)

1.3.2 Depolymerization/Decomposition

When nitrogen in biota is returned to soils during decay, it is in the form of large polymeric organic molecules (Figure 1.1). As plants cannot immediately use these large organic N forms, they must be broken down into more soluble forms such as amino acids, nucleotides or amino sugars. As mentioned earlier, this breakdown is known as depolymerization, and it is thought to regulate the overall available N in many ecosystems (Schimel & Bennett, 2004).

1.3.3 Mineralization and Immobilization

When plant, animal, or bacterial cells die, their contained organic N becomes mineralized (converted into inorganic N) by heterotrophic fungi and bacteria (Figure 1.1; Raven *et al*., 1999). Mineralization is usually done by heterotrophic soil bacteria that convert urea or amino acids into NH_4^+. If microorganisms themselves are N limited,

they may incorporate the N into their own cells, leading to immobilization (Miller & Cramer, 2004). Immobilization depends on the C:N ratio of organic matter. As a general rule, if the C:N ratio is above 30 it will lead to net immobilization of N in soils, while if the C:N ratio is below 20 it will lead to net mineralization of N in soils. Soil protozoa and fauna can also feed on bacterial decomposers, and excrete excess N as NH_4^+; thus, these organisms may also act to increase rates of mineralization (Clarholm, 1985). Immobilization is the conversion of N from inorganic to organic forms; hence, immobilization can also refer to assimilation of inorganic N by plants. As the rate of mineralization in forest ecosystems is typically low, plants and microbes usually immobilize any NH_4^+ produced. Thus, most forests are said to have a tightly regulated N cycle (Miller *et al*., 1979).

The assimilation of NH_4^+ into organic N in tissues is direct through the glutamine synthase-glutamine2-oxoglutarate aminotransferase (GS-GOGAT) pathway. The assimilation of NO_3^- into organic nitrogen requires four enzymatic steps. Nitrate reductase (NR) converts NO_3^- into nitrite (NO_2^-) (Campbell, 1988). Then, nitrite reductase (NiR) converts nitrite into NH_4^+. During immobilization, glutamine synthase (GS) then uses ATP to combine NH_4^+ and glutamate into glutamine. Lastly, glutamine2-oxoglutarate aminotransferase (GOGAT) combines glutamine and alpha-ketoglutarate to give the final product, 2-glutamate (Campbell, 1988).

1.3.4 Nitrification

Nitrate may be produced by the conversion of organic N by soil heterotrophs; however, more commonly NO_3^- is produced by the oxidation of NH_4^+ by soil chemolithoautotrophic, nitrifying bacteria (Figure 1.1). The mineralization of N is followed by nitrification, where NH_4^+ is oxidized into hydroxylamine, in a reaction catalyzed by the enzyme: ammonia monooxygenase (AMO) (Reaction 2). Hydroxylamine is then converted into NO_2^- by ammonium oxidizers such as *Nitrosomonas spp*. and *Nitrosospira spp*. that contain the enzyme: hydroxylamine oxidoreductase (Miller & Cramer, 2004; Reaction 3). NO_2^- is then rapidly converted into NO_3^- by nitrite oxidizing bacteria such as *Nitrobacter spp*. and *Nitrospira spp*. (Miller & Cramer, 2004; Reaction 4). Nitrate is readily taken up by plants or immobilized by

heterotrophic soil organisms; however, due to its negative charge, NO_3^- is not retained well on negatively charged soil surfaces; thus, it is more mobile in soils and is easily lost due to leaching. Recently, nitrifiers have been discovered, which include members of Archaea such as the anerobic *Nitrosopumilus maritimus* (Konneke *et al*., 2005).

Reaction 2*: Conversion of ammonia to hydroxylamine*

$NH_4^+ + O_2 + H^+ \rightarrow NH_2OH + H_2O$ (catalyzed by ammonia monoxygenase)

Reaction 3*: Conversion of hydroxylamine to nitrite*

$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+$ (catalyzed by hydroxylamine oxidoreductase)

Reaction 4*: Conversion of nitrite to nitrate*

$NO_2^- + ½\ O_2 \rightarrow NO_3^-$

NO_2^- rarely accumulates in soils, as the second reaction of nitrification possesses a high Gibbs free energy (Reaction 4). Furthermore, NO_2^- is toxic to plants and is not thought to be of value to plant nutrition (Campbell, 1988).

Nitrification produces hydrogen ions, which can lead to soil acidification (Reaction 3). However, nitrification is inhibited by low pH (< 3.7); thus, this reaction may cause the self-regulation of NO_3^- in soils. Furthermore, nitrification is also reduced in anaerobic or dry conditions, and in cool or hot temperatures (Lewis, 1986).

1.3.5 Denitrification

Denitrification, which occurs under anaerobic conditions, is the reduction of NO_3^- into NO, N_2O, and atmospheric nitrogen. During denitrification, NO_3^- (rather than oxygen) is used as the terminal electron acceptor of respiration; thus, NO_3^- is converted into NO_2^-, NO, N_2O, and N_2 gasses (Figure 1.1; Miller & Cramer, 2004). Denitrification is positively correlated with NO_3^- supply, warm temperatures, anaerobic conditions, and carbon availability (Luo *et al*., 2000; Strong & Fillery, 2002).

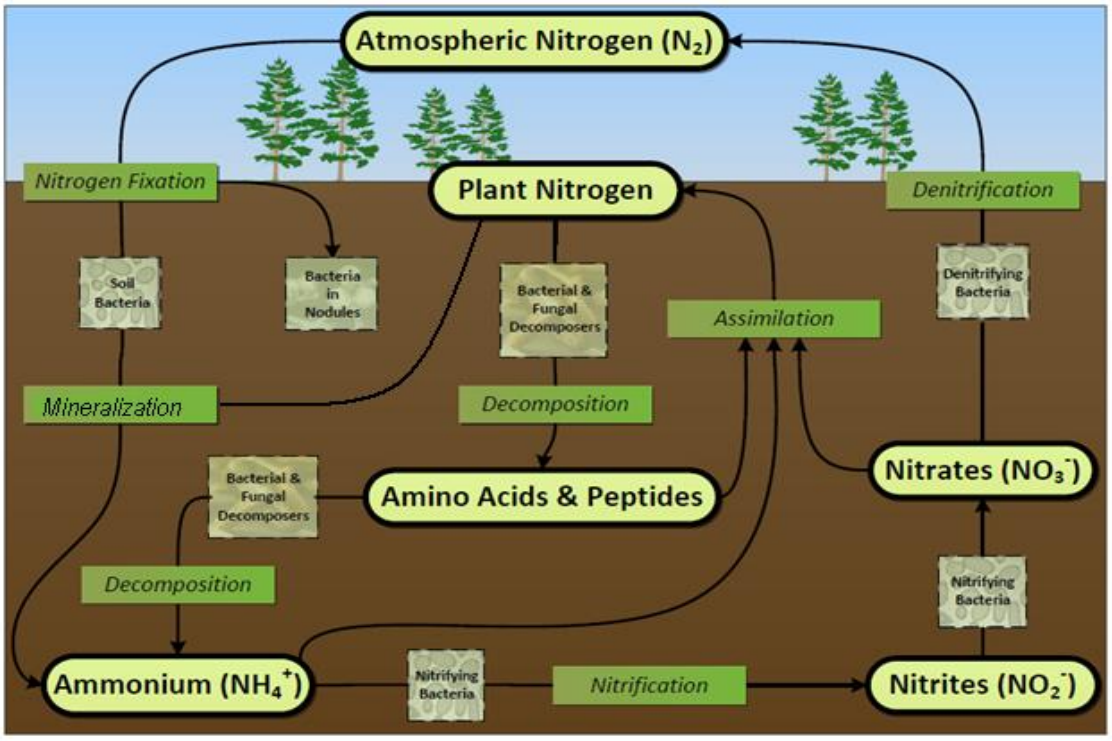

Figure 1.1. Schematic of the nitrogen (N) cycle.

1.4 Effect of Temperature on Soil Cycling of Nitrogen

As plants generally increase rates of growth and photosynthesis with increasing temperature, they consequently require greater amounts of macronutrients, such as N, from the soil. Changes in soil cycling by microbial communities caused by temperature can interact with plant demand, affecting growth. Many aspects of the N cycle are altered by changes in the climate, and microbial activity is directly affected by temperature (Brookshire *et al.*, 2011). Soil microbial activity and organic matter decomposition is negligible at temperatures less than 5 °C however, microbial activity generally doubles for every 10 °C increase in temperature up to an optimum of approximately 40 °C (Brady & Weil, 2002).

In cold, acidic soils, both nitrification and mineralization are slowed; however, mineralization is less sensitive to the temperature change, and nitrification is highly reduced in cool (< 5 °C) and hot (> 40 °C) temperatures (Lewis, 1986). Hence, cooler soils tend to contain relatively more NH_4^+ than NO_3^- than warm soils (Bloom and Chapin, 1981; von Wiren *et al.*, 1997). Below 14 °C soil N availability predominantly

switches to NH_4^+ (Bloom, 1981). Kapulnik (1981) demonstrated that the process of biological nitrogen fixation is also impaired at low temperatures, decreasing available N to plants.

With warmer temperatures, nitrogen mineralization and nitrification generally increase (Miller & Cramer, 2004; Cookson *et al*., 2007; Andresen *et al*., 2010). Thus, overall availability of inorganic N to plants should increase with temperature. Myers (1975) concluded that temperature was positively correlated with mineralization and nitrification, and at soil temperatures above 10 °C, microbes start to oxidize NH_4^+ ions into NO_3^-. Thus, with increasing temperatures, soils contain relatively higher NO_3^- concentrations. However, NO_3^- is easily leached from the soil; thus, variable NO_3^- concentrations are seen in differing climates (Table 1.1). The proportion of N in organic form is also thought to increase as temperatures decline, as mineralization of amino acids into NH_4^+ and NO_3^- by microbial species is inhibited (Atkin, 1996).

It is important to study ecosystems independently as not all systems show the same patterns of change of relative availability of N forms with temperature (Hovenden *et al.*, 2008; Miller *et al*., 2007; Cookson *et al.*, 2007; Dessureault-Rompré *et al*., 2010).

1.5 Other Factors that Affect N Cycling

1.5.1 Moisture and Oxygen: Usually a decrease in soil moisture leads to a decline in microbial activity (Sylvia *et al*., 1999). Low soil moisture can inhibit microbial activity by lowering water potential, reducing the hydration and consequently the growth of specific bacterial cells and the activity of the enzymes involved in the N cycle (Stark & Firestone, 1995).

Related to soil moisture, oxygen plays a large role in the bacterial activity of the N cycle. Nitrifiers are almost exclusively aerobic microorganisms while denitrifiers are anaerobic (Sylvia *et al*., 1998). Aerobic microorganisms usually have optimal activity at a soil water holding capacity of approximately 60%, but more water reduces O_2 availability and consequently decreases the rates of nitrification in soils (Sylvia *et al*., 1998).

1.5.2 pH: Nitrification is low below pH 4.5. At high pH, NO_3^- usually accumulates because denitrifiers are more inhibited by high pH than NH_4^+ oxidizers. Therefore autotrophic nitrifiers are considered neutrophilic (Sylvia et al., 1998)

1.5.3 Substrate Availability and Enzyme Activity: Perhaps the most important factor controlling the N cycle is substrate availability (Vitousek, 1982; Sylvia, 1999). As N transformations are often energetically expensive, it is the pools of N that control related processes (Sylvia *et al*., 1998). For example, the amount of NH_4^+ in soils controls the amount of nitrification and the amount of organic matter controls decomposition, etc.

Enzymes of the N cycle themselves can limit N cycling if they are not being produced or if enzyme activity is being affected. Brockett *et al*. (2012) found that all forms of available N were negatively correlated with N-acetyl-beta-D-glucosaminidase (*NAGase*) activity (an enzyme of depolymerisation); total available N and available NH_4^+ were negatively correlated with peroxidase activity; while available NH_4^+ was also negatively correlated with glucosidase activity.

1.5.4 Other Dynamics of the N cycle: Each pool of N in soils is controlled both by its source and sink. Thus, an increase or decrease in any one process of the N cycle can have effects on all processes of the N cycle that are involved dynamically. These dynamics make studying changes in the N cycle challenging.

1.5.5 Ecosystem: Studying N cycling can be complicated by interactions between the bacteria of the N cycle and other members of the ecosystem. For N cycling to occur, the appropriate microbial communities must be both present and active. The presence/activity of microbial functional groups can be affected by any of the factors, described above, but also by disturbance and other biological communities such as plants and bacteria that secrete allelopathic chemicals (Sylvia *et al*., 1998). Plants contain variable amounts of N in their foliage. Plant species (Brockett *et al.,* 2012), the retention of N in plants and consequent litter quality all affect soil composition and nutrition (Kranabetter *et al*., 2009); and influence soil microbial communities. Prescott et al. (2000) found that N mineralization was affected by understory plants of the forest

floor, but surprisingly not by litter chemistry. In addition, mycorrhizae play an important role in the depolymerization process through the action of extracellular proteases and enzymes, and thus influence other soil microorganisms. Competition for soil N may also exist between microbial communities. For example, removal of the forest canopy can increase nitrogen mineralization by decreasing resource competition between bacterial heterotrophs and mycorrhizae (Vitousek, 1982).

1.6 Conifer-Soil Interactions

Nitrogen availability usually limits plant growth in forests, thus plants compete for soil nitrogen. Besides competition for N among and within plant species, plants and soil microbes can be in competition for soil nutrients (Rennenberg *et al.*, 2009; Mansson *et al.*, 2009). This competition is mainly attributed to the compostability of soils, bacterial activity, and the C:N ratio (Mansson *et al.*, 2009). Competition may also be influenced by temperature due to the differences in physiological optima of the competing organisms (Öhlund & Näsholm, 2004).

The environment of a plant may lead to adaptation to certain N forms (Kronzucker *et al.*, 1997); conversely, plants can also affect the N forms present in the soils through selective uptake (Houlton, 2007). At lower temperatures, a plant's metabolic demand for N is reduced; this can lead to higher amounts of inorganic N left in the soils (Bloom, 1981).

Although there is a relatively high proportion of organic N in temperate soils, the pool of amino acids is highly dynamic with high turnover rates ($t_{1/2}$= 5 hours) (Jones & Kielland, 2012; Öhlund & Näsholm, 2004). Thus an increase in a N form in a soil may not indicate increased long-term availability, but instead indicate that the use of the N form by plants is constant or low, and turnover by bacteria is raising soil N levels.

1.7 Conifer Species of Study: Ecology and Background

1.7.1 Douglas -fir

Douglas-fir (*Pseudotsuga menziesii*) is a member of the family Pinaceae, and is not a true fir (genus *Abies*). It is widespread (latitudinal range from 55 °N in central B.C. to 19 °N in Mexico over a distance of 4500 km) and occurs from dry, low elevations to moist, montane areas (Figure 1.2). This species is long lived and can reach ages of thousands of years, and heights of 90 m. Douglas-fir possesses flat, linear leaves that encircle the branches. It can be identified by the mature cones that hang downwards with 3 lobed bracts protruding from between the scales (Figure 1.2). There are two varieties of Douglas-fir in B.C (1) coastal (*Pseudotsuga menziesii* (Mirb.) France var. *menziesii*) and (2) interior/montane (*Pseudotsuga menziesii* (Beisssn.) Franco var *glauca*). The two varieties differ in growth rate as the coastal variety grows faster and obtains a larger size, while the interior variety is more cold-hardy and slow growing (Hermann & Lavender, 1990). The coastal variety is said to be early successional while the interior variety is more shade-tolerant and is considered a late successional tree.

Douglas-fir is moderately shade tolerant, and seedlings grow best in partial shade (Hermann & Lavender, 1990). After the first year of growth, Douglas-fir requires more light to outcompete shrubs of the forest floor. Douglas-fir is considered a seral species, establishing after fire. Without fire, coastal Douglas-fir is often replaced by other species throughout its range such as western hemlock, Sitka spruce, western red cedar, amabilis fir, and grand fir (Farrar, 1995; Pojar & MacKinnon, 2004). Highly productive Douglas-fir stands are usually found on deep soils that are well aerated and acidic (pH 5-6), and growth of stands is often solely limited by N (Hermann & Lavender, 1990). Douglas-fir has shown a preference towards NO_3^- when only inorganic N forms were tested(Kronzucker *et al.*, 2003). When supplied with NO_3^-, growth of Douglas-fir is improved, and Douglas-fir has been shown inefficient at regulating NH_4^+ uptake (Kronzucker *et al.*, 2003). However, uptake of NH_4^+ has shown to be both higher (Kamminga-van & Prins, 1993) and lower (Hawkins *et al.*, 2008) than NO_3^- uptake in single source solutions.

Douglas-fir is often used in silviculture, as it forms even aged stands after clear cutting and replanting. The trees are important to the timber industry as well as for sale as Christmas trees.

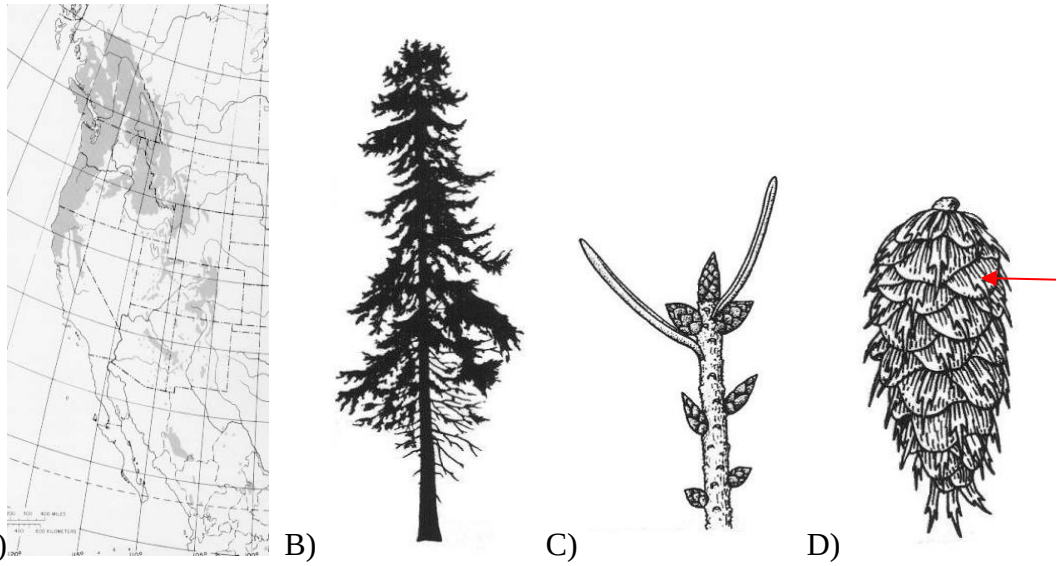

Figure 1.2. A) Range of Douglas-fir (*Pseudotsuga menziesii*) along the west coast of North America (From Burns & Honkala, 1990), B) mature tree growth form, C) branch form, D) cone form showing 3-pronged bract (red arrow) (B, C, & D from Farrar, 1995).

1.7.2 Sitka spruce

Sitka spruce (*Picea sitchensis*) is a member of the family Pinaceae named after the town 'Sitka' in Alaska. Its native range spans 61 °N in southern Alaska to 19 °N in California (Figure 1.3). Sitka spruce grows 55-95 m (average 70 m) tall and is recorded as the tallest native conifer in Canada (Farrar, 1995). It possesses thin scaly bark, thin long cones, a cylindrical crown, and sterigmata for support of leaves (Figure 1.3). It occurs in moist, low to mid elevation soils. It is common on nutrient rich sites of floodplains and marine terraces of B.C. (Pojar & MacKinnon, 2004).

Sitka spruce is a later seral species, and has a greater tolerance of shade than Douglas-fir (Farrar, 1995). It is able to establish on exposed soils near the ocean due to its tolerance to salt spray (Harris, 1990). Sitka spruce prefers deep, moist, well-aerated acidic soils (pH 4-5.7) rich in calcium, magnesium, phosphorus, and nitrogen.

Sitka spruce produces valuable, high quality timber (Harris, 1990). Due to the high strength:weight ratio, Sitka spruce is commonly used in sounding boards for high quality instruments, ladders, turbine blades, boat masts, and planks (Harris, 1990).

Other species of spruce such as Norway spruce, have shown a preference for NH_4^+ over NO_3^- when given a solution of both ions (Marschner *et al.*, 1991; Lumme, 1994). White spruce also preferentially takes up NH_4^+ rather than NO_3^- from soils

(Kronzucker *et al*., 1995). However, as Sitka spruce is a coastal species, adapted to a mild climate, the preference for NH_4^+ may not be as strong as other spruces.

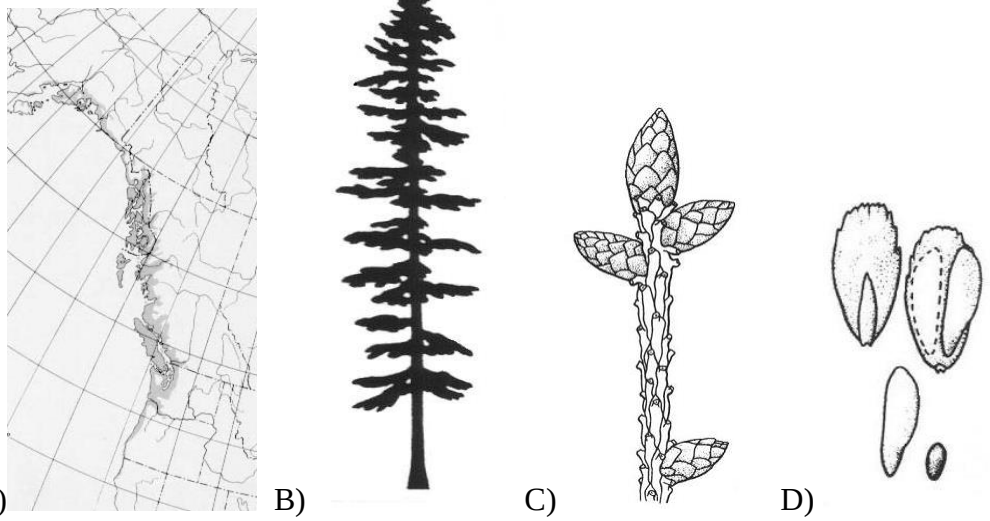

Figure 1.3. A) Range of Sitka spruce (*Picea sitchensis*) along the west coast of North America (From Burns & Honkala, 1990), B) mature tree growth form, C) branch showing sterigma (pl. sterigmata) and bud morphology, D) mature female cone scale, bract and seed (B, C, & D from Farrar, 1995).

1.7.3 Engelmann spruce

Engelmann spruce (*Picea engelmannii*) is also a member of the Pinaceae. Engelmann spruce is a major component of the Rocky Mountains flora and a minor component of the high elevation east slope forests of the Coastal B.C. mountain range (Alexander and Shepard, 1990; Figure 1.4). It is a medium sized evergreen with scaly bark and a cylindrical crown that can live up to 600 years (300 average) (Alexander and Shepard, 1990). Engelmann spruce is commonly found in humid climates characterized by heavy snowfall, with long winters and short cool summers (Alexander and Shepard, 1990).

Engelmann spruce seedlings grow well in areas of adequate soil moisture, cool temperatures and shade. At higher elevations, full shade is favourable to growth (Alexander, 1990). The conifer is known to have a low tolerance to high temperatures and drought, and possesses a shallow root system; thus, it is restricted to high elevations (Alexander and Shepard, 1990).

Engelmann spruce is often used in silviculture; however, cone/seed insects and rodents cause high seed mortality in the conifer, and trees are quite susceptible to

windthrow due to shallow roots (Alexander, 1990). The timber is often used in home construction, prefabricated wood products, and pulp (Alexander and Shepard, 1990).

Engelmann spruce forms a hybrid –known as interior spruce- with white spruce in the interior of B.C.; thus, it is likely that the N form preference of white spruce would be similar to that of Engelmann spruce. Kronzucker *et al*., (1995) showed that the uptake of NH_4^+ was 20 times greater than the uptake of NO_3^- in white spruce, indicating a very strong preference for NH_4^+.

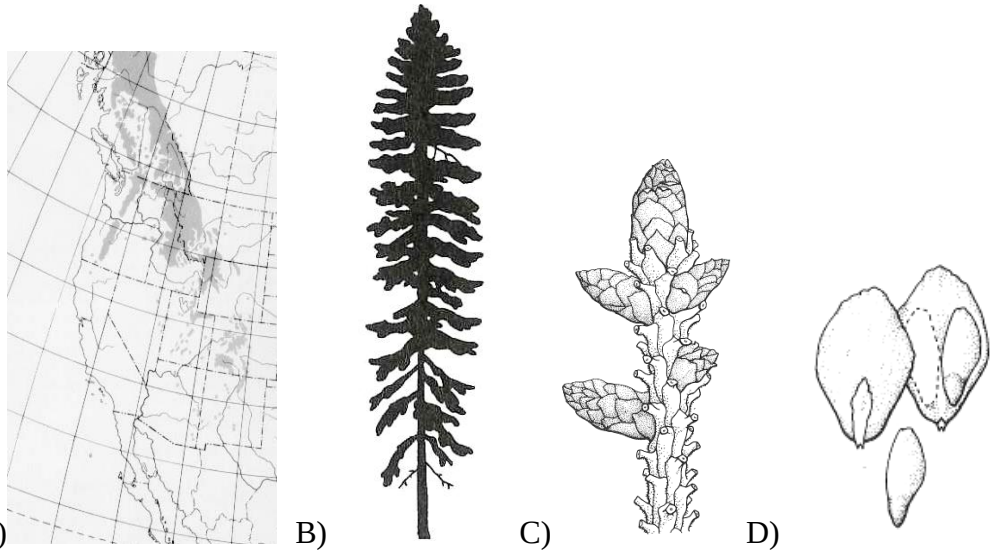

Figure 1.4. A) Range of Engelmann spruce (*Picea engelmannii*) along the west coast of North America (From Burns & Honkala, 1990), B) mature tree growth form, C) branch and bud morphology, D) bract and seed (B, C, & D from Farrar, 1995).

1.8 Study Objective

The objective of this thesis is to determine N form preference and uptake of three conifer species from contrasting environments, and how N preference and uptake rates respond to increases in soil temperature. I will also study how temperature affects N forms in forest soils from different climatic conditions. Understanding how conifer species utilize available soil nutrient resources at different soil temperatures will help to predict their relative success as climates change. Species' relative competitive abilities for N can then be compared to predict changes in species composition, abundance, and diversity in B.C. forests.

I hypothesize that increases in soil temperature will increase the proportion of NO_3^- available to plants; whereas, at cooler temperatures, the proportion of NH_4^+ available will be higher, and at very cold temperatures the relative availability of amino acids will increase in soils, leading to preferential uptake of these forms (based on previous literature). Because of previous adaptations, I expect that low elevation soils will contain relatively more NO_3^- and exhibit higher rates of decomposition than high elevation soils. I also hypothesize that there will be differences in N preference, uptake, and utilization between different conifer species, as there will be a preferential uptake of NO_3^- by Douglas-fir, a weak preference for NH_4^+ by Sitka spruce and a strong preference for NH_4^+ uptake by Engelmann spruce (based on literature). As temperatures increase, I expect to see warmer adapted species (Douglas-fir and Sitka spruce) having higher N uptake and assimilation than cold-adapted species (Engelmann spruce), especially when supplied with their preferred N form.

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CHAPTER 2. The Effect of Temperature on Nitrogen Transformations in Two Soils from Vancouver Island B.C.

2.1 Introduction

2.1.1 Nitrogen Availability in Soils

Insufficient avaiable N in soils limits plant growth in most natural ecosystems. Nitrogen limitation is, in part, a result of the high N requirements of plants. However, in temperate or boreal forests, a large amount (95-98%) of N in soils is held within large organic molecules, which are unavailable for plant uptake (Vitousek & Howarth, 1991). Thus, nitrogen limitation is also caused by a lack of nutrient availability to plant roots.

A main focus of plant nutrition research has been to characterize what affects N availability of soils, especially in relation to management practices such as fertilization, tillage, and forestry harvest methods. Soils poor in available N can result in reduced growth and productivity in both plants and the animals that consume them. Excess soil N can lead to high levels of N in ground and surface water. For these reasons, nitrogen has been studied more extensively than any other mineral element on Earth (Brady & Weil, 2002).

2.1.2 Nitrogen Cycling

As described in Chapter 1, in the nitrogen cycle (Figure 2.1), N is primarily present in the atmosphere as dinitrogen gas (N_2). Plants cannot take up N_2 as it is held together by a very stable triple covalent bond, so they rely on microbial symbionts or free-living bacteria that can fix N. Through the N cycle, fixed N is converted into other forms, including organic nitrogen (amino acids, small peptides proteins, nucleic acids, and metabolic intermediates), NH_4^+, and NO_3^-. Microbial populations control the conversion of N from one form to another.

Nitrogen cycling occurs in three general steps (Leake & Read, 1989; Sylvia, 1998; Persson *et al*., 2003; Schimel & Bennett, 2004). First, organic N polymers (*eg.* proteins, large peptides and DNA) from plant or microbial turnover are broken down by proteases (extracellular, hydrolytic enzymes excreted by bacteria and other soil

organisms) into smaller organic compounds such as amino acids, nucleic acids, or small peptides. This makes N available to plants and microbes. Next, small organic molecules can be further mineralized into NH_4^+ during microbial metabolism. Lastly, NH_4^+ is oxidized to form NO_2^-, which is rapidly transformed to NO_3^- by bacteria. Plants are capable of taking up any free N form from soils at each step after polymer breakdown. The proportion of inorganic to organic N in soils changes by several orders of magnitude with environmental and seasonal gradients due to changes in N cycling.

Plants acquire the bulk of their N from the soil. However, 95% of the N in soils is held within complexed organic forms, which results in a slow release of N into more dynamic N pools available to plants for assimilation (Mengel, 1996). It was traditionally accepted that the mineralization of amino acids into inorganic N forms was the rate limiting step for the production of plant available N (Tamm, 1991); however, it is now suggested that the conversion of proteins into amino acids may be the limiting reaction for certain ecosystems (Jones & Kielland, 2002).

The amount and form of nitrogen available within a soil is driving on the microbial communities of soils. Microbial communities are heavily influenced by pH, soil type and structure, moisture, disturbance, aeration, substrate availability and temperature (Mengel, 1996; Sylvia, 1998).

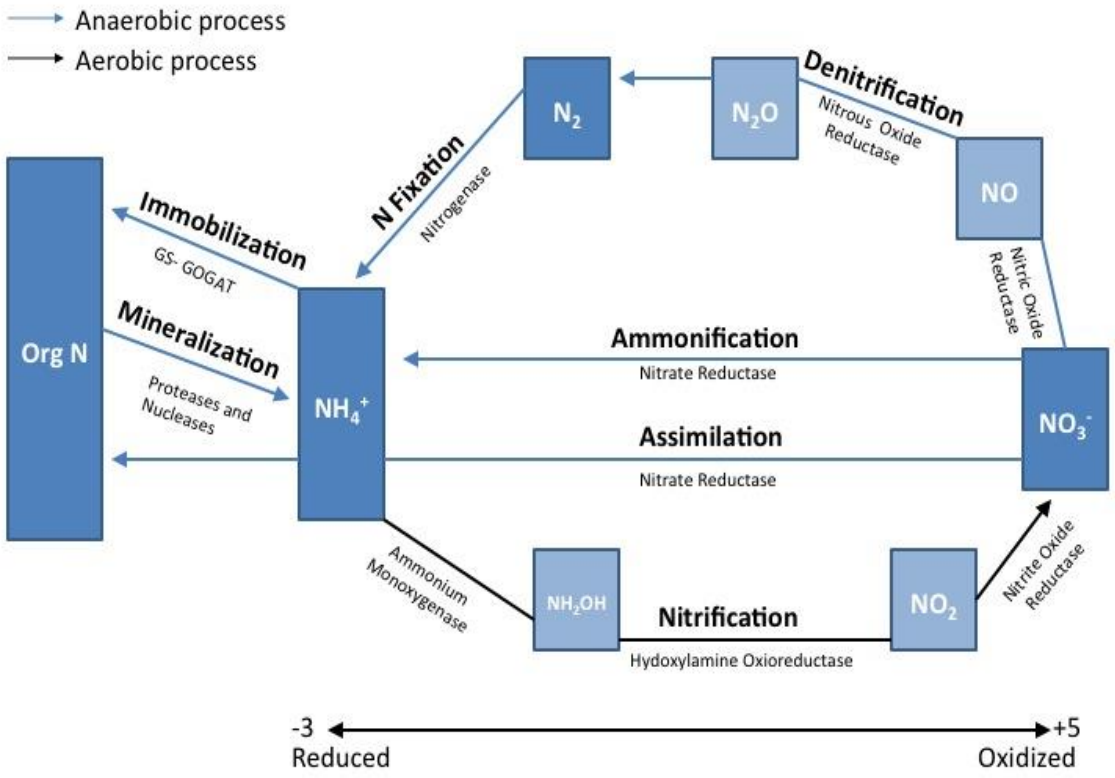

Figure 2.1. Schematic of the nitrogen (N) cycle with the oxidation states of inorganic N forms. Adapted from Knowles (1996).

2.1.3 The Effect of Temperature on Nitrogen Cycling

In many ecosystems, temperature controls N cycling in soils. Microbial activity is directly affected by temperature (Brookshire *et al.*, 2011). This is because temperature affects enzymatic reactions and soil moisture. Soil microbial activity and organic matter decomposition are negligible at temperatures less than 5 °C; however, microbial activity generally doubles for every 10 °C increase in temperature up to an optimum of approximately 40 °C (Brady and Weil, 2002).

In cold, acidic soils, most microbial processes are slowed; however, each process of the N cycle is affected to a different extent. NO_3^- dominates in soils that are disturbed (early successional soils), and this may be because these soils possess a higher pH and are warmer (Prescott, 1997; Kronzucker, 1997). Nitrifiers are chemolithotrophs, utilizing NH_4^+ to produce energy for the reduction of CO_2 to sugars, and mineralizers are heterotrophs requiring organic C for energy. Chemolithotrophs are slow growing due

to a relatively inefficient metabolism, thus nitrification usually limits the N cycle. In cool soils, mineralization is slowed, eliminating the substrate for nitrification (Warren, 2009). In general, cooler soils tend to contain more NH_4^+ than NO_3^- (Bloom, 1981; von Wiren *et al.*, 1997), and below 14 °C, soil N generally is dominated by NH_4^+ (Bloom, 1981). Kapulnik (1981) demonstrated that the process of nitrogen fixation by soil bacteria is impaired at low temperatures, decreasing the overall N availability to plants. However, the proportion of N in the form of proteinaceous materials (such as amino acids) is also thought to increase as temperatures decline (Sowden *et al*., 1977), as mineralization of amino acids into NH_4^+ and NO_3^- by microbial organisms is inhibited but depolymerization is marginally affected, causing a buildup of organic N (Atkin, 1996; Warren, 2009). Maithani *et al.* (1998), determined that the relative amount of organic N increased in soils that were held below the freezing point for part of the year, and Kranabetter *et al.* (2007) stated that the significance of organic N is thought to be considerably more than inorganic N in arctic, alpine, and boreal sites.

In warm soils, nitrification and mineralization (Miller & Cramer, 2004; Cookson *et al*., 2007; Andresen *et al.,* 2010). Thus, availability of inorganic N to plants generally increases with temperature. Higher temperatures increase ammonification and nitrification, with the optimum temperatures in a tropical soil being approximately 50 °C and 35 °C, respectively (Myers 1975). However in soils found in cool climates in Alberta, Canada, temperature optimum for nitrification was considerably lower (20-25 °C) (Mahli & McGill, 1982), and in sludge, nitrifying bacteria maximum specific growth rate was found to be optimum at 15–25 °C (Antoniou *et al*., 1990), indicating that environmental adaptations can affect the bacterial functional groups of a particular soil. Stark & Firestone (1996) found the temperature optimum for N mineralizing bacteria to be 32-36 °C, similar to Myers' (1975) results. At soil temperatures above 10 °C, microbes start to oxidize NH_4^+ ions into NO_3^-, increasing NO_3^- concentrations. However, NO_3^- is also easily lost from the soil via leaching (and also by denitrification); thus, diverse NO_3^- concentrations are seen in differing climates.

Denitrification increases with increasing soil temperature; thus, very high temperatures may lead to losses of N from forest ecosystems. Although the increase of atmospheric N_2O is also largely related to nitrification rather than denitrification

(Conrad, 1996), denitrification can also lead to increases of N_2O released. As N_2O is a powerful greenhouse gas and is effective at depleting ozone, it can cause a positive feedback loop, further increasing temperatures and consequently denitrification (Bardgett, 2005). Most heterotrophic denitrifiers require organic carbon and low oxygen environments to convert NO_3^- to atmospheric N.

Not all ecosystems show the same patterns of change in the relative availability of N forms with temperature. Hovenden *et al.* (2008) found no changes in soil NO_3^-, NH_4^+, or organic N concentrations with warming of grassland soils. D'Orangeville *et al.* (2013) also found that three years of increased soil temperature (4 °C increase) had no effect on the N status of a mature balsam fir forest. Miller *et al*. (2007) demonstrated that mineralization and ammonification can actually increase as temperatures cool and approach 0 °C. Other studies have found that the effects of temperature changes are highly reduced or almost negligible in forest soils compared to agricultural soils (Cookson *et al.,* 2007; Desureault-Rompré *et al.,* 2010). Thus, it is important to study each ecosystem of interest independently.

2.1.4 Soils of Vancouver Island, British Columbia

The forests of Vancouver Island are primarily temperate rainforests, an extremely rare ecoregion that is characterized by cool temperatures and high precipitation. Currently, the mean soil temperature during the growing season at 15 cm depth on B.C. coastal forest sites is 10 °C (Spittlehouse & Stathers, 1990).

Concentrations of N forms across different forest ecotypes vary greatly in the literature (Tables 1.1 and 1.2); and there are few typical N concentrations reported for B.C. temperate forests. A previous study has shown that the N content of soils near Jordan River, B.C. ranged from 0.2 to approximately 10 µmol- NH_4^+/g-soil, with approximately 10 times less NO_3^-, and 10 times more amino acid-N using water extractions (Metcalfe, 2005). Prescott *et al.* (2000) found that NH_4^+ and NO_3^- concentrations were 6-42 µmol/g and 21-50 µmol/g, respectively in coniferous B.C. coastal forests. Kranabetter *et al.* (2007) measured 4-17 kg/ha DON, 0.5- 5 kg/ha NH_4^+, and 0-2 kg/ha NO_3^- when testing soils from four ecosystem types.

The IPCC (2007) projects a 1.1 to 6.4 °C global surface temperature increase in 100 years. Thus, mean growing season soil temperatures on the B.C. coast in 100 years may rise as high as 16 °C. With global projected increases in temperatures, both N form availability in soils and N form uptake by forest conifers may change, affecting the competitive advantages among species and perhaps the species composition of the area.

2.1.5 Objective and Hypothesis

The objective of this study is to determine how temperature affects the relative availability of N forms in two forest soils from contrasting climatic conditions (low elevation with lower precipitation and warmer temperatures *vs*. high elevation with higher precipitation and cooler temperatures). I aim to determine how various pools of plant available N change with temperature. Understanding how the availability of B.C. forest soil-N changes with temperature will help to predict the relative performance of forest species as our climates change.

Based on previous research, I hypothesize that at warmer temperatures, the proportion of available NO_3^- will increase, whereas, at cooler temperatures, the proportion of available NH_4^+ will increase, and at very cool temperatures the relative availability of soluble organic N may increase in soils. I also predict that soil communities are adapted to the conditions of their particular ecosystem; therefore, soils from warm areas will be more responsive to warming compared to soil communities adapted to cooler areas, and cool soil communities may even show inhibition of metabolic processes at warm temperatures.

2.2 Materials and Methods

2.2.1 Soil Sites

Soils were collected from two sites at different elevations near Jordan River, B.C. on October 27th 2011 (High = high elevation site - 48^o 30.65' N, 124^o 8' W, 830 m elev., CWHvm2-03; Low = low elevation site - 48^o 29.17' N, 124^o 15.9' W, 210 m elev., CWHvm1-04). Mean annual precipitation in Jordan River ranges from 1413-3611 mm

(Environment Canada, 2011). Climate data for each site was calculated using the ClimateBC web-based program (Wang *et al*., 2006). Mean annual temperature (MAT) of the high elevation site is lower than at the low elevation site (WNADATA; Table 2.1). The mean annual precipitation (MAP) of the high elevation site is higher than at the low elevation site (WNADATA; Table 2.1). The area is comprised of ferro-humic podzolic soils with sandy-loam textures (Jungen, 1985).

Table 2.1. Calculated climate indices for the two forest sites studied: mean annual temperature (MAT; °C), mean warmest month temperature (MWMT; °C), mean coldest month temperature (MCMT; °C), mean annual precipitation (MAP; mm), mean summer precipitation (MSP; mm), annual heat:moisture index (AHM; (MAT+10)/(MAP/1000)), summer heat: moisture index (SHM; (MWMT) / (MSP/1000)), and degree days below 0 °C (DD<0) (Wang *et al*., 2006).

Site ID	MAT	MWMT	MCMT	MAP	MSP	AHM	SHM	DD<0
Low	8.7	15.1	2.9	3133	465	6	32.5	107
High	7.2	15.2	1	3682	555	4.7	27.4	202

The dominant vegetation of the high elevation site consisted of the species *Abies amabilis* (amabilis fir), *Chamaecyparis nootkatensis* (yellow cedar), *Pseudotsuga menziesii* (Douglas-fir), *Menziesia ferruginea* (false azalea), *Vaccinium alaskaense* (Alaskan blueberry), *V. parvifolium* (red huckleberry) and *Sambucus racemosa* (red elderberry). The low elevation site was dominated by *Pseudotsuga menziesii* with mosses and *Polystichum munitum* (sword fern) in the understory.

2.2.2 Nitrogen Availability

At each site, a plot of forest ground approximately 5 X 5 m^2, without obvious coarse woody debris or heavy shrub cover was selected, litter was removed, and soil cores were collected to a depth of 7.5 cm and put into pots measuring 6.5 X 6.5 X 7.5 cm^3, keeping all soil horizons intact. In total, 432 soil samples were collected, 216 from each site. To monitor N transformations in the two forest soils, we measured N availability in the pots of soil incubated in temperature-controlled units over 16 weeks. Pots of soil were incubated at 10 °C, 16 °C or 20 °C (treatments 10, 16, 20) in temperature-controlled chambers from November 2011 to February 2012 in a greenhouse at the Pacific Forestry Centre, Victoria, B.C. There were nine temperature chambers in total, three for each temperature treatment. Amounts of soluble NO_3^-N and

NH_4^+-N were determined after 1, 2, 3, 4, 6, 8, 12, and 16 weeks (eight sampling periods) of soil incubation, whereas total N was determined on weeks 1, 8, 12, and 16. Water soil extractions were done at each sampling point to determine concentrations of plant-available (soluble) NO_3^-, NH_4^+ and total N in each of the incubated soils. Soluble organic N was estimated by subtracting soluble inorganic N forms from the total soluble N value.

During incubation, soil moisture was maintained by the use of a control soil for each of the sites in each of the nine temperature chambers. These pots of soil were weighed after field collection at their initial moisture contents. The pots were then weighed two to three times a week to determine water lost. The number of pots of soils in the chamber was multiplied by the amount of water lost from control soil pots. The total amount of water was then sprayed evenly and slowly over the soils.

Soil extractions for each treatment (site and temperature combinations) were prepared from three pots in each of the temperature chambers (nine pots for each treatment) on each of the sampling weeks. To prepare soils for extraction, individual pots of soil were mixed well using a food processor. Approximately five grams of soil were added to a centrifuge tube with 25 ml ultrapure water (18.2 MΩ·cm) purified by a Milli-Q Gradient water polisher. A subsample of soil was weighed and dried to calculate percent moisture. Soil slurries were shaken for one hour and then centrifuged at 3000 rpm for 15 minutes (2740 X g). Supernatant was then transferred into a clean centrifuge tube and frozen at -20 °C until analysis. Soil water extractions were frozen until analysis, which occurred within the 16-week duration.

Colorimetric determination of NH_4^+ and NO_3^- was carried out using the Analytical Segmented Flow system and FASPac® software (Astoria-Pacific). Colorimetric NH_4^+-N determination is based on the Berthelot reaction, where the concentration of NH_4^+ is proportional to the intensity of the blue indophenol dye produced by the reaction (Mulvaney, 1996). Absorbance for NH_4^+-N was measured at 660 nm. Colorimetric determination of soil NO_3^--N is based on the Griess-Ilosvay method where NO_3^--N was first reduced to NO_2^--N for analysis (Mulvaney, 1996). N was reduced by passing the sample through a copperized cadmium column. NO_2^--N concentration was then determined colorimetrically by the intensity of the red-purple

azo-chromophore produced. Consequently, NO_3^--N plus NO_2^--N concentration was determined with this method (Mulvaney, 1996). Absorbance for NO_3^--N was measured at 540 nm.

The concentrations of N in samples was determined by running a standard curve using water standards containing NH_4^+-N/ NO_3^--N concentrations ranging from 0.1/0.01 mg-N/L-water to 500/50 mg/L on the Analytical Segmented Flow system before each analysis. In cases where a larger amount of N was in extracts, a standard of 1000/100 mg/L was run. Quality assurance standard checks were placed between every 8 samples. All samples were above the detection limit of 0.2 mg-N/L-soil for NH_4^+-N and 0.01 mg/L for NO_3^--N.

With a subsample of soil extracts, total soluble nitrogen and organic nitrogen (calculated as the difference between total soluble N and inorganic N) were determined using the method outlined in Qualls (1989). A water persulphate digest was applied to water samples to sweep the nitrogen from all N compartments into NO_3^- with the modification of adding 3:1, rather than 5:1, sample:oxidizing reagent to ensure that high N samples were also oxidized. In this method, digests were analyzed by automated colorimetry with the Analytical Segmented Flow system and FASPac®.

2.2.3 Autotrophic Nitrification Potential

With a method modified from Schmidt & Belser (1994), using aseptic techniques, we took 3 pots of soil from high and low elevation soon after collection and mixed soils using a food processor. Sixty grams of homogenized soil from each site were added to 600 ml of water. The slurries were then spiked with ammonium sulphate to a final concentration of 10 μmol $(NH_4)_2SO_4$/g soil. The soil slurries were each shaken and 60 ml was poured into 12, 150 ml jars. Jars were then closed with a lid fixed to contain a butyl rubber stopper. Jars containing soil slurries were kept on a shaker throughout the experiment to maintain aeration. One percent (v/v) or 1.5 ml of acetylene was added to half of the jars (n = 6) as a control. The addition of acetylene inhibited the nitrification process by autotrophic bacteria as acetylene binds to ammonia monooxygenase (it is a suicide substrate), inhibiting the AMO enzyme that converts

NH_4^+ to NH_2OH (Hyman & Wood, 1985; Hynes & Knowles, 1989). Acetylene is commonly used in long-term (weeks or more) studies of acidic soils. In these long-term studies, nitrification was either nearly completely or totally inhibited by acetylene in almost all soils (De Boer *et al*. 1989; Stams *et al*. 1990; Roy & Knowles, 1995; Zhu & Carreiro, 1999).

Seven milliliter aliquots of soil slurries were sampled using a syringe at 0, 0.5, 2, 4, 8, 12, and 16 weeks and transferred into 15 ml centrifuge tubes. Aliquots were centrifuged at 3000 rpm for 20 minutes (2740 X g). Supernatant was then drawn out of tubes using a 16 gauge syringe fitted with a 40 um cellulose filter. Samples were transferred into a clean centrifuge tube and frozen until analysis. To maintain a constant pressure in sample bottles, after each 7 ml aliquot was removed, 7 ml of air, or 6.5 ml of air and 0.5 ml of acetylene (controls) was added to the bottles to maintain at least a one percent acetylene concentration in the control jars. Colorimetric determination of NH_4^+-N, NO_3^--N, and total N was carried out using the same methods described previously.

2.2.4 Soil Chemistry

Soil organic content was measured by combusting 10 g (dry weight) of each soil at 375 °C for 2 hours. Ash was weighed and the difference between final and initial weight was counted as organic material. The amount of organic material was very high (80-90%), thus soil C, C:N, and organic:inorganic ratios were not quantified, as differences in measurements would likely be masked by sampling variability. The inorganic layers (10-20% of soils) from a subsample of five soils from high elevation and low elevation were sieved to determine particle size.

A sub-sample of soil extractions from the beginning and end of the study was taken to determine soil pH in soils initially and after 16 weeks, using a Fisher Scientific AR50 Accumet glass pH electrode. The same subsample of initial soils was also extracted with 2 M KCl (1:10 w/vol) to determine the amount of N bound to soils (indicates cation exchange capacity of soils). KCl is a very common extraction method for N in soils, as K^+ ions can replace NH_4^+ bound onto soil particles; however, it can overestimate the amount of plant available N in soils; thus, we chose to use water for extractions of the majority of our samples.

2.2.5 Statistical Analysis

Data were tested for normality using a Shapiro-Wilk test, which showed all data sets to be normal. For both the nitrogen availability of the soils and the potential nitrification ability, a repeated measures analysis was done with a significance value of $\alpha \leq 0.05$. The effect caused by time in our soils was determined by the tests: Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace, and Roy's Greatest Root. In the rare case that the values of these tests did not agree, significance values were taken from the tests that held the majority (*i.e.* In some cases, one test would not show significance while the other three did and vice versa. This usually occurred when significance values were very close to $\alpha = 0.05$). As all analyses contained a significant effect of N caused by time that was not the same for all treatments *(i.e.* the lines of each treatment were not parallel), an ANOVA was run for each individual week to determine the significance ($\alpha \leq 0.05$) of N form concentration. Nitrogen concentration was considered as the response variable, while temperature and site/elevation were considered fixed factors. Analyses described above were conducted using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

Data comparing pH of soils, and differences in water or KCl extraction methods were analyzed with a one-sample t test. Correlations between concentrations of N forms were also analyzed for the nitrogen availability experiment. For correlations, total soluble N, NH_4^+, and organic soluble N concentrations were transformed to linear data by taking the square root of values as they showed an exponential relationship with time. Again a significance value of $\alpha \leq 0.05$ was used, and correlations were considered strong if $R \geq 0.6$ (*i.e.* $R^2 \geq 0.35$). Analyses described above were conducted using R version 2.15.3 (R Core Team, 2012).

2.3 Results

2.3.1 Site Characteristics

(a) Soil Properties

Soil organic matter ranged from 80 to 90%. Parent material from both sites contained mainly (85-100%) sand (0.5-2mm). Occasionally some gravel (<5 mm) was found. pH of high and low elevation soils was not significantly different at the time of collection (df=4, p=0.073) mean ± standard error (5.30 ± 0.06 and 5.2 ± 0.06 respectively). There was a significant difference in pH between initial (5.20 ± 0.06) and final (4.70 ± 0.06) samples in the low elevation soil (df=4, p<0.001), but not between initial (5.30 ± 0.06) and final (5.24±0.09) samples in the high elevation soil (df=4, p=0.729) (Figure 2.2).

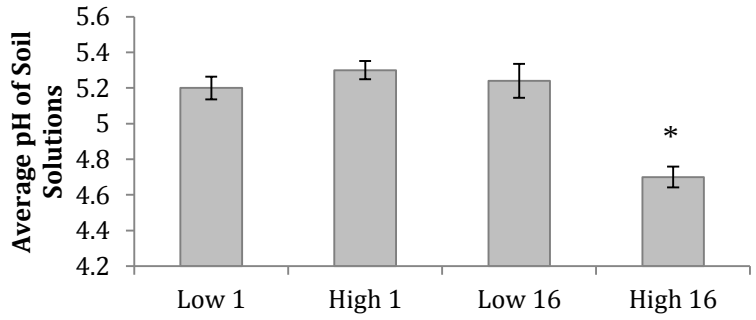

Figure 2.2. Average pH of soil solutions initially and after 16 weeks of incubation. pH was tested on five soils from a high elevation and a low elevation at time of incubation and after 16 weeks. Error bars represent standard error. Asterisk (*) indicates a significantly different mean ($\alpha \leq 0.05$).

(b) Comparison of KCl Extraction with Water

The amount of NH_4^+ in soil extractions with KCl increased significantly by 99.8% in low elevation soils compared to extractions with water (df=2, p= 0.038), but only by 55.7% in high elevation soils (df=2, p=0.046; Figure 2.3). There was no difference in NO_3^- concentrations when extractions were done with KCl or water in either the high (df=2, p=0.111) or low (df=2, p=0.167) elevation soil (Figure 2.3).

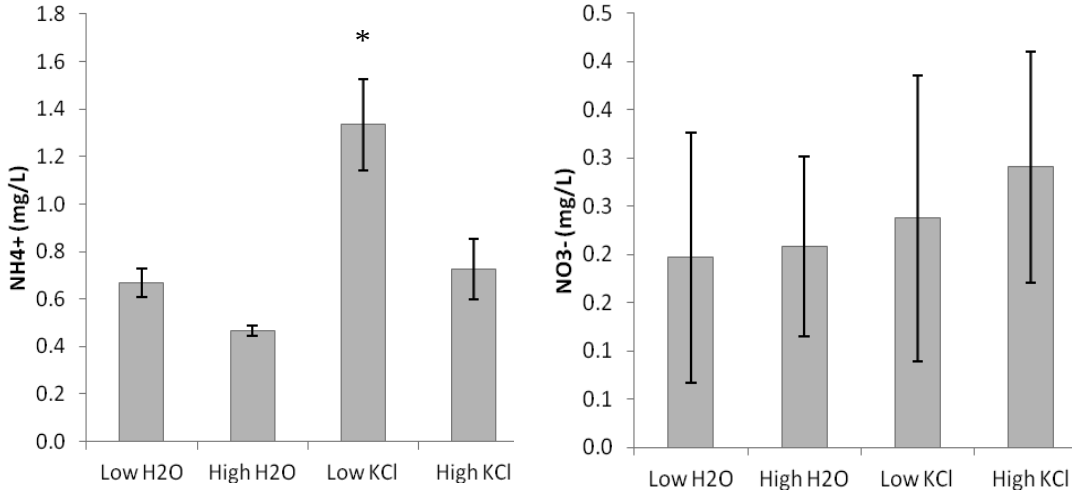

Figure 2.3. Average ammonium and nitrate concentrations (mg/L) in initial soils. Soils were collected from two sites, one at a high elevation and one at a low elevation. Extractions using water or KCl were done on three soil samples from each site. Error bars represent standard error. Asterisk (*) indicates a significantly different mean ($\alpha \leq 0.05$).

2.3.2 The Effect of Temperature on Nitrogen Availability

The concentration of all forms of N changed significantly with time (Table 2.2). Furthermore, the slopes of the treatment lines were significantly different when comparing elevations and temperature treatments; thus, there were significant interactions between elevation and temperature (Table 2.2).

Table 2.2. Results of repeated measures analysis of nitrogen availability. Degrees of freedom (df), F-values (F), and significance values at $\alpha \leq 0.05$ (p) are given for concentration (mg/L) of total nitrogen (Tot N), ammonium (NH_4^+-N), nitrate (NO_3^--N), and organic N (Org N) in solutions sampled from soils collected from two sites (high and low) and incubated at three temperatures (10 °C, 16 °C and 20 °C) over 16 weeks.

Nitrogen	Time			Elevation			Temperature			El * Temp		
Form	df	F	P	df	F	p	df	F	p	df	F	p
Tot N	2	54.07	<0.0001	2	6.91	0.0114	4	10.70	<0.0001	4	3.96	0.0144
NH_4^+-N	7	18.42	<0.0001	7	6.30	<0.0001	14	3.41	<0.0001	14	2.33	0.0415
NO_3^--N	7	3.39	0.0058	7	3.33	0.0065	14	2.21	0.0138	14	2.23	0.0126
Org N	2	17.54	0.0004	2	6.07	0.0167	4	4.86	0.0058	4	2.77	0.0499

(a) Total soluble N

Trend

Initial total soluble N concentrations of soils were (mean ± standard error) 10.08 ± 1.79 mg/L and 10.74 ± 1.97 mg/L for the low and high elevation sites, respectively (Figure 2.4), which was not significantly different (df=5, p= 0.639). After 8 weeks, soil N concentrations had differentiated between temperature treatments, and after the full 16-week experimental period, Low 20 soils contained the most soluble N followed by Low 16, High 16, High 20, Low 10, and finally High 10 (Figure 2.4).

Significance

There was a significant difference in total soluble soil N concentrations between the two sites and between temperatures at all sampling dates; however, there was also a significant interaction between temperature and elevation/site (Table 2.3; Figure 2.4). At week 8 and 12, the Low 20 soils contained significantly more total soluble N than the Low 10 treatment ($p<0.05$; Figure 2.4), but the temperature effect was not significant for the high elevation soil. By week 16, both the high and low elevation soils contained significantly more total soluble N when kept at 16 °C and 20 °C than when kept at 10 °C ($p<0.05$; Figure 2.4), but the 16 °C and 20 °C treatments were not significantly different from each other. During digestion, high N samples (over 200 mg/L) were only 80% oxidized. Thus, high average soluble N and organic N values may be underestimated.

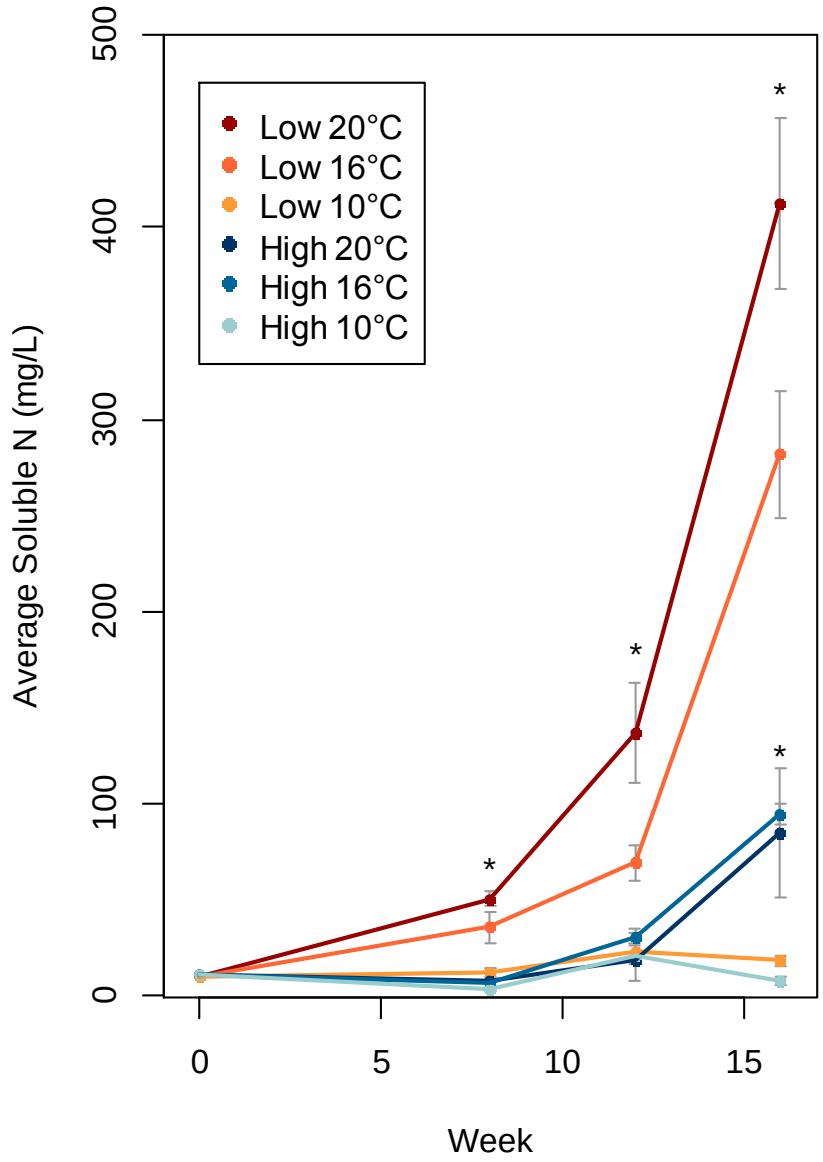

Figure 2.4. Average total soluble nitrogen concentrations (mg/L) in incubated soils. Soils were collected from high or low elevation and incubated at: 20 °C, 16 °C, or 10 °C for four months. Soil extractions were done using water. Error bars represent standard error of the mean, n=9. Asterisk (*) indicates a significant interaction between elevation and temperature, and a significant difference in one or both soils from differing elevations ($\alpha \leq 0.05$).

Table 2.3. P-values ($\alpha \leq 0.05$) for ANOVA of total soluble N concentrations (mg/L) in incubated soils on three sampling dates over 16 weeks with elevation and temperature as fixed factors. Degrees of freedom (df), F-values (F), and significance values (p) are given for concentration (mg/L) of total soluble N sampled in soils collected from two sites (high and low) and incubated at three temperatures (10 °C, 16 °C and 20 °C) over 16 weeks.

Sampling	Elevation			Temperature			El * Temp		
Week	df	F	p	df	F	P	df	F	p
8	1	23.11	<0.0001	2	5.33	0.0081	2	3.2	0.0495
12	1	17.67	0.0001	2	6.45	0.0033	2	7.24	0.0018
16	1	54.1	<0.0001	2	35.23	<0.0001	2	14.86	<0.0001

(b) Ammonium

Trend

Initial NH_4^+-N of soils was significantly different (df=5, p=0.036) being 0.42 ± 0.06 mg/L and 0.59 ± 0.04 mg/L for the low and high elevation sites, respectively (Figure 2.5). After 6 weeks incubation, soil concentrations started to differentiate between treatments, and after the full 16-week experimental period, Low 20 soils contained the most soluble NH_4^+-N followed by Low 16, High 16, High 20, Low 10, and finally High 10 (Figure 2.5). This is the same trend shown in the total soluble N of the samples (Figure 2.4).

Significance

There was a significant difference between sites/elevations at all sampling periods and a significant interaction between elevation and temperature at weeks 8, 12 and 16 (Table 2.4; Figure 2.5). At weeks 8 and 12, the High 16 treatment contained significantly more NH_4^+-N than the High 10 ($p<0.05$; Figure 2.5). At week 12, the Low 20 and Low 16 treatments contained significantly more NH_4^+-N than the Low 10 treatment ($p<0.05$; Figure 2.5). By week 16, both the high and low elevation soils in the 20 °C and 16 °C treatments contained more NH_4^+ than the 10 °C treatment ($p<0.05$; Figure 2.5), but were not significantly different from each other.

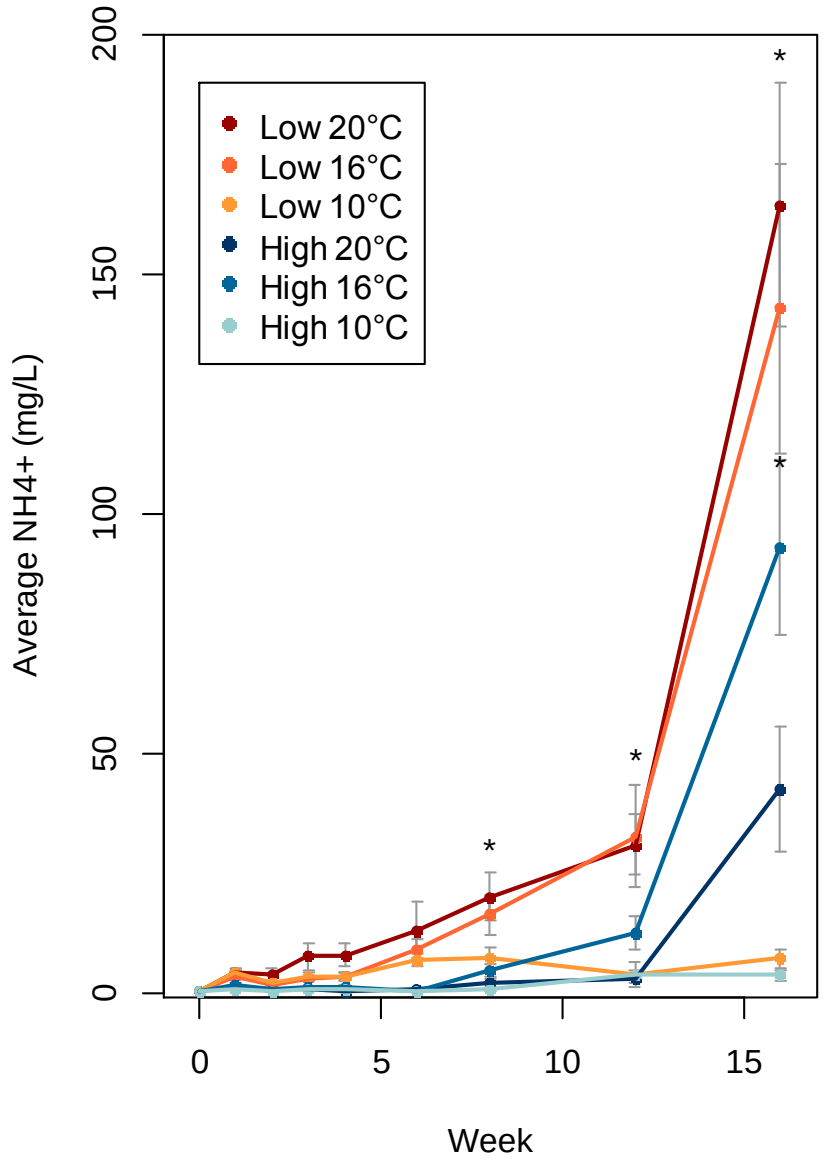

Figure 2.5. Average ammonium-N (NH_4^+) concentrations (mg/L) in incubated soils. Soils were collected from high or low elevation and incubated at 20 °C, 16 °C or 10 °C for four months. Soil extractions were done using water. Error bars represent standard error of the mean, n=9. Asterisk (*) indicates a significant interaction between temperature and elevation, and a significant difference in nitrogen concentration caused by temperature in one or both soils from differing elevations ($\alpha \leq 0.05$). Elevation caused a significant difference in nitrogen concentrations at all sampling times.

Table 2.4. P-values ($\alpha \leq 0.05$) for ANOVA of NH_4^+-N concentrations (mg/L) in incubated soils on eight sampling dates over 16 weeks with elevation as a fixed factor. Degrees of freedom (df), F-values (F), and significance values (p) are given for concentration of NH_4^+-N sampled in soils collected from two sites (high and low) and incubated at three temperatures (10 °C, 16 °C and 20 °C) over 16 weeks.

Sampling	Elevation			Temperature			El * Temp		
Week	df	F	P	df	F	p	df	F	p
1	1	63.89	<0.0001	2	0.43	0.6536	2	2.07	0.1375
2	1	19.93	<0.0001	2	2.26	0.1149	2	1.75	0.1845
3	1	14.95	0.0003	2	2.15	0.1280	2	1.96	0.1518
4	1	21.56	<0.0001	2	1.95	0.1531	2	3.40	0.0415
6	1	18.60	<0.0001	2	0.77	0.4709	2	0.65	0.5245
8	1	26.24	<0.0001	2	3.95	0.0257	2	2.42	0.0097
12	1	13.26	0.0007	2	6.52	0.0031	2	3.61	0.0346
16	1	14.82	0.0003	2	21.67	<0.0001	2	5.15	0.0094

(c) Nitrate

Trend

Initial NO_3^--N concentrations of soils were 0.16 ± 0.04 mg/L and 0.13 ± 0.06 mg/L for the low and high elevation sites, respectively (Figure 2.6), which was not significantly different (df=5, p=0.358). After 4 weeks, soil NO_3^--N concentrations started to differentiate between temperature treatments and at 4 to 6 weeks, low elevation soils kept at 10 °C contained similar NO_3^--N concentrations as the other low elevation soils (range: 0.19 ±0.08 mg/L to 3.53 ±2.00 mg/L); however, the amount of NO_3^--N in this treatment dropped after the eighth week (Figure 2.6). This slight increase in NO_3^- in low elevation soils kept at 10 °C during weeks 4-6, is mirrored in NH_4^+ concentrations (Figure 2.5). Nitrate-N concentrations in high elevation soils remained similar to initial levels at all incubation temperatures.

Significance

There was a significant difference in NO_3^--N concentration between sites at all sampling dates (Table 2.5; Figure 2.6), and low elevation soils consistently had greater NO_3^--N concentrations than high elevation soils, on average. There were no interactions between elevation and temperature for analyses of NO_3^--N concentration, except at week 2. After the 8 week experimental period, temperature had a significant effect on soil

NO_3^--N concentrations (df=2, F=4.20, p=0.0136). After 16 weeks, low elevation soils in the 20 °C and 16 °C treatments contained more NO_3^--N than in the 10 °C treatment ($p<0.05$; Figure 2.6). On other dates, all treatments contained approximately the same NO_3^--N concentrations as at the initial sampling (Figure 2.6).

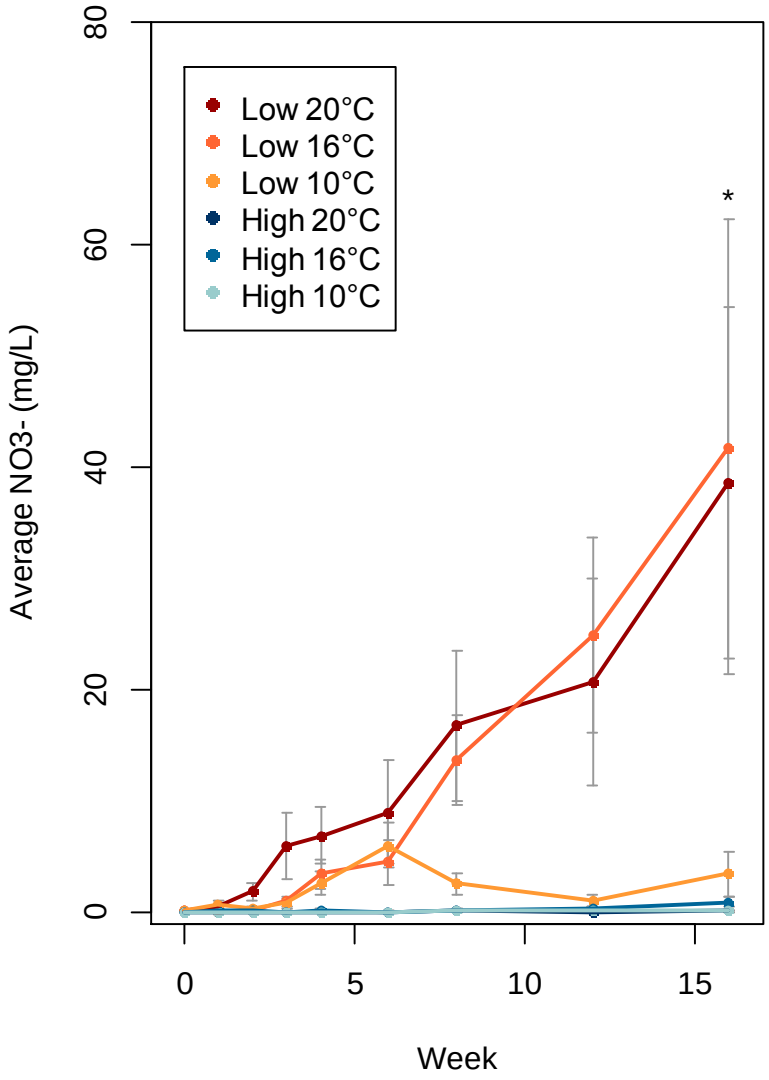

Figure 2.6. Average soluble nitrate-N (NO_3^-) concentrations (mg/L) in incubated soils. Soils were collected from high or low elevation and incubated at 20 °C, 16 °C or 10 °C for four months. Soil extractions were done using water. Error bars represent standard error of the mean, n=9. Asterisk (*) indicates a significant interaction between temperature and elevation, and a significant difference in nitrogen concentration caused by temperature in the low elevation soil ($\alpha \leq 0.05$). Elevation caused a significant difference in nitrogen concentrations at all sampling times.

Table 2.5. P-values ($\alpha \leq 0.05$) for ANOVA of NO_3^--N concentrations (mg/L) in incubated soils on eight sampling dates over 16 weeks with elevation as a fixed factor. Degrees of freedom (df), F-values (F), and significance values (p) are given for concentration of NO_3^--N sampled in soils collected from two sites (high and low) and incubated at three temperatures (10 °C, 16 °C and 20 °C) over 16 weeks.

Sampling	Elevation			Temperature			El * Temp		
Week	df	F	p	df	F	p	df	F	p
1	1	5.76	0.0203	2	1.5	0.2446	2	2.2	0.1239
2	1	7.13	0.0103	2	3.4	0.0416	2	4.1	0.0222
3	1	6.57	0.0136	2	2.9	0.0657	2	2.8	0.0719
4	1	17.1	0.0001	2	1.6	0.2114	2	1.7	0.1936
6	1	12.8	0.0008	2	0.5	0.588	2	0.5	0.6108
8	1	16.8	0.0002	2	2.7	0.0754	2	2.6	0.0822
12	1	12.9	0.0008	2	3.0	0.0607	2	2.9	0.0625
16	1	10.2	0.0025	2	2.1	0.136	2	2.0	0.1491

(d) Organic N

Trend

Initial soluble organic N concentrations of soils were 3.07 ±0.11 mg/L and 3.15 ±0.13 mg/L for the low and high elevation sites, respectively (Figure 2.7), which was not significantly different (df=5, p= 0.714). Between 8 and 12 weeks, soil concentrations started to differentiate between treatments and after the full 16 week experimental period, low elevation soils kept at 20 °C and 16 °C contained the most organic N (160.98 ±53.66 mg/L and 148.83 ±49.61 mg/L, respectively) while all other treatments contained approximately the same concentrations as initial soils (Figure 2.7).

Significance

There was a significant difference in soil organic N concentration between sites and temperatures at all sampling periods (Table 2.6; Figure 2.7). Low elevation soils consistently had greater organic N concentrations than high elevation soils. At weeks 8 and 12, the 20 °C treatment contained significantly more organic N than the 16 °C and 10 °C treatments in the low elevation soil ($p<0.05$; Figure 2.7). There were no interactions between elevation and temperature for organic N at weeks 12 and 16. At

week 16, high elevation soils contained significantly more organic N when kept at 20 °C than when kept at 16 °C or 10 °C ($p<0.05$; Figure 2.7).

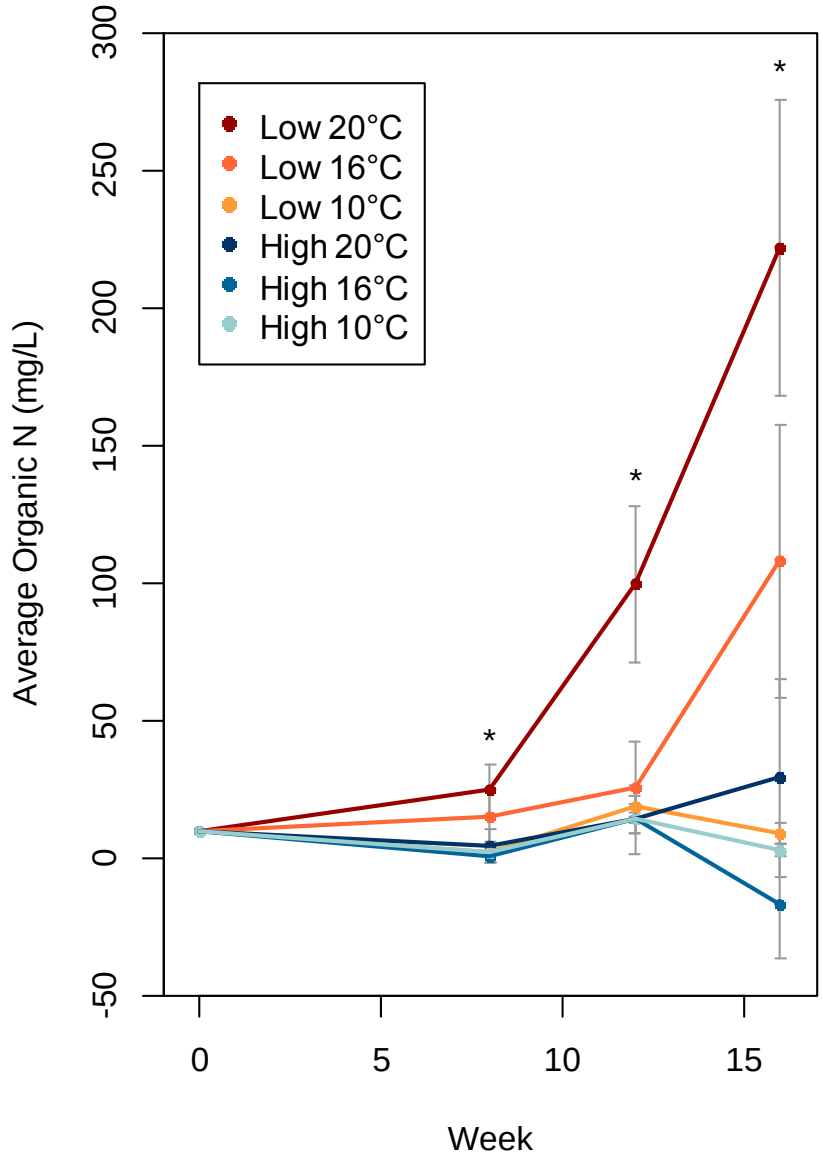

Figure 2.7. Average organic nitrogen concentrations (mg/L) in incubated soils. Soils were collected from either high or low elevation and incubated at either: 20 °C, 16 °C, or 10 °C for four months. Soil extractions were done using water. Error bars represent standard error of the mean, n=9. Asterisk (*) indicates a significant interaction between N and elevation, and a significant difference in nitrogen concentration caused by temperature in the one or both soils ($\alpha \leq 0.05$). Elevation caused a significant difference in nitrogen concentrations at all sampling times.

Table 2.6. P-values ($\alpha \leq 0.05$) for ANOVA on organic N concentrations (mg/L) in incubated soils on three sampling dates over 16 weeks with elevation and temperature as fixed factors. Degrees of freedom (df), F-values (F), and significance values (p) are given for concentration (mg/L) of organic N sampled in soils collected from two sites (high and low) and incubated at three temperatures (10 °C, 16 °C and 20 °C) over 16 weeks.

Sampling	Elevation			Temperature			El * Temp		
Week	df	F	p	df	F	p	df	F	p
8	1	3.69	0.0501	2	5.55	0.0068	2	2.36	0.1366
12	1	7.08	0.0105	2	7.71	0.0012	2	3.8	0.0516
16	1	59.9	<0.0001	2	37.8	<0.0001	2	3.22	0.0757

Often the increases in soluble N forms over time were correlated to other N forms when treatments were separated (Table 2.7). In particular, NH_4^+ was the most correlated to total soluble N as all treatments showed high R and low P values except in the Low10 treatment.

Table 2.7. P-values ($\alpha \leq 0.05$) and R-values ($R \geq 0.6$) for correlations between concentration (mg/L) of N forms in soils (df=31). P and R-values are given for correlations between all combinations of total soluble N, NH_4^+, NO_3^-, and organic soluble N in soils collected from two sites (high and low) and incubated at three temperatures (10 °C, 16 °C and 20 °C) over 16 weeks. Strong correlation values (R=0.6 or R^2=0.35) are in bold, and non-significant correlations (α>0.05) are crossed out.

		NH_4^+		NO_3^-		Organic N	
	Treatment	p value	R value	p value	R value	p value	R value
Total N	**Low 10**	0.010	0.44	~~0.266~~	~~0.20~~	0.005	0.47
	Low 16	**<0.001**	**0.94**	**<0.001**	**0.71**	**<0.001**	**0.81**
	Low 20	**<0.001**	**0.95**	0.002	0.52	**<0.001**	**0.95**
	High 10	**<0.001**	**0.69**	**<0.001**	**0.64**	**<0.001**	**0.95**
	High 16	**<0.001**	**0.92**	**<0.001**	**0.61**	~~0.255~~	~~0.20~~
	High 20	**<0.001**	**0.94**	0.023	0.39	**<0.001**	**0.97**
NH_4^+	**Low 10**			**<0.001**	**0.67**	0.002	-0.312
	Low 16			**<0.001**	**0.68**	**<0.001**	**0.61**
	Low 20			<0.001	0.41	**<0.001**	**0.86**
	High 10			**<0.001**	**0.74**	0.011	0.43
	High 16			**<0.001**	**0.61**	~~0.457~~	~~-0.13~~
	High 20			0.009	0.45	**<0.001**	**0.84**
NO_3^-	**Low 10**					<0.001	-0.36
	Low 16					0.04	0.36
	Low 20					0.033	0.37
	High 10					0.005	0.47
	High 16					~~0.339~~	~~-0.17~~
	High 20					0.048	0.35

The ratio of N-form concentration on a given sampling date, relative to the N form found at the lowest concentration (NO_3^-), showed the proportion of NH_4^+ to increase with temperature in both high and low elevation soils (Table 2.8). The predominance of NH_4^+ was more pronounced in the high elevation soil. The relative amount of organic N showed no clear trend with temperature for the high elevation soil (Table 2.8). However, the relative amount of organic N dropped substantially, compared to initial values, at all incubation temperatures in the low elevation soils (Table 2.8).

Table 2.8. Ratios of N forms (NH_4^+: NO_3^-: Organic N) in the high and low elevation soils. Ratios are shown for the initial soil collection, and after incubation at 10 °C, 16 °C or 20 °C for 16 weeks.

		NH_4^+		NO_3^-		Org N
Low	Initial	5	:	1	:	19
	10 °C	15	:	1	:	15
	16 °C	102	:	1	:	0
	20 °C	210	:	1	:	145
High	Initial	2	:	1	:	32
	10 °C	2	:	1	:	3
	16 °C	3	:	1	:	4
	20 °C	4	:	1	:	4

2.3.3 Potential Autotrophic Nitrification Ability

(a) Total soluble N

Total soluble N in soil slurries increased significantly with time (df=7, F=629.51, p=0.0016; Figure 2.8) and although not significant, total soluble N was observably higher in low than high elevation soil slurries. However, there were no differences in slopes when comparing acetylene treatments, and elevation treatments (df=7, F=2.62 and 15.06, respectively; p=0.3041 and 0.0637, respectively; Figure 2.8). There was no interaction between acetylene levels and elevation (df=7, F= 0.43, p=0.8335).

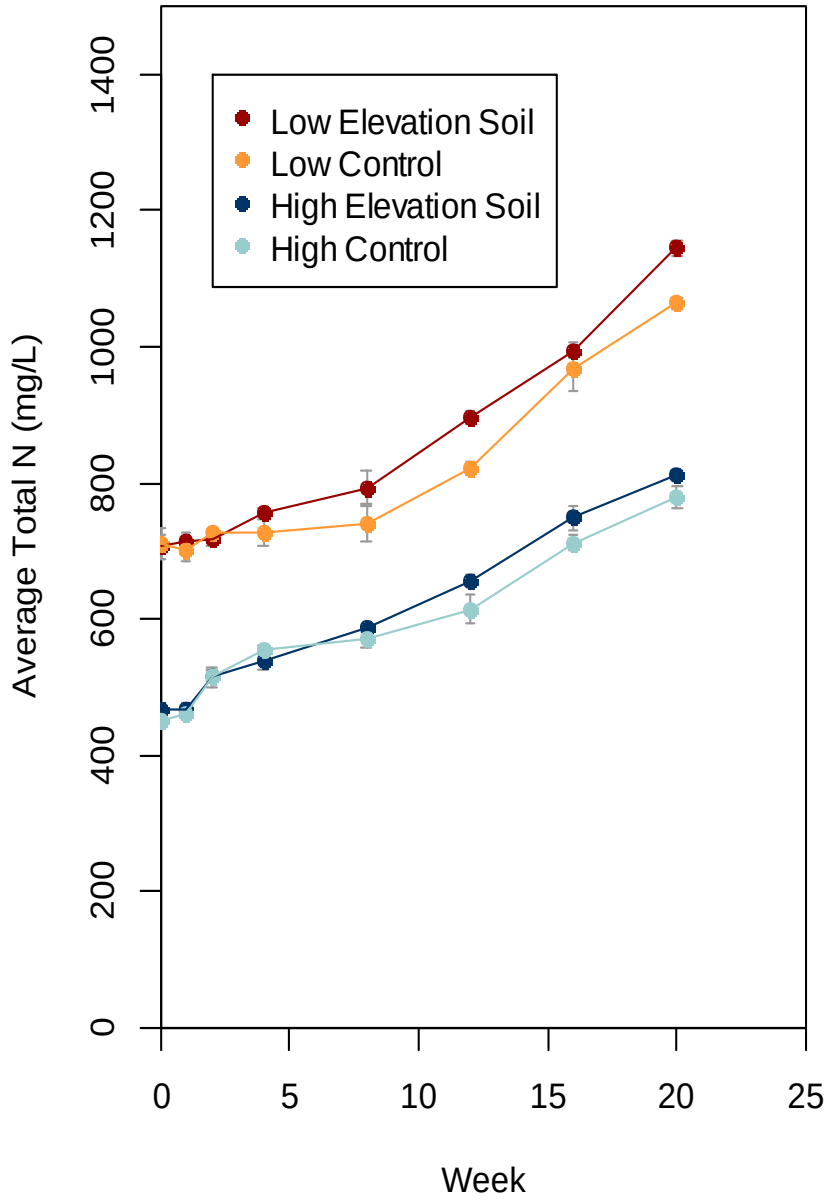

Figure 2.8. Average total soluble nitrogen concentrations (mg/L) in spiked soil slurries. Soils for slurries were collected from either high or low elevation and spiked with ammonium sulphate to a final concentration of 10μmol $(NH_4)_2SO_4$/g soil. Control soils had 1% acetylene added to inhibit nitrification. Slurries were then incubated for 20 weeks. Error bars represent standard error of the mean, n=3.

(b) Ammonium

Ammonium in soil slurries did not change significantly with time (df=7, F=10.36, p=0.0909; Figure 2.9) but was observably higher in slurries from low than high elevation soils. There were no significant differences in slopes when comparing

acetylene levels and elevation treatments (df=7, F=0.95 and 1.81, respectively; p=0.6004 and 0.4020, respectively; Figure 2.9). There was no interaction between acetylene levels and elevation (df=7, F=1.25, p=0.5127).

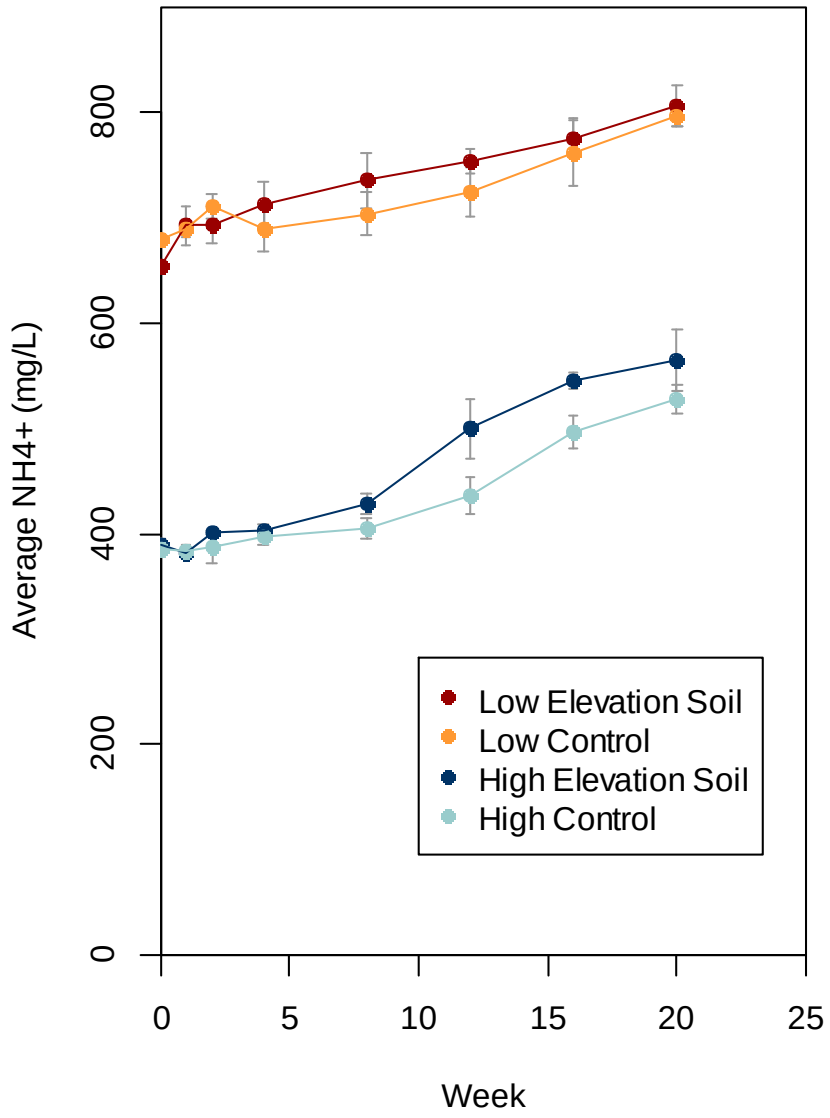

Figure 2.9. Average nitrogen concentrations (mg/L) for ammonium (NH_4^+) in spiked soil slurries. Soils for slurries were collected from either high or low elevation and spiked with ammonium sulphate to a final concentration of 10µmol $(NH_4)_2SO_4$/g soil. Control soils had 1% acetylene added to inhibit nitrification. Slurries were then incubated for 20 weeks. Error bars represent standard error of the mean, n=3.

(C) Nitrate

Nitrate in the low elevation soil slurries increased significantly with time (df=7, F=64864, p<0.0001; Figure 2.10). A significant difference existed between slopes of both acetylene levels and elevations (df=7, F=65710 and 65934, respectively, p<0.0001 for both; Table 2.9, Table 2.10, and Figure 2.10). There was a significant interaction between acetylene levels and elevation (Table 2.9). The significant interaction was caused by the dramatic increase in NO_3^- -N in the low elevation soil slurry from the second week until the end of the experiment. In all other treatments, there was little change in NO_3^- -N over time.

Table 2.9. P-values ($\alpha \leq 0.05$) for ANOVA of nitrate (NO_3^-) concentrations in spiked soil slurries with elevation and acetylene levels as fixed factors. Soil samples were incubated over 20 weeks.

Sampling	Elevation			Acetylene			Elevation*Acetylene		
Week	df	F	p	df	F	p	df	F	p
0	1	105.31	<0.0001	2	38.48	0.0003	2	35.17	0.0003
1	1	25.15	0.0010	2	18.63	0.0025	2	7.37	0.0264
2	1	119.37	<0.0001	2	41.68	0.0002	2	62.62	<0.0001
4	1	289.70	<0.0001	2	209.45	<0.0001	2	229.61	<0.0001
8	1	650.11	<0.0001	2	587.81	<0.0001	2	590.61	<0.0001
12	1	272.94	<0.0001	2	251.18	<0.0001	2	249.47	<0.0001
16	1	11169	<0.0001	2	10361.6	<0.0001	2	10367.3	<0.0001
20	1	433.69	<0.0001	2	420.95	<0.0001	2	420.95	<0.0001

Table 2.10. P-values ($\alpha \leq 0.05$) for ANOVA of nitrate (NO_3^-) concentrations in spiked soil slurries from a low elevation soil with acetylene level as a fixed factor. Soil samples were incubated over 20 weeks.

Sampling	Acetylene (Low El Soil)		
Week	df	F	p
2	1	62.62	<0.0001
4	1	229.61	<0.0001
8	1	590.61	<0.0001
12	1	249.47	<0.0001
16	1	10337	<0.0001
20	1	420.95	<0.0001

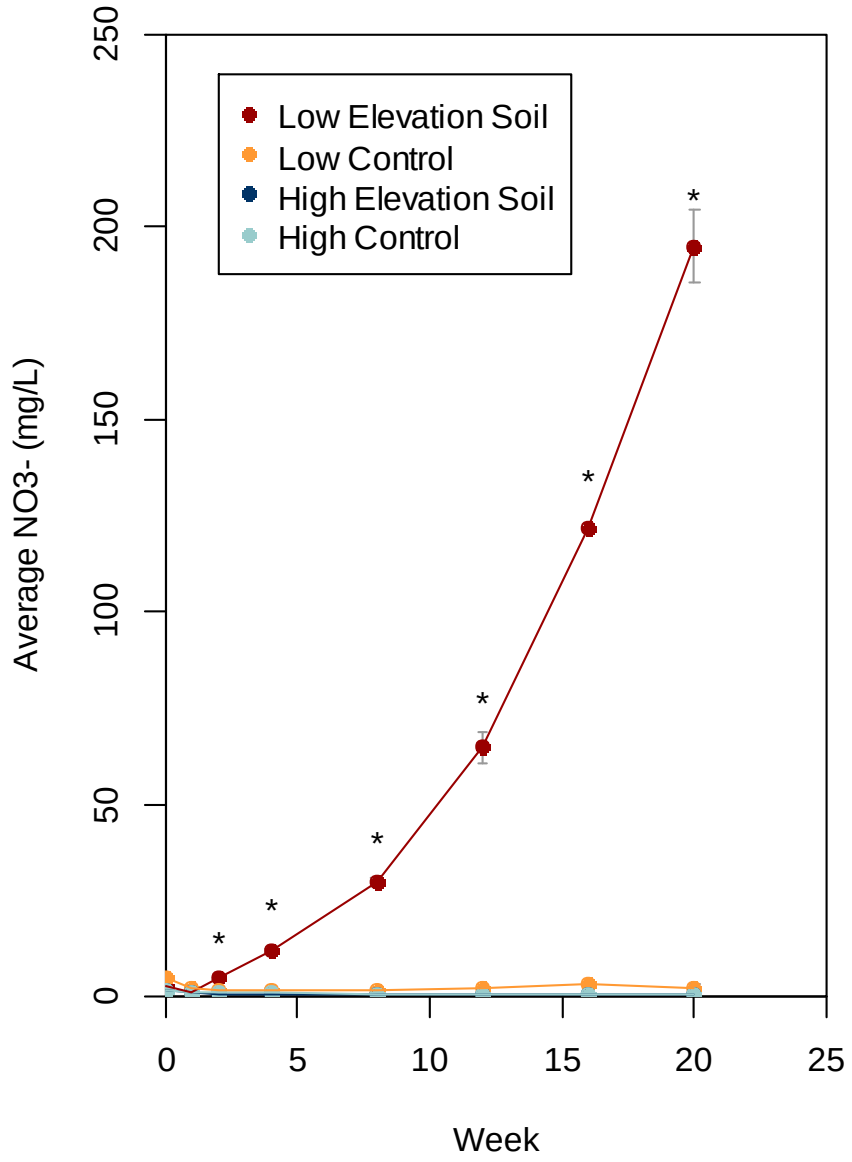

Figure 2.10. Average nitrogen concentrations (mg/L) for nitrate (NO_3^-) in spiked soil slurries. Soils for slurries were collected from either high or low elevation and spiked with ammonium sulphate to a final concentration of 10µmol $(NH_4)_2SO_4$/g soil. Control soils had 1% acetylene added to inhibit nitrification. Slurries were then incubated for 20 weeks. Error bars represent standard error of the mean, n=3. Asterisk (*) indicates a significant difference in nitrogen concentration in low elevation soils caused by the addition of acetylene ($\alpha \leq 0.05$).

(D) Organic N

Soluble organic N in soils significantly increased with time (df=7, F=26.00, p=0.0375; Figure 2.11). However, there were no significant differences between acetylene levels or elevations/sites (df=7, F=6.98 and 7.84 respectively, p=0.1310 and 0.1177 respectively; Figure 2.11). There was also no interaction between acetylene levels and elevation (df=7, F=0.48, p=0.8059).

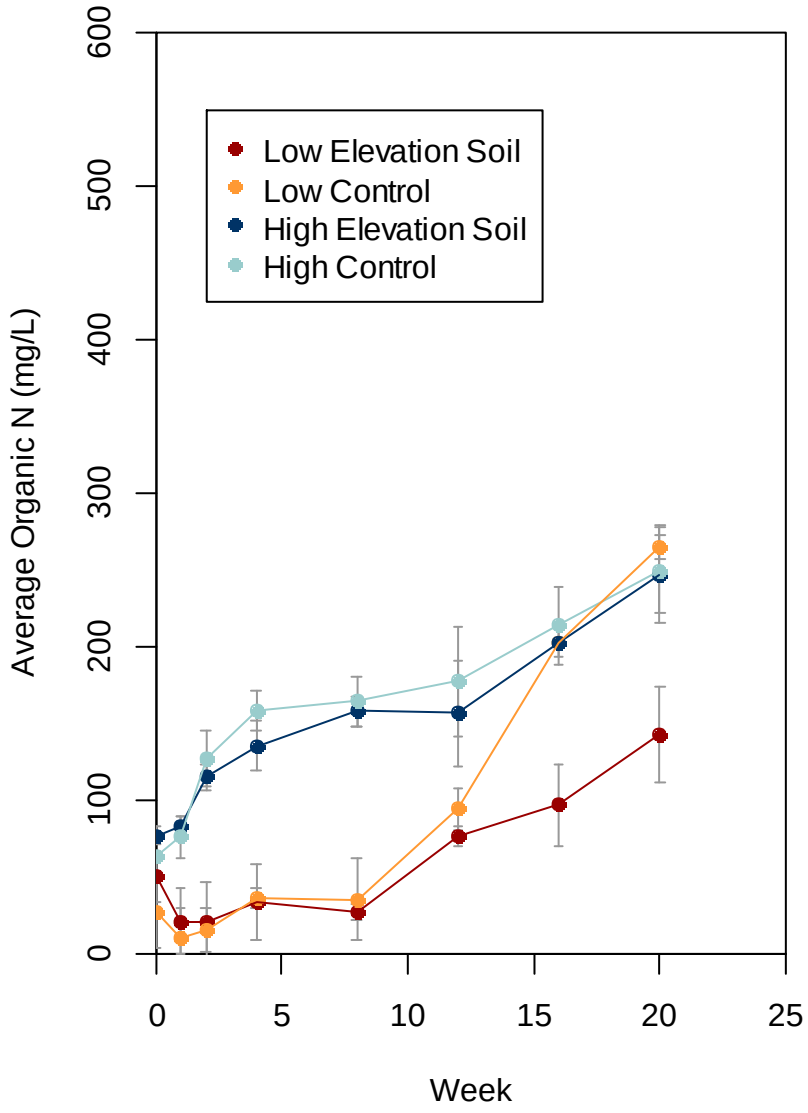

Figure 2.11. Average nitrogen concentrations (mg/L) for soluble organic N in spiked soil slurries. Soils for slurries were collected from either high or low elevation and spiked with ammonium sulphate to a final concentration of 10µmol $(NH_4)_2SO_4$/g soil. Control soils had 1% acetylene added to inhibit nitrification. Slurries were then incubated for 20 weeks. Error bars represent standard error of the mean, n=3.

2.4 Discussion

My objective was to determine if and how N cycling in two forest soils is affected by temperature. I chose two soils with similar characteristics, located near each other, but subject to different climates. Based on the forms of N in study soils, I saw differences between N concentrations initially and after incubation. However, the two soils differed in their response to temperature. The forms of N in soils are a result of many interacting pathways of the N cycle. As processes can either increase or decrease the amount of N in any one pool simultaneously, what I saw in this study is only a snapshot of a dynamic cycle; and the processes of decomposition, mineralization, and nitrification described below refer to the net process.

2.4.1 Changes to N Pools

The amount of N forms in the two soils was affected by temperature. When the soils from two sites at Jordan River were incubated at warmer temperatures, I saw an increase in most soluble N forms in the soils. The N forms did not reach a state of equilibrium throughout this study soils, however, the response of total soluble N, NH_4^+, NO_3^- and soluble organic N was generally greater in soils collected from the low elevation site, which has a warmer climate (+1.1 °C) with less precipitation (-155 mm/yr). This discrepancy in response indicates that there are differences in soil bacterial functional groups at my two sites. I hypothesized that raising soil temperatures would increase the proportion of available NO_3^- relative to NH_4^+; however, I did not see this trend, as the warmer incubation temperatures resulted in a relatively larger increase in NH_4^+ than NO_3^- (although the overall availability of both N-forms increased). My results contrast with those of other studies that found no change in the NH_4^+:NO_3^- ratio, or an increase in NO_3^- with warming (Hovenden *et al.*, 2008; Butler *et al.* 2012; D'Orangeville *et al*., 2013). I also expected that the relative availability of soluble organic N would increase in soils kept at the coolest temperatures due to a lack of N mineralization. There was no evidence of this trend within my 16-week sampling period (Figure 2.7), indicating that my coolest temperature treatment was not cool enough to

reduce mineralization. Thus, soluble inorganic N accumulated in soils at all temperatures.

The application of this study was to understand how N form availability to plants might change with increases in soil temperature. Although I did not directly study the effect of temperature on the specific processes (mineralization, decomposition, nitrification etc.) of the N cycle in my two soils, and I did not determine which bacterial species were causing the changes to N forms, I can make inferences on how various processes of the N cycling changed (and why) as I increased soil temperature. Initial organic content (non-soluble), particle size, and pH values were similar between soils (Figure 2.2) and both soils were maintained at about 60% moisture; thus, any differences in N cycling between soils are unlikely to be due to differences in soil physical, chemical, or biotic characteristics.

2.4.2 Possible Changes to N Cycle Processes

(a) Total N

Overall Effect of Temperature

There was an increase in total soluble N in both soils of study at 16 and 20 °C (Figure 2.4). The increase in total soluble N can be due to a number of processes. The soils included in this study contained a large portion of organic matter, and organic matter decomposition is highly sensitive to increasing temperature, as it follows Michaelis–Menten kinetics (Davidson & Janssens, 2006); thus, this is a likely cause of the increase in total soluble N in study soils with temperature. Total soluble N of soils was most highly correlated with NH_4^+ and organic N, further indicating that the breakdown of organic N is likely the cause of total soluble N increases (Table 2.7).

Other studies have also seen an increase in total N with warming. In agricultural soils, higher temperatures cause decreases to soil organic matter, as heat leads to more rapid and complete organic matter decomposition to soluble products (Haas *et al.*, 1957). In a more recent study, Butler *et al.* (2012) documented that in forest soils

warmed 5 °C in vivo for 7 years there was a 34% average annual increase in total soluble nitrogen, which was sustained over the entire period of the study.

Nitrogen fixation takes atmospheric N and adds it to the soluble N pool in soils. This process usually occurs in symbiotic organisms with plant roots or in free-living soil bacteria; however, nitrogen fixation can also occur in the rhizoids of mosses (Bay *et al.*, 2013) or in the bark of lodgepole pine (Bent and Chanway, 1998) However, N fixation requires a lot of energy and C, and is dependent on the availability of N in soils (the presence of NH_4^+ will inhibit the transcription of nitrogenase and all other nitrogen fixation (nif) genes); thus, as NH_4^+ of soils increases, N fixation will stop (Brill, 1980; Sylvia *et al*., 1999).

A lack of denitrification can also increase the total N of a system. However, as denitrification should increase with temperature, the increased total soluble N is likely attributable to increased N decomposition/depolymerization; converting the large organic N polymers found in organic matter into smaller organic N forms that are soluble.

There was no change in soil total soluble N during the first 2 months of study, but after this apparent lag period, there was an exponential increase in decomposition processes (Figure 2.4). Maynard (1983) found in agricultural soils that a lag-time of only seven days occurred for nitrification when soils high in N were incubated at 20 °C. However, Maynard (1993) also found that there was a considerable lag-time (63 days) for nitrification in the organic layer of forest soils collected from Alberta, regardless of N applications. This difference is likely due to the composition of the non- soluble organic materials, as agricultural soils would contain less recalcitrant organic matter, and would likely decompose more rapidly than forest soils. Vitousek *et al.* (1982) reviewed lag times in the N cycle after disturbance to forest stand soils (trenching of soil surfaces) and found that it took about two months for any changes in NH_4^+ to be seen. Thus, it takes time for substrate to become available for microbial functional groups of the N cycle, and even with available substrate, it takes considerable time for soil bacteria to process N. Thus it is necessary to study nutrient-deficient soils over an extended period of time to gauge any changes in the N cycle caused by external factors.

Differences Between Soils

Although I saw an overall increase in total soluble N at higher temperatures, I saw more drastic changes to the soil collected from low elevation than the soil collected from high elevation. Increased temperature can affect bacteria (and other decomposers) in many different ways: the overall chemical reactions of decomposition can increase with increased kinetic energy input into the reaction, there may be different optimal temperatures for different enzymes, or there may be adaptations of a functional group to a certain temperature (Sylvia *et al.,* 1999). Decomposition depends on the metabolism of microbes. Because the low elevation soil was collected from a warmer climate it would likely support a more active initial decomposer community (larger population size, larger species richness, or higher metabolic activity (Leckie *et al.,* 2004)), whereas the same community/adaptations may not have been present in the high elevation soils, due to cooler climates that are less favourable to bacterial growth or to a difference in stand histories. Furthermore, the species of bacteria and other decomposers in the two soils may have differed, and as different species within a bacterial functional group are adapted to different temperatures, this may have caused the discrepancy between soils. Adaptation to temperature has previously been seen in mineralizing bacteria found in sediments (Thamdrup and Fleischer, 1998) where sediments from cool coastal areas had an optimum mineralization temperature of 19 °C while warm temperate sediments had an optimal temperature of 30-40 °C. Brockett *et al.*, (2012) also found that a large difference in soil microbial communities existed between relatively warm- dry sites and cool-wet sites, which supports the suggestion of differences in N cycling communities in study soils.

Other factors can affect mineralization in soils. The differences seen between elevations may have been a result of differing lag times for the two differing populations of decomposers, as I did not reach an equilibrium in N cycling in this study. Lag times for decomposition can fluctuate between 1.5 and 2.5 months in soils from different forest stands (Vitousek *et al.*, 1982). Lastly, the amount of carbon in study soils was very high, but I did not do any analyses on the organic portion of soils; thus, soils from a high elevation may have contained more recalcitrant organic matter, which would slow the decomposition of N.

(b) Organic N

Differences Between Soils

The amount of soluble organic N in low elevation soils increased with time (Figure 2.7), which was most evident in the warmest temperature treatment. Furthermore, the proportion of soluble organic N in soil extracts increased in the highest temperature incubation, but decreased (relative to initial values) when soils were incubated at 16 °C (Table 2.8). Perhaps at 16 °C the amount of soluble organic N being produced is low enough for most of it to be consumed by increasing rates of mineralization, but at 20 °C soluble organic N production may exceed rates of mineralization (Table 2.8). In high elevation soils, there was little change in the amount of organic N in soil extracts with temperature (Figure 2.7), but the overall proportion of organic N decreased. This decrease is likely due to slower depolymerization than mineralization.

Soluble organic N increased after 8 weeks with increasing temperature in low elevation soil extracts. This supports the idea that there was increased organic matter breakdown over the 16-week study period due to favourable conditions for soil heterotrophs. The pool of soluble organic N in the low elevation soils was similar to the pool of NH_4^+ (Figure 2.5, Figure 2.7). Thus it is likely that soon after decomposition occurred in study soils, mineralization occurred, and N was further broken down into other forms. Decomposition should also have occurred in the high elevation soil, but perhaps soluble organic N molecules were produced at a slower rate than they were consumed, causing no build-up of this N pool.

(c) NH_4^+

Overall Effect of Temperature

NH_4^+ concentration of soils increased with temperature (Figure 2.5). Ammonia and nitrite oxidizers must oxidize many molecules of NH_4^+ or NO_3^- in order to fix a single molecule of CO_2, and as both ammonia oxidation and nitrite oxidation must occur

for complete nitrification, nitrification usually limits the N cycle. Due to the inefficient metabolism and slow growth of nitrifying bacteria, increases in NH_4^+ of soils are to be expected when there is increased mineralization. Prescott (1997) also found that mineralization increased in clear cuts (which are warmer and more disturbed than closed forest), causing concentrations of soluble inorganic N in soils to increase.

Ammonification is also a source of NH_4^+, but it is unlikely to cause the observed increase in NH_4^+ since ammonifying, heterotrophic bacteria metabolize little N, they primarily use NO_3^- as an electron acceptor when they break down organic C. Furthermore, if ammonification occurred in study soils, NH_4^+ and NO_3^- would not be correlated in low elevation soils kept at 10 °C *i.e.* a decrease in NO_3^- would cause a subsequent increase in NH_4^+ with ammonification (Figure 2.5, Figure 2.6; Table 2.7).

I did not exceed the optimum temperature for mineralization in this study (~35 °C); thus, increased NH_4^+ with increased temperature was expected. An increase in N mineralization in response to warming has also been observed in other forest climate change studies (Peterjohn *et al.*, 1994; Rustad *et al.*, 2001; Melillo *et al.*, 2011).

The lag time for a response to soil warming for NH_4^+ was similar to the lag time for increased total soluble N (about 8 weeks), and NH_4^+ concentrations showed strong correlations with total soluble N (Figure 2.3). Thus, I can conclude that mineralization to NH_4^+ is not the limiting step, but it is the breakdown of large, organic N-containing molecules to soluble organic N compounds that is limiting to the N availability of soils or the activity of nitrifying bacteria, and thus to nitrification. Short lag times for mineralization have also been seen in a forest study in Western Canada (Maynard, 1993); however, another study showed a relatively long lag time (1-2 months) when meadow soils were incubated at 20 °C (Simard and Adrian, 1993). A long lag time may indicate that the presence or activity of mineralizing bacteria is not uniform across landscapes, and my short lag time for mineralization may indicate that a readily available substrate (org N) existed in study soils.

Differences Between Soils

There was a greater response to warming in low elevation soils. This again indicates that mineralizing microbes in the low elevation soil are adapted in some way to

their warmer habitat. The difference in response may mean that differences in species composition exist in my two soils, or perhaps differences in genes or enzymes in bacterial communities of the two soils exist, giving the low elevation soil a greater ability to mineralize organic N.

In high elevation soils from cool habitats, there appeared to be inhibition of mineralization at the highest temperature of 20 °C (Figure 2.5). Thamdrup & Fleischer (1998), who studied arctic and temperate coastal sediments, found that an adaptation to lower temperatures was evident in N mineralizing communities. Each enzyme has an optimum temperature. It is possible that the enzymes or genes in the high elevation bacterial community are adapted to low temperatures and cannot tolerate temperatures above 16 °C.

(d) NO_3^-

Overall Effect of Temperature

At the start of the nitrification assay (soil slurry experiment), there was no net nitrification occurring in the acetylene/ control soils incubated in the lab. No significant differences between initial and final soil NO_3^- concentrations in acetylene treated soil slurries were observed. This suggests that any gross nitrification in spiked soils slurries was likely from classical chemolithotrophic nitrifiers (or some archaean nitrifiers) whose ammonium monooxygenase activity would have been blocked by the addition of acetylene. If nitrification in the soil slurry experiment been from heterotrophic nitrifiers, acetylene would have had no effect (Figure 2.10; Hynes & Knowles 1982; De Boer *et al.* 1991).

If mineralization increases, increases in nitrification should follow, and my correlations between NH_4^+ and NO_3^- reflect this (Table 2.7). In an experiment that warmed forest soils by 5 °C in vivo for 7 years, Butler *et al.* (2012) documented that soils exhibited a 45% average annual increase in net nitrogen mineralization and 25% of the nitrogen mineralized was then nitrified. Jerabkova *et al.* (2011) found that clear cutting increased the proportion of soil NO_3^-, but this increase was delayed in conifer soils, which may explain why increases in NO_3^- were relatively slow in my study.

I hypothesized that although both nitrification and mineralization would increase with temperature, the proportion of NH_4^+: NO_3^- would decrease due to increased availability of substrate for nitrification (mineralization makes NH_4^+ available for nitrification). However, in both the high and low elevation soils, the relative amount of NH_4^+ increased compared to NO_3^- when soils were incubated at higher temperatures (Figure 2.5; Figure 2.6; Table 2.8) indicating that nitrification was affected by temperature to a lesser degree than mineralization, which differs from my hypothesis.

Nitrifiers are CO_2 fixers and tend to grow slowly. Thus, this increase in NH_4^+: NO_3^- may be due to **(1)** losses of NO_3^- from soil (NO_3^- is fairly easily leached from negatively charged soils, whereas NH_4^+ is held in soils by covalent bonds). Leaching was minimized in this study by maintaining the moisture content of soils equal to that at the time of collection. Thus, the increase in NH_4^+: NO_3^- is more likely a result of **(2)** an extended lag time for the nitrification process due to slow growth of bacteria. As a lag time in the nitrification process has been seen in numerous studies (reviewed in Vitousek *et al.,* 1982), perhaps with more time, I would see the expected relative increases in NO_3^- in high elevation soils and at higher temperatures (Table 2.8). The lag time for nitrification is longer than for mineralization, especially in organic soils (Vitousek *et al.,* 1982), which was the case for study soils. The increase in NH_4^+: NO_3^- may also occur if **(3)** nitrification has a higher temperature optimum than mineralization. By increasing soil temperature, I may have brought mineralization very close to optimal temperatures, but nitrification not as close. If this third scenario is true for my study soils, I can expect that, with extended warming, these soils will show the same trend and contain more NH_4^+. This experiment should be repeated for a longer time period to test if the second or third scenario is most likely.

On top of low production of NO_3^-, some of the NO_3^- produced could have been rapidly removed by immobilization (bacterial growth or population increase) or by denitrification causing low amounts of NO_3^- to build in soils. However, immobilization is less likely to be responsible for the removal of NO_3^- from soils, since soil heterotrophs exhibit a stronger preference for NH_4^+ vs. NO_3^- as a nitrogen source (Vitousek *et al.*, 1982). Denitrification may have removed a portion of NO_3^- from soils, but as soil total

soluble N increased with time, it is likely that net denitrification is relatively low.

Differences Between Soils

Both nitrate content and nitrification increased only in the low elevation soils when incubated at warmer temperatures, even though the initial concentrations of the two soils were similar. (Figure 2.6, Figure 2.10). The increase in NO_3^- in low elevation soils during the nitrification assay suggests that nitrification was an important source of NO_3^- in the low elevation soils (Figure 2.10). Nitrification was higher (Figure 2.10), and the amount of NO_3^- was higher in low elevation soils than in high elevation soils (Table 2.8). As nitrification produces H^+, it is likely that these differences in nitrification caused the decrease in pH in low elevation soils after incubation (Figure 2.2).

There are a few possibilities as to why the two soils behaved differently. An increased lag time for nitrification may be more prominent in the high elevation soil, as cellular physiological factors may differ that might cause changes in bacterial population size, diversity or activity. Wertz *et al.* (2011) determined that AOB and nitrobacter-like NOB abundances were correlated with both NO_3^- in soils and potential nitrifiers, supporting the idea that microbial abundances may differ in my 2 soils. This imbalance of nitrification and mineralization in my two study soils may also be attributed to the bacterial communities. As my high elevation soils are subject to a cooler climate, perhaps the nitrifiers of this soil have adapted to the low substrate (NH_4^+) concentrations usually found in these soils, and do not have the cellular mechanisms or the numbers to process NH_4^+ at higher levels. A study that compared ammonium oxidization in three soils from various latitudes in the northern hemisphere found that more oxidation occurred in soils from Egypt (most south/warmest climate) than from Donau, and Bavaria (most north/coolest climate) due to low activity of nitrifiers (Saad and Conrad, 1993). Although little work has been done on the biogeography of microbes, Frier *et al.* (2009) surveyed 23 soils from across North America, finding that about 80% of ammonia oxidizing bacteria (AOB) were *Nitrosospira spp*., while the rest were *Nitrosomonas spp*., and temperature affected the community composition of these two genera by selecting for different AOB lineages. As both community structure and function affect processes of the N cycle, another

possibility for low NO_3^- is poor colonization by nitrifiers, which would prevent the amount of NO_3^- in soils from increasing with temperature (Brockett *et al.*, 2012).

Lastly, there may have been more non-soluble soil organic content containing N in the low elevation soils (although overall amounts of organic matter in soils were similar, KCl extractions show that more NH_4^+ was bound to soils in the low elevation site (Figure 2.7)); thus, there could have been less substrate for nitrifiers of high elevation soils to work on. This last idea is supported by the greater depolymerization of N in low elevation soils (Figure 2.4).

I have determined that the nitrifying functional groups on my two sites may differ, so why were NO_3^- concentrations for both sites similar at time of collection (Figure 2.6)? In the low elevation soil, nitrification controlled the amount of NO_3^-, but perhaps it was the lack of substrate (NH_4^+) in initial conditions that caused low soil NO_3^-. This idea is supported as the amount of NO_3^- in study soils appeared to be correlated with NH_4^+ (Figure 2.7). In the high elevation soil, I determined nitrification was not an important source of NO_3^- to the soils; thus, perhaps these soils still contain some NO_3^- in vivo due to less NO_3^- loss. Denitrification is a processes resulting in N loss that I would expect to be low in high elevation soils. Immobilization by plants or bacteria would also likely be low in a cool climate, with a short growing season; however, leaching should increase with the increased slope and MAP of my high elevation site.

2.4.3 Implications and Future Studies

Although it is difficult to extrapolate these results to soils of forest ecosystems, with predicted changes in climate, the N cycling of forest soils is likely to change in many ways. If future MATs of forest soils increase by 6 °C or more (as climate models project), this study suggests a large increase in the amount of NH_4^+ available to temperate forest plants. Organic N availability to plants should also increase due to the breakdown of large organic polymers, and this would increase available amino acids for plant uptake. Although the amount of NO_3^- in soils is expected to increase with temperature, the relative proportion of NO_3^- in soils may decrease due to the slow growth of this functional group limiting the N cycle.

When looking at the high elevation soils alone, there is some evidence of the inhibition of certain N cycling processes by temperature. For NH_4^+, the highest concentration was at 16 °C. This suggests that optimum temperatures for certain processes depend on the environment to which these communities are adapted. As high elevation soils may possess microbial communities subject to cooler environments, the optimum for processes of the N cycle may reflect an adaptation to this environment. My results suggest that increases in global temperature may have different effects on N cycling processes in microbial communities from contrasting environments.

Very little is known about the patterns or controls of microbial distribution over major habitats, and only recently has work begun to model the biogeography of soil microbes (Bradford & Frier, 2012; Frier & Ladau, 2012). Thus, I can only speculate what might be happening to functional groups in this study and what species comprise these groups, which would cause the trends I saw. By understanding how bacterial communities are structured in different ecosystems, I will be better able to understand the controls on soil N processes and the consequent effects of community composition and function. To properly conclude what was happening in study soils, molecular studies to identify size and diversity of functional groups within soils from differing climates should also be done.

This study used one high elevation site and one low elevation site. Other factors than climate could have contributed to the differences seen in N cycling of my two soils. To determine how soil N forms, N cycling, microbial communities, and microbial activity are affected by elevation and associated climactic factors, further studies of a greater range of soils subject to different climates should be undertaken. As both of these soils would be classified as colluvial Ferro-Humic Podzols (Lord & Valentine, 1979), it may also be interesting to investigate how soils of different classes respond to temperature.

2.5 Summary and Conclusions

Studies are lacking on the effect of warming on N forms in natural soils from Vancouver Island. The purpose of this study was to determine how/if pools of plant

available N would change in response to soil warming. I determined that soil-warming causes large increases in N to most plant available N pools, but the increase of each N form is not uniform, and is highly dependent on the soil's history. In low elevation soils, the amount of total soluble N increased 40 times over 16 weeks when incubated at high temperatures, while in soils from a high elevation, the increase was about 9 times. It is possible that the microbial populations of the low elevation soil are larger with many species filling each niche of the N cycle, while high elevation soils may not experience the necessary climate to support a large or diverse microbial community, and consequently may have low rates of N cycling. Other possibilities as to why there is a greater response in low elevation soils are that microbial metabolic activity may be higher in bacteria adapted to warm conditions (differently adapted enzymes), a larger community, or perhaps, a more diverse organic matter composition exists in these soils. Mineralization was a driving force for NH_4^+ in both soils, but more mineralization occurred in low elevation soils. Nitrification was an important source of NO_3^- in low elevation soils only. If a difference of 1.1 °C and/or 155 mm/yr precipitation in a soil's history can cause such drastic variation to a soil's response to environmental change, it is likely that the alterations in soil N cycling due to climate change will be considerable.

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CHAPTER 3. The Effect of Temperature on N Form Preference and Net Flux of NH_4^+, NO_3^-, and H^+ in Seedling Roots of Three Members of Pinaceae: *Pseudotsuga menziesii*, *Picea sitchensis*, and *Picea engelmannii*

3.1 Introduction

Nitrogen (N) is found in a variety of forms from N_2 gas in the atmosphere to large complex organic molecules in the soil. However, only certain forms of N are available for plant use. Nitrogen (N) is the most abundant mineral element in many plant tissues (Miller & Cramer, 2004); however, it is estimated that only 0.00024% of N on earth is available to plants (Miller & Cramer, 2004). In natural (uncultivated) systems, including temperate conifer forests, available N is usually the most significant environmental factor limiting plant growth and productivity (Wollum & Davey, 1975; Prescott *et al.*, 1992; Marschner, 2002). Thus, N uptake is usually positively correlated with photosynthesis and plant biomass, and when soluble forms of N are available to plant roots, they are readily taken up and either used immediately or stored by plants (Rennenberg & Schmidt, 2010).

3.1.1 Nitrogen Uptake

Nitrogen in the soil is present in various inorganic and organic forms resulting in a heterogeneous distribution over geographical space that undergoes seasonal changes (Miller & Cramer, 2004). Most plants can only take up certain forms of nutrients in soils. For N, it has been demonstrated that plants can take up the inorganic forms of NH_4^+ and NO_3^- as well as small organic nitrogen forms such as amino acids and small peptide chains (Kronzucker *et al.*, 1995; Näsholm *et al.*, 1998; Persson, 2003; Öhlund & Näsholm, 2004; Miller & Cramer, 2004; Näsholm *et al.*, 2009). The uptake of N by trees occurs either by fungal, symbiotic associations (mycorrhizae) or the direct uptake through root tips or root hairs (Figure 3.1). The roots of many northern temperate and boreal trees only associate with ectomycorrhizal fungi to increase N uptake from soils. Ectomycorrhizal fungi are able to secrete hydrolytic enzymes, which break down

proteins into amino acids; influencing amino acid uptake by plants (Miller & Cramer, 2004; Frank & Groffman, 2009).

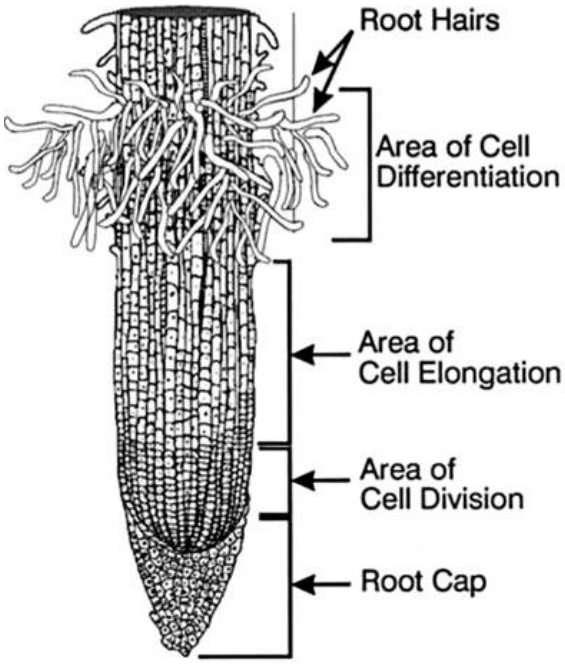

Figure 3.1. A typical plant root tip. http://ecologyadventure2weeds.edublogs.org/botany-expert/

Nutrients, such as ammonium (NH_4^+) or nitrate (NO_3^-), must first reach the plant root before they can be taken up. Nutrient movement towards the root can either be driven against a concentration gradient (by mass flow, transpiration, or gravity), or more commonly, movement can follow a concentration gradient (by diffusion between the root surface and the soil (Miller & Cramer, 2004)). Molecules or atoms diffuse through the soil at various rates. Nitrate usually diffuses 10- 100 times faster in soils than NH_4^+ (Miller & Cramer, 2004) or amino acids (Owen & Jones, 2001). However, Inselsbacher & Näsholm (2012) found that diffusion rates of the amino acids glycine and glutamine were higher than NH_4^+, while diffusion rates of NO_3^- were about 30% lower than those of NH_4^+ and similar to those of various other amino acids, possibly because of soil structure.

Nutrients must be transported across the plasma membrane to become available for plant metabolism. NH_4^+ and NO_3^- uptake by the root is aided by proton pumps. The

NO_3^- ion can enter the cell cytoplasm via active transport after the establishment of a proton gradient, while NH_4^+ uptake is primarily passive through ion channels. The pumping of protons by roots displaces NH_4^+, and other positively charged ions, from soils, increasing the availability for uptake (Brady & Weil, 2002; Taiz & Zeiger, 2011).

Organic N uptake has not been extensively studied as the importance of this N form to plant nutrition has only been recently recognized. The uptake of organic N occurs by a different mechanism than inorganic N. The transport of amino acids (a.a.) across the plasma membrane is a controversial topic. It was previously thought to occur by a proton-coupled symporter with a broad substrate specificity (Neelam *et al*., 1999); but recently a debate has arisen over whether a single or multiple transporter system exists and their identities (Näsholm *et al.,* 2009). Amino acids or small peptides are both transported through the xylem and phloem and between cellular organelles where they are directly assimilated into proteins.

Once N is in the root, it is transported to the shoot via the xylem (Marshner *et al.,* 1996; Miller & Cramer, 2004). Through nutrient cycling between the roots and shoots, plants can detect the nutrient content of their tissues. Internal plant concentrations act as signals and influence how much of a mineral is taken up (Marshner *et al.,* 1996).

3.1.2 Nitrogen Preferences

Plants can show plasticity as to which N form they take up. Due, in part, to the variability in soil nutrient availability, plants have evolved mechanisms to control the uptake of NO_3^- and NH_4^+. Preference towards a N form can be shown by either increased uptake or enhanced growth when supplied with that N form. The preference a plant shows for a N form may be related to the most abundant N form in soils where that plant grows (the plant may be adapted to take up available N forms). In cool climate areas such as arctic, alpine, and boreal regions where N-mineralization rates are very low, amino acid uptake is comparable to that of inorganic N (Raab *et al.*, 1996; Öhlund & Näsholm, 2001; Persson, 2003). Many crop species perform better when supplied with some NO_3^- (in addition to NH_4^+), and agricultural soils tend to contain relatively high amounts of NO_3^- (Clarkson *et al.*, 1986; Martins-Loução & Cruz, 1999). Soils of

coastal Vancouver Island often contain a large proportion of organic N and NH_4^+ (Metcalfe, 2005), and most temperate conifers prefer NH_4^+ to NO_3^-.

The relative availabilities of NO_3^-, NH_4^+, and amino acids and the uptake of N forms vary along productivity gradients. Highly productive sites where plants have high biomass production tend to have relatively more inorganic N available, and plants growing in these sites tend to take up more inorganic N than organic N (Nordin *et al.*, 2001; Weigelt *et al.*, 2005, Kranabetter *et al.*, 2007). Low productivity sites tend to have soils dominated by amino acid-N and the plants in these sites are slow growing and take up amino acids at rates comparable to inorganic-N uptake (Nordin *et al.* 2001). It has been demonstrated that root uptake of N can also be affected by the N form to which a plant was previously acclimated (Bassisirad *et al.*, 1993; Bassisirad, 2000). Plasticity in preferences was demonstrated in ryegrass where the ratio of NH_4^+:NO_3^- uptake decreased when plants were pre-treated with NO_3^- (Clarkson & Warner 1979). Thus, plants may show acclimation (vs. adaptation) to their available N source. However, preference for a certain nutrient can also be related to energy required for uptake, assimilation, and storage.

Kronzucker *et al.* (1997) suggested that nutrient preferences may be a prediction tool for fitness in certain ecosystems. For example, if a plant shows high preference for NH_4^+ as a N source, it is less likely to do well on agricultural soils than other species that prefer NO_3^-. Thus, information on N preference and uptake can be used to predict productivity or competitive ability of a species. If there is a change in the environment, I can use these predictions to forecast changes in species composition.

3.1.3 N Niche Theory

It has been suggested that differences in uptake capabilities or N preferences may be due to niche partitioning (McKane *et al.*, 2002; Weigelt *et al.*, 2005; Harrison, 2007). N niche partitioning would occur in soils with limited N availability. As there are a number of N forms available to plants in soils, different plant species competing for N in the same rooting zone may diverge in their N form preference to decrease competition (McKane *et al.*, 2002; Harrison, 2007). The use of a wider variety of N sources available in soils, including organic N, could increase plant diversity in N-limited environments

(McKane *et al.*, 2002). McKane *et al.* (2002) showed that species partition N both in terms of form, and in terms of timing and depth of uptake.

3.1.4 The Effect of Temperature on N Uptake and Preference

Soil temperature can affect plant N form preference (Bonan, 1991; Scholberg *et al.*, 2002). Warming increases the overall availability of N in soils (Lukac *et al.*, 2011) while increasing plant uptake of N by increasing membrane fluidity (Reece *et al.*, 2011); thus, the uptake of most N forms is positively correlated with temperature (Dong *et al.*, 2001). Although temperature and N uptake are correlated, the rates of change may not be uniform across N forms, as the uptake of NO_3^- is more sensitive to temperature than the uptake of NH_4^+ (Clarkson & Warner, 1979; Clarkson *et al.*, 1986). There are few studies of temperature effects on N form preference, and little work has been done to determine the current preferences of British Columbian conifers that include organic N as a N source. Furthermore, it is inconclusive if species' preference for certain N forms is a result of acclimation or adaptation. Thus, it is worthwhile to investigate these questions.

3.1.5 Objective and Hypothesis

The objective of this study was to search for evidence of adaptation and/or acclimation in N form preference and uptake of three conifer species from contrasting environments (interior low elevation —Douglas-fir, coastal low elevation —Sitka spruce, and interior montane—Engelmann spruce), and determine how/if conifer N preference and uptake respond to increases in soil temperature.

I investigated these issues by conducting two experiments. The first experiment determined the short-term *in situ* N form preference of three conifer species. Conifer root ion flux measurements were taken on young seedling roots bathed in nutrient solutions of different temperatures containing different N forms. The second experiment determined long term N form preferences. Conifer seedlings were planted at three test temperatures and given three N forms in a full factorial design. Various growth measurements were used to determine N form preference and how/if preference changed with temperature. Studies on the effect of temperature on root length, diameter, and

branching patterns may also help determine how nutrient uptake of conifers will change in an altered climate.

I hypothesized that there would be preferential uptake of NO_3^- by Douglas-fir, a weak preference for NH_4^+ by Sitka spruce and a strong preference for NH_4^+ uptake by Engelmann spruce (based on previous literature). As incubation temperatures increased, I expected species from warmer climates (Douglas-fir and Sitka spruce) to have higher N uptake and assimilation than species from cooler climates (Engelmann spruce). Furthermore, I hypothesized that if conifers are adapted to a certain N form, preference (in terms of uptake) will be more evident shortly after the first supply of that N form (experiment 1) and the same preference (in terms of growth) will be shown after continuous supply of that N form to which the conifer is adapted (experiment 2); whereas, if conifers acclimate to the N form they are given, long term growth trends (experiment 2) would not reflect the initial N uptake preferences of the conifer (experiment 1). I also hypothesized that higher increases in root temperature will increase overall N uptake, and result in lower root:shoot due to higher nutrient availability.

3.2 Materials and Methods

3.2.1 Seed Germination

Seed of interior Douglas-fir, Sitka spruce, and Engelmann spruce were sown in a greenhouse on February 13, 2012 in a 1:1 sand:peat moss mix at ambient temperature (approximately 20 °C) after being stratified at 4 °C for 3 weeks. Two seedlots of each species was selected with similar latitude, elevation, biogeoclimactic zone, and germination percent (Table 3.1).

Table 3.1. Summary of seedlots used in study.

Species	Seedlot	Elevation (m)	Latitude (°N)			Longitude (°W)		
Douglas-fir	5062	1000	50°	51'	20"	118°	3'	30"
	5129	1080	51°	1'	50"	118°	3'	30"
Sitka spruce	7758	750	51°	9'	0"	125°	41'	0"
	7761	750	51°	13'	0"	125°	34'	0"
Engelmann spruce	4319	1585	50°	58'	0"	121°	47'	0"
	8136	1585	51°	0'	0"	118°	49'	0"

3.2.2 Short-term N form Preference – N Ion Flux Measurement

Relative uptake of NH_4^+ and NO_3^- at root temperatures of 10 °C, 16 °C and 20 °C were compared in seedling roots of three contrasting conifer species. Seedlings received no nutrient applications during the initial seven weeks of growth. After seven weeks after germination, seedlings were selected for ion flux measurement. Every day for five consecutive days, a random subsample of seedlings was selected. Seedlings were removed from soil, roots were carefully washed and seedlings were tied onto Plexiglas strip holders. Seedlings on holders were placed into large test tubes filled up to the root collar with 100 μM NH_4NO_3 solution with a pH of 7 as pH highly affects plant growth and nutrient uptake (Rengel, 2002). Test tubes were aerated and incubated overnight (~ 18 hr) at the respective solution test temperature (10, 16, or 20 °C). In total, 108 seedlings were assessed (6 seedlots, 3 temperatures, 6 replicates). A half hour before flux measurements were taken, seedlings tied to Plexiglas holders were transferred into Plexiglas chambers to acclimate. Solution was replenished in the chambers every 10 minutes to maintain solution concentration and treatment temperature. Seedlings remained in the chambers for 30 minutes before root flux measurements were done.

Short term NH_4^+ and NO_3^- flux in seedling roots was measured using a microelectrode ion flux measurement system, MIFE® (MIFE, Unitas Consulting, Hobart, Australia). Ion flux measurements were made in solutions of the same temperature as the acclimation solution temperature. The MIFE® system uses ion concentrations or electrochemical potentials to create a voltage, which is measured at

different distances from root surfaces to determine flux of specific ions, in this case NH_4^+, NO_3^-, and H^+, in or out of the tissue (Newman, 2001).

Measurements using the MIFE system are as described in Shabala *et al.*, (1997) and Shabala & Newman (1997). Electrode blanks were pulled from 1.5 mm borosilicate glass capillaries, dried in an oven at 220 °C for 4 h, and silanized with tributylchlorosilane (catalog no. 90796, Fluka). Cooled microelectrodes were backfilled with 200 mM NH_4Cl for NH_4^+, 500 mM KNO_3 plus 100 mM KCl for NO_3^-, and 15 mM NaCl plus 40 mM KH_2PO_4 (adjusted to pH 6.0 using 0.1 M NaOH) for H^+. Electrode tips were then filled with commercially available ion-selective H^+ or NH_4^+ resin cocktails (Fluka catalog no. 95297 and 09882, respectively), or an NO_3^- selective cocktail containing 0.5% methyltridodecylammoniumnitrate (MTDDA NO_3^-), 0.084% methyltriphenylphosphonium bromide (MTPPB) and 99.4% n-phenyloctylether (NPOE) (Plassard *et al.*, 2002). Electrodes were calibrated in a set of known standards.

The electrodes were mounted on an electrode holder (MMT-5, Narishige, Tokyo, Japan) providing three-dimensional positioning. Electrodes were positioned in a line approximately 20 μm above the seedling root surface with their tips spaced 3 to 4 μm apart. The chamber was attached to a computer-controlled micromanipulator (PatchMan NP2, Eppendorf AG, Hamburg, Germany). During flux measurements, the MIFE computer gently moved the chamber up and down, providing virtual movement of the Aelectrode tips between two positions, 20 μm and 60 μm above the root surface, in a 10 s square-wave cycle. The concentration of each ion was calculated from its electrochemical potential at each position. The flux of each ion was later calculated from the measurements of the difference in the electrochemical potential between the two positions (Shabala *et al.* 1997). For analysis, the first 2 minutes of each cycle was ignored.

Root fluxes were measured 1 cm up from the root tip. Average ion concentrations changed by a maximum of 3 μM or 0.1 pH units during measurements, and average temperature changed by a maximum of 2.5 °C. Flux measurements were discarded if the data indicated that the conifer was stressed (unusually large H^+ fluxes), or if electrical interference was evident. Fluxes were measured from 5 plants per treatment, but only 3-5 replicates were used in analyses.

3.2.3 Long-term N form preference

(a) Seedling Growth

The effect of three root temperatures (10 °C, 16 °C and 20 ^{o}C (ambient)) on NH_4^+, NO_3^-, and amino acid (a.a.) uptake, growth and root morphology of three species of conifer seedlings was measured using controlled root temperature chambers at Pacific Forestry Center in Victoria, British Columbia. Six weeks after germination, a subsample of seedlings sown on February 13, 2012 was replanted in 2-inch diameter styroblock containers fitted for the root temperature chambers and containing a 50:50 mix of peat and sand. Temperature chambers were freezers modified to house a thermostat and a thermocouple to monitor and manipulate the rhizosphere temperatures, and were located in a greenhouse. There were four root temperature chambers at each of the test temperatures of 10 °C, 16 °C, and 20 °C (ambient) but shoots of all seedlings were exposed to ambient greenhouse temperature. The 18 treatments (three species, 2 seedlots per species, and three N treatments) were assigned randomly among the cells of styroblocks placed in the four replicate chambers per root temperature treatment. Seedlings were grown in the greenhouse for four months.

During the first two months of growth, seedlings were watered twice a week and nutrients were added to seedlings during every second watering. Nutrient solution was added separately to the water so same amount of N was applied in each treatment despite different water usage among root temperature treatments. During the 3rd and 4th month of growth, when seedlings were more resilient to drying, the conifer seedlings were only watered and fed with nutrient solution once a week. Soil moisture was maintained between 60-80%. The amount of distilled water required for the styroblock in each chamber was determined by weighing the styroblock at initial moisture content (between 60-80%) and then subtracting the weight after drying. using the following formula: *Water added semi weekly=initial styroblock weight at field capacity - weight after drying period*

Soils kept in cooler temperatures exhibited less evaporation, thus they required less water to maintain soil moisture.

Nitrogen was applied weekly to the soil. 5000 ppm N solutions were prepared (Table 3.2), and from these concentrated solutions, 10 ml was added to the water for each of the styroblocks. As there were 45 conifers per styroblock, each conifer received approximately 0.001g N per week. Macronutrients were also applied (in the same watering solution as N treatments) in a ratio of 5N:1P:5K. Micronutrients were added from Plant–prod chelated micronutrient mix (Plant Products Co. Ltd. Brampton, ON, Canada) applied at 0.015g/styroblock (0.0003 g/plant). Ammonium was supplied as $(NH_4)_2SO_4$, NO_3^- as $Ca(NO_3)_2$, organic N as 1:1 arginine:alanine, P as H_3PO_4, and K as K_2SO_4 (Table 3.2). As plant growth is significantly decreased when only one N form is available to a plant, 90:10 ammonium:nitrate and nitrate:ammonium treatments were applied.

Table 3.2. Amount of N, P and K sources used to make up 1 L of nutrient concentrate that was diluted and applied to conifer seedlings.

Chemical	Molecular weight	g/L	
$(NH_4)_2SO_4$	132.14	23.59	(5000ppm-N)
$Ca(NO_3)_2$	134.09	29.29	(5000ppm-N)
Alanine	89.09	15.90	(2500 ppm-N)
Arginine	174.20	7.77	(2500ppm-N)
K_2SO_4	174.26	10.66	(5000 ppm-K)
HPO_3	97.99	3.16	(1000 ppm-K)

(b) Growth/ Biomass Measurements

Seedling biomass, root architecture and total N uptake were measured by harvesting the seedlings after the 4 months of growth. Seedlings were carefully removed from pots and lightly shaken to remove soil; separated at the soil line into roots and shoots; and roots were rinsed with lukewarm water to remove any remaining bound soil. Seedling height and number of primary branches were measured. Root collar diameter was determined using an electronic caliper to the nearest 0.01 mm. Root architecture was measured using the Epson perfection v750 scanner (Epson Canada Ltd., Markham ON, Canada) and WinRHIZO® software to determine average root length, average root diameter, average root surface area, and number of root tips. Roots and shoots were then

dried for 48 h in an oven at 60 °C and biomass was measured as dry weight (g) of roots and shoots to the nearest 0.0001g.

(c) ^{15}N Uptake

^{15}N labeled N forms were applied at the end of the four-month experiment, 24 hours prior to seedling harvest, to a sub sample of conifer seedlings. The amount of ^{15}N in seedlings after 24 h was measured to determine net N uptake at each temperature at the end of the experiment. For each styroblock, one tree per N form treatment per species were selected and ^{15}N-labelled $Ca(NO_3)_2$ ($^{15}N_2$, 98%$^+$), ^{15}N-labelled $(NH_4)_2SO_4$ ($^{15}N_2$, 98%$^+$) and ^{15}N-labelled arginine ($^{15}N_4$, 98%$^+$) were applied to the respective N-form treatment seedlings at 50 ppm N by the use of a syringe through the entire soil column. 24 hours after ^{15}N injections, roots were extracted and washed with water. Roots were then soaked in a 500 µMol $CaCl_2$ solution for 5 minutes to remove any ^{15}N bound to the root surface. Conifer tissues were dried, ground, and 7 mg of whole-plant sample was packaged in (6 x 4 mm) tin capsules and analyzed for ^{15}N and total N content on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility, Davis, California.

(d) Growing Medium Experiment

To assess if the N forms applied to seedlings could have been transformed to another N form over the course of the study, a second experiment with the same set up as the long-term growth experiment was conducted, excluding conifer seedlings. To monitor N transformations in the soil, I measured N form availability in soil incubated in root temperature units over 16 weeks. 40 pots of soil were incubated at each of 10 °C, 16 °C, and 20 °C (ambient) treatments in temperature controlled chambers from September 2012 to December 2012. There were nine temperature chambers in total, and 3 were kept at each temperature treatment. During incubation, soil N and water applications were the same as the seedling growth experiment. Amounts of soluble NO_3^--N, NH_4^+-N, as well as total N were determined on days 0, 1, 3 and 7 during weeks 1, 5, 8, of soil incubation, and on the very last day of the entire incubation. Water soil

extractions were done at each sampling period to determine concentrations of plant-available (soluble) NO_3^-N and NH_4^+-N in each of the incubated soils.

Soil extractions for each treatment (N form and temperature combinations) were prepared from four pots, randomly sampled from the three temperature chambers for each temperature treatment on each of the sampling weeks (4 replications per temperature and N form). To prepare soils for extraction, a 1-inch (diameter) by 3-inch (length) cylindrical core of soil was taken and homogenized from each of the samples. Approximately five grams of homogenized soil was added to a centrifuge tube with 25 ml ultrapure water (18.2 MΩ·cm) purified by a Milli-Q Gradient water polisher. A subsample of soil was weighed and dried to calculate percent moisture. Soil slurries were shaken for one hour and then centrifuged at 3000 rpm for 15 minutes (2740 X g). Supernatant was then transferred into a clean centrifuge tube and frozen at -20 °C until analysis. Soil water extractions were frozen until analysis, which occurred within the 16-week duration.

Colorimetric determination of NH_4^+ and NO_3^- was carried out using the Analytical Segmented Flow system and FASPac® software (Astoria-Pacific). Colorimetric NH_4^+-N determination is based on the Berthelot reaction, where the concentration of NH_4^+-N is proportional to the intensity of the blue indophenol dye produced by the reaction (Mulvaney 1996). Absorbance for NH_4^+-N was measured at 660 nm. Colorimetric determination of soil NO_3^--N is based on the Griess-Ilosvay method where NO_3^--N is first reduced to NO_2^--N for analysis (Mulvaney, 1996). N reduction was done by passing the sample through a copperized cadmium column. NO_2^--N concentration was then determined colorimetrically by the intensity of the red-purple azo-chromophore produced. Consequently, NO_3^--N plus NO_2^--N concentration was determined with this method (Mulvaney, 1996). Absorbance for NO_3^--N was measured at 540 nm.

Total nitrogen and organic nitrogen (calculated via the difference between total N and inorganic N) were determined using the method outlined in Qualls (1989). A water persulphate digest was applied to water samples to sweep the nitrogen from all N compartments into NO_3^- with the modification of adding 3:1, rather than 5:1, sample:oxidizing reagent to ensure that high N samples were fully oxidized. In this

method, digests were analyzed by automated colorimetry with the Astoria Analytical Segmented Flow system and FASPac®.

3.2.4 Statistical Analysis

Data were tested for normality using a Shapiro-Wilk test, by which all data sets were shown to be normal. For all analyses a significance value of $\alpha \leq 0.05$ was used. ANOVA analysis was used for root influx /efflux (MIFE) data, atom $\%^{15}N$, total N of plants, and soil N data. Plant growth parameters were tested with a MANOVA means comparison. As species interactions were significant in the MANOVA, I redid the analysis by species using ANOVA. Tukeys post hoc tests were done for any means that showed significant differences. For ANOVAS of root influx/efflux, flux rate was considered the response variable, while temperature and species were considered fixed factors. For ANOVAS of ^{15}N and total N data, concentration was considered the response variable, while temperature, N treatment, and week were considered fixed factors. All analyses above were conducted using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

For ANOVAS of soil data, nitrogen concentration was considered the response variable, while temperature and week were considered fixed factors. Mean NH_4^+, NO_3^- and organic N (as a percent of total N, not concentration) were compared, and percents were transformed by arcsin square root. A principal component analysis (PCA) was done on plant growth measurements. PCA and growing medium data were analyzed using R version 2.15.3 (R Core Team, 2012).

3.3 Results

3.3.1 Short-term N-form preference

(a) H^+ flux

Proton flux was significantly affected by the temperature of the solution surrounding the roots (Table 3.3; Figure 3.2). However, there was also a significant interaction between species, seedlot, and temperature (Table 3.3). Generally, proton efflux increased with increasing temperatures, and conifers held at 10 °C showed significantly less efflux of H^+ than conifers held at 20 °C (Figure 3.2 inset). This trend was driven by Douglas-fir where the greatest proton efflux occurred at 20 °C. In the two spruce species, there was no significant effect of temperature in the two seedlots. There were no significant differences in H^+ flux between species or seedlots, on average (Table 3.3; Figure 3.2).

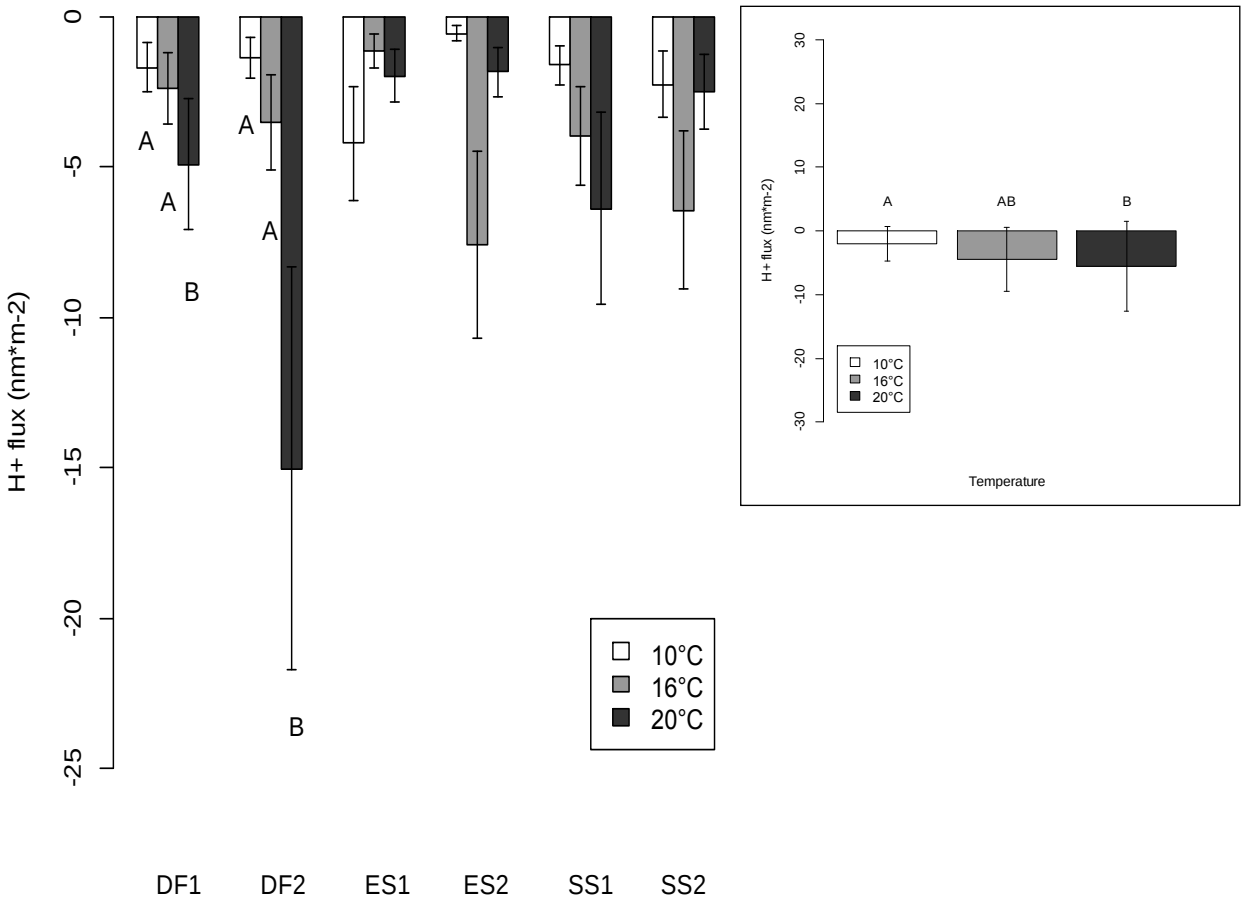

Figure 3.2. Mean proton flux (nmol/m^2) of two seedlots of Douglas-fir (DF1 and DF2), Engelmann spruce (ES1 and ES2), and Sitka spruce (SS1 and SS2). Negative flux indicates efflux. Inset figure is average proton flux of all trees (species and seedlots) combined. Error bars represent standard error about the mean. Sample size (n) ranged from 4 to 6 seedlings. Seedling roots were bathed in a 100µM NH_4NO_3 solution with a pH of 7 at either: 10, 16, or 20 °C. Different lettering indicates significant differences between treatment temperatures found within that seed lot ($\alpha \leq 0.05$).

Table 3.3. Anova table for proton flux including degrees of freedom (df), F values and p values for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedling roots were bathed in a 100µM NH_4NO_3 solution with a pH of 7 at either: 10, 16, or 20 °C. Species (Spp), seedlot (SDlot) within species, and temperature (Temp) effects were examined.

	df	F value	p value
Spp	2	1.40	0.2529
SDlot(Spp)	3	1.72	0.1718
Temp	2	4.11	0.0205
Spp*Temp	4	3.77	0.0079
SDlot(Spp)*Temp	6	2.52	0.0288

(b) NH_4^+ flux

Ammonium flux was also significantly affected by root temperature (Table 3.4; Figure 3.3). However, there was again a significant interaction between species, seedlot, and temperature (Table 3.4). In most treatments, ammonium efflux was observed, and generally, efflux increased with increasing temperatures (Figure 3.3). The only instances of ammonium influx were in roots incubated at 10 °C, and in one Douglas-fir seedlot, at 16 °C (Figure 3.3). There were no significant differences in NH_4^+ flux among species (Table 3.4).

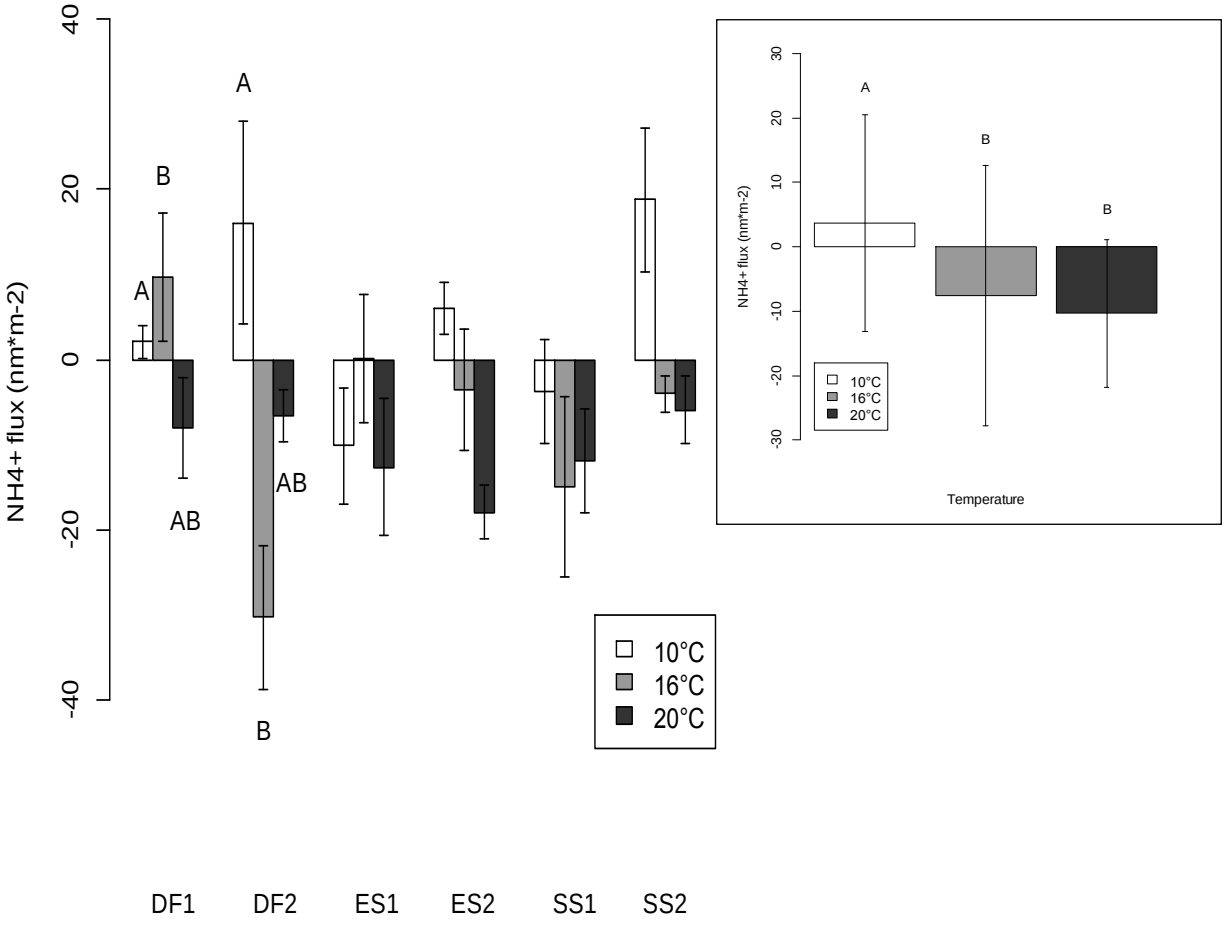

Figure 3.3. Mean ammonium flux (nmol/m^2) of two seedlots of Douglas-fir (DF1 and DF2), Engelmann spruce (ES1 and ES2), and Sitka spruce (SS1 and SS2). Negative flux indicates efflux. Inset figure is average ammonium flux of all trees (species and seedlots) combined. Error bars represent standard error about the mean. Sample size (n) ranged from 4 to 6 seedlings. Seedlings were bathed in a 100μM NH_4NO_3 solution with a pH of 7 at either: 10, 16, or 20 °C. Different lettering indicates significant differences found within that seed lot ($\alpha \leq 0.05$).

Table 3.4. Anova table for ammonium flux including degrees of freedom (df), F values and p values for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedling roots were bathed in a 100μM NH_4NO_3 solution with a pH of 7 at either: 10, 16, or 20 °C. Species (Spp), seedlot (SDlot) within species, and temperature (Temp) effects were examined.

	df	F value	p value
Spp	2	0.27	0.7646
SDlot(Spp)	3	2.35	0.0810
Temp	2	7.20	0.0015
Spp*Temp	4	1.20	0.3189
SDlot(Spp)*Temp	6	3.13	0.0095

(c) NO_3^- flux

Nitrate flux was significantly affected by root temperature (Table 3.5; Figure 3.4). However, there was a significant interaction between species and temperature (Table 3.5). On average, NO_3^- uptake was highest at 16 °C (Figure 3.4 inset). Conifer roots at 10 °C possessed a significantly lower uptake of NO_3^- than seedling roots in 16 °C or 20 °C solution (Figure 3.4). There were no significant differences in NO_3^- flux between species (Table 3.5) nor between seedlots of the three species (Table 3.5).

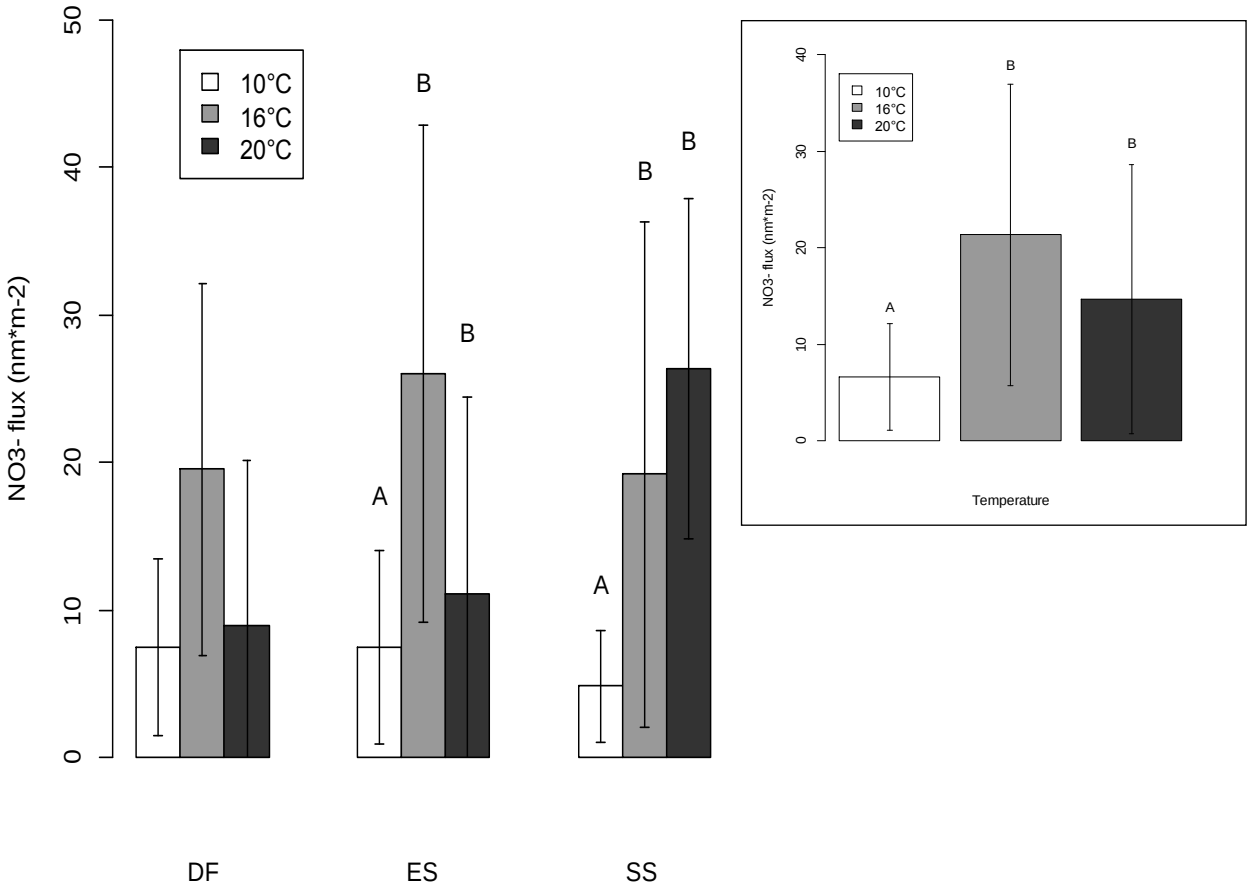

Figure 3.4. Mean nitrate flux (nmol/m^2) of two seedlots of Douglas-fir (DF1 and DF2), Engelmann spruce (ES1 and ES2), and Sitka spruce (SS1 and SS2). Inset figure is average nitrate flux of all trees (species and seedlots) combined. Error bars represents standard error about the mean. Sample size (n) ranged from 8 to 12 seedlings. Seedlings were bathed in a 100µM NH_4NO_3 solution with a pH of 7 at either: 10, 16, or 20 °C. Different lettering indicates significant differences found within that species ($\alpha \leq 0.05$).

Table 3.5. Anova table for nitrate flux including degrees of freedom (df), F values and p values for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedling roots were bathed in a 100µM NH_4NO_3 solution with a pH of 7 at either: 10, 16, or 20 °C. Species (Spp), seedlot (SDlot) within species, and temperature (Temp) effects were examined.

	df	F value	p value
Spp	2	1.15	0.322
SDlot(Spp)	3	1.03	0.3858
Temp	2	11.89	<0.0001
Spp*Temp	4	3.09	0.0211
SDlot(Spp)*Temp	6	1.78	0.1155

3.3.2 Long-Term N-form Preference

In the MANOVA analyses of all data, some interaction terms for the main factors temperature or N treatment, with species were significant for most growth parameters (Table 3.6); therefore significant data were reanalyzed by species using ANOVA. On average, Engelmann spruce seedlings were the smallest followed by Douglas-fir, while Sitka spruce seedlings grew the most. On average, growth of seedlings increased with temperature and was greater with inorganic forms of N than with amino acids.

Table 3.6. MANOVA table for major growth parameters including degrees of freedom (df), F values and p values for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. Temperature (Temp), nitrogen treatment (NTrtmt), species (Spp), and seedlot (SDlot) within species effects, and their interactions were examined.

	df	F Value	p value	df	F Value	p value	df	F Value	p value	df	F Value	p value
	Root Collar Diameter			***Dry Weight of Shoot***			***Root Surface Area***			***Number of Root Tips***		
Temp	2	192.55	<0.0001	2	201.59	<0.0001	2	5.70	0.0174	2	8.23	<0.0001
NTrtmt	2	10.69	<0.0001	2	4.41	0.0127	2	1.26	0.2827	2	0.02	0.9835
Spp	2	125.68	<0.0001	2	165.93	<0.0001	2	4.27	0.0146	2	12.01	<0.0001
SDlot(Spp)	3	4.18	0.0063	3	2.56	0.0545	3	0.50	0.6833	3	1.57	0.1943
Temp*NTrtmt	4	3.11	0.0458	4	3.10	0.0462	4	0.67	0.5109	4	4.57	0.0108
Temp*Spp	4	37.09	<0.0001	4	35.00	<0.0001	4	3.10	0.0406	4	6.59	0.0015
NTrtmt*Spp	4	11.85	<0.0001	4	9.46	<0.0001	4	1.34	0.7388	4	0.48	0.7500
NTrtmt*Sdlot(Spp)	6	1.38	0.2199	6	0.96	0.4530	6	1.23	0.2952	6	1.59	0.1892
Temp*SDlot(Spp)	6	0.16	0.9876	6	0.84	0.4697	6	0.54	0.7796	6	1.64	0.1330
Temp*NTrtmt*Spp	8	1.31	0.2654	8	1.18	0.3168	8	3.15	0.0142	8	0.78	0.5377
	Shoot Height			***Total Weight of Seedling***			***Root Diameter***			***Number of Secondary Branches***		
Temp	2	82.89	<0.0001	2	173.99	<0.0001	2	109.39	<0.0001	2	48.07	<0.0001
NTrtmt	2	11.26	<0.0001	2	1.68	0.1868	2	0.67	0.5125	2	1.86	0.1695
Spp	2	165.42	<0.0001	2	157.64	<0.0001	2	123.87	<0.0001	2	235.52	<0.0001
SDlot(Spp)	3	3.33	0.0197	3	2.62	0.0508	3	2.51	0.0588	3	0.88	0.3481
Temp*NTrtmt	4	6.64	<0.0001	4	0.96	0.3817	4	0.17	0.9559	4	0.09	0.3629
Temp*Spp	4	31.32	<0.0001	4	31.59	<0.0001	4	29.49	<0.0001	4	30.35	<0.0001
NTrtmt*Spp	4	14.29	<0.0001	4	2.97	0.0194	4	0.30	0.8799	4	0.35	0.9399
NTrtmt*Sdlot(Spp)	6	0.80	0.0254	6	0.70	0.6433	6	4.55	0.0002	6	0.33	0.9201
Temp*SDlot(Spp)	6	1.79	0.9244	6	1.48	0.2182	6	0.19	0.9799	6	0.88	0.5077
Temp*NTrtmt*Spp	8	2.73	0.0062	8	1.08	0.3661	8	0.51	0.8456	8	0.46	0.8403
	Dry Weight of Root			***Root: Shoot***			***Root Length***			***Number of Tertiary Branches***		
Temp	2	167.66	<0.0001	2	2.50	0.1146	2	15.11	<0.0001	2	2.52	0.2738
NTrtmt	2	2.34	0.0969	2	1.84	0.1608	2	0.63	0.5310	2	2.71	0.0639
Spp	2	129.96	<0.0001	2	13.15	<0.0001	2	2.38	0.0930	2	28.88	<0.0001
SDlot(Spp)	3	2.06	0.0519	3	1.45	0.2283	3	0.90	0.4393	3	0.99	0.3968
Temp*NTrtmt	4	4.67	0.0099	4	1.58	0.2055	4	2.20	0.1121	4	0.17	0.5347
Temp*Spp	4	20.47	<0.0001	4	0.01	0.9907	4	12.10	<0.0001	4	2.65	0.1726
NTrtmt*Spp	4	6.28	<0.0001	4	16.34	0.9568	4	0.99	0.4120	4	2.55	0.0388
NTrtmt*Sdlot(Spp)	6	0.91	0.4860	6	0.75	0.6070	6	1.01	0.4128	6	1.73	0.1127
Temp*SDlot(Spp)	6	1.68	0.9164	6	0.06	0.9780	6	1.73	0.1588	6	0.66	0.6820
Temp*NTrtmt*Spp	8	0.54	0.7053	8	0.04	0.9975	8	0.32	0.8655	8	0.23	0.9239

Data Analyzed by Species

(a) Interaction Between N Treatment and Temperature

There was an interaction between N treatment, and temperature when data was analyzed by species for root collar diameter, shoot height, shoot dry weight, root dry weight, total dry weight, and root:shoot ratio (Table 3.7). When looking at total biomass, at 10 and 16 °C, Douglas-fir (DF) showed fairly equal biomass accumulation (*i.e.* preference) with NH_4^+ and NO_3^- but at higher temperatures, the growth with NO_3^- was greater (Figure 3.5). At lower temperatures, Engelmann spruce showed equal growth with NH_4^+ and amino acids, but as temperatures increased the growth with amino acids decreased (Figure 3.5). Sitka spruce showed consistently greater growth with NH_4^+ (the increase of growth with AA with temperature in Sitka spruce was unique to the growth parameter of biomass, whereas, shoot height, RCD growth remained low with AA at every temperature (data not shown), but as temperature increased so did the relative growth with NO_3^- (Figure 3.5). Other growth parameters showed a similar trend to total biomass (data not shown). Although there was an interaction between N treatment and temperature, most preferences exhibited by species towards N form did not change with temperature, and trends were mainly amplified with temperature, except where previously mentioned.

Table 3.7. Anova table values for the N-form treatment x Temperature interaction term for analyses of major growth parameters including degrees of freedom (df), F values and p values for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. Nitrogen*temperature effects were examined by species.

Douglas-fir	**df**	**F**	**p**
Root Collar Diameter (mm)	4	2.27	0.0652
Shoot Height (cm)	4	8.11	<0.0001
Root Dry Weight (g)	4	929	<0.0001
Shoot Dry Weight (g)	4	6.27	0.0001
Total Weight (g)	4	7.33	<0.0001
Root: Shoot	4	10.45	<0.0001
Sitka spruce	**df**	**F**	**p**
Root Collar Diameter (mm)	4	1.71	0.1513
Shoot Height (cm)	4	2.95	0.0228
Root Dry Weight (g)	4	4.13	0.0036
Shoot Dry Weight (g)	4	6.89	<0.0001
Total Weight (g)	4	7.16	<0.0001
Root: Shoot	4	3.46	0.0102
Engelmann spruce	**df**	**F**	**p**
Root Collar Diameter (mm)	4	1.26	0.2891
Shoot Height (cm)	4	4.22	0.0031
Root Dry Weight (g)	4	0.11	0.9785
Shoot Dry Weight (g)	4	1.18	0.3240
Total Weight (g)	4	0.91	0.4624
Root: Shoot	4	1.43	0.2273

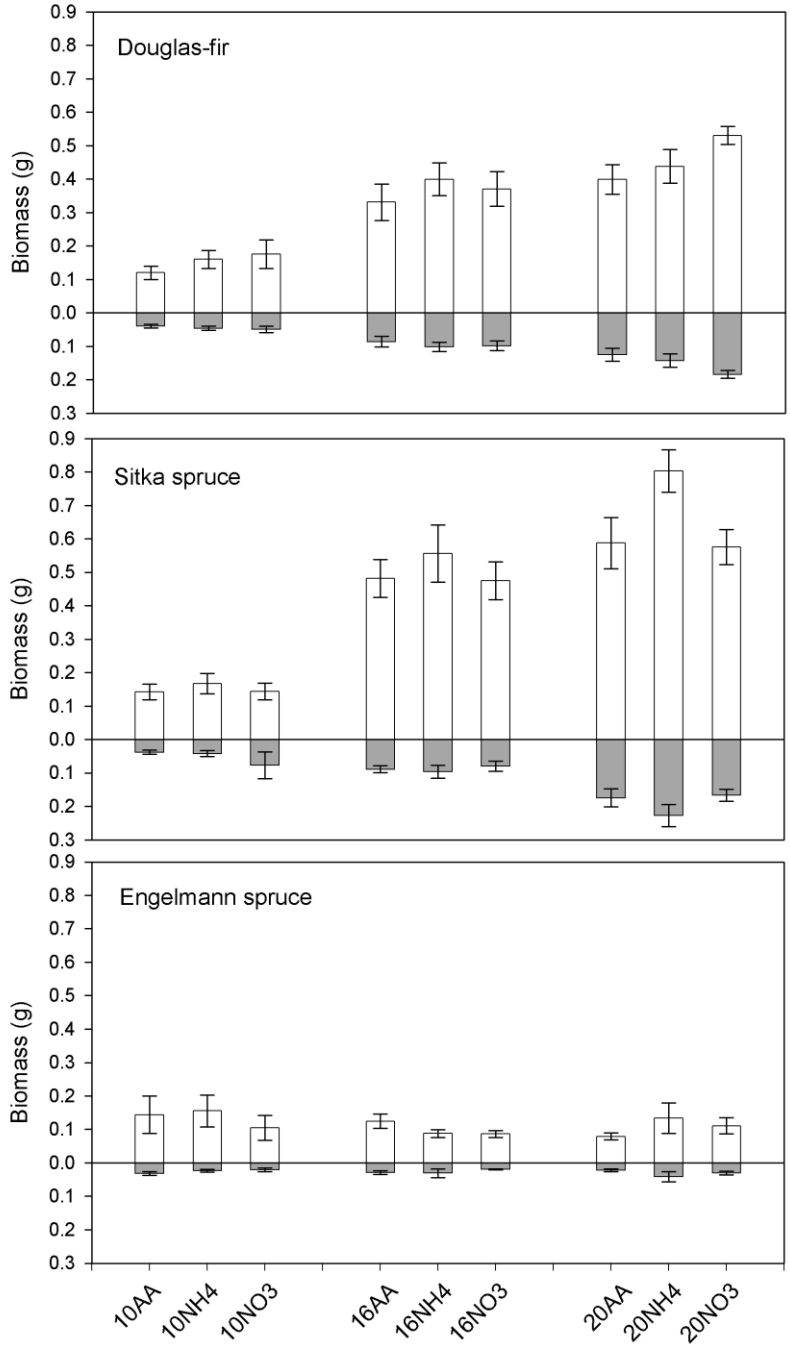

Figure 3.5. Total biomass of conifer species in different temperature and N-form treatments. Plant dry weight (g) was measured in two seedlots of three species (Douglas-fir, Engelmann Spruce, and Sitka Spruce), given various nitrogen treatments (arginine/alanine (AA), NH_4^+ or NO_3^-) incubated at three rhizosphere temperatures (10, 16 or 20 °C) for four months. Bars represent mean values, of root (grey bars) and shoot (white bars) dry weight and error bars represent standard error of the mean. Sample size (n) ranged from 11 to 19.

(b) Effect of N Treatment

There was a significant effect of N treatment on species for shoot height, root collar diameter, dry weight of conifer shoots, dry weight of conifer roots, and total conifer weight (Table 3.8). Averaged over the three temperatures, Douglas-fir showed the highest biomass when supplied with NO_3^-, followed by NH_4^+ and amino acids (Figure 3.6; Table 3.9). Engelmann spruce showed highest biomass when supplied with NH_4^+ followed by amino acids and finally NO_3^- (Figure 3.6; Table 3.9). Sitka spruce showed the highest biomass when supplied with NH_4^+ followed by either amino acids or NO_3^- (Figure 3.6; Table 3.9). Other major growth parameters followed a similar trend (Table 3.9). Root allocation was significantly lower in NH_4^+ treatment for Douglas fir, NO_3^- treatment for Englemann spruce, and for AA in Sitka spruce (Table 3.8, Table 3.9).

Table 3.8. Anova table for values for the N-form treatment term for analyses of major growth parameters including degrees of freedom (df), F values and significance values at $\alpha \leq 0.05$ (P) for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. Nitrogen treatment effects were examined by species.

Douglas-fir	**df**	**F**	**p**
Root Collar Diameter (mm)	2	17.58	<0.0001
Shoot Height (cm)	2	39.35	<0.0001
Root Dry Weight (g)	2	22.33	<0.0001
Shoot Dry Weight (g)	2	25.86	<0.0001
Total Weight (g)	2	26.7	<0.0001
Root: Shoot	2	3.56	0.0315
Sitka spruce	**df**	**F**	**p**
Root Collar Diameter (mm)	2	15.67	<0.0001
Shoot Height (cm)	2	28.83	<0.0001
Root Dry Weight (g)	2	7.25	0.0011
Shoot Dry Weight (g)	2	23.31	<0.0001
Total Weight (g)	2	19.02	<0.0001
Root: Shoot	2	7.41	0.0009
Engelmann spruce	**df**	**F**	**p**
Root Collar Diameter (mm)	2	4.56	0.0122
Shoot Height (cm)	2	20.03	<0.0001
Root Dry Weight (g)	2	3.54	0.032
Shoot Dry Weight (g)	2	3.11	0.0482
Total Weight (g)	2	3.87	0.0234
Root: Shoot	2	2.5	0.0864

Table 3.9. Mean ($\bar{x}$) and standard error (SE) of growth parameters for seedlings of conifer species in three N-form treatments, averaged over three temperatures. Post hoc test (Tukey) results are shown where a difference in lettering indicates a significant difference in means.

Douglas-fir		**AA**			**NH4**			**NO3**	
GROWTH PARAMETER	$\bar{x}$	**SE**	**Tukey**	$\bar{x}$	**SE**	**Tukey**	$\bar{x}$	**SE**	**Tukey**
Root Collar Diameter (mm)	1.257	0.056	**A**	1.584	0.080	**B**	1.756	0.082	**B**
Shoot Height (cm)	11.749	0.652	**A**	14.765	0.811	**B**	20.58	1.402	**C**
Root Dry Weight (g)	0.073	0.009	**A**	0.092	0.009	**A**	0.151	0.018	**B**
Shoot Dry Weight (g)	0.251	0.029	**A**	0.314	0.028	**B**	0.52	0.053	**AB**
Total Weight (g)	0.325	0.037	**A**	0.406	0.037	**B**	0.671	0.056	**AB**
Root: Shoot	0.317	0.018	**A**	0.287	0.018	**B**	0.313	0.017	**A**
Sitka spruce									
GROWTH PARAMETER									
Root Collar Diameter (mm)	1.641	0.074	**A**	1.865	0.098	**B**	1.422	0.091	**C**
Shoot Height (cm)	16.056	0.756	**A**	19.889	0.886	**B**	13.147	0.884	**C**
Root Dry Weight (g)	0.099	0.010	**A**	0.182	0.033	**B**	0.108	0.015	**A**
Shoot Dry Weight (g)	0.439	0.041	**A**	0.766	0.082	**B**	0.437	0.048	**A**
Total Weight (g)	0.525	0.048	**A**	0.935	0.109	**B**	0.549	0.062	**A**
Root: Shoot	0.271	0.029	**A**	0.287	0.044	**B**	0.292	0.046	**B**
Engelmann spruce									
GROWTH PARAMETER									
Root Collar Diameter (mm)	0.962	0.052	**AB**	1.068	0.064	**A**	0.840	0.043	**B**
Shoot Height (cm)	8.815	0.505	**A**	9.529	0.538	**A**	5.942	0.267	**B**
Root Dry Weight (g)	0.032	0.004	**AB**	0.039	0.008	**A**	0.019	0.002	**B**
Shoot Dry Weight (g)	0.137	0.024	**A**	0.152	0.025	**A**	0.083	0.011	**B**
Total Weight (g)	0.170	0.026	**AB**	0.191	0.030	**A**	0.103	0.012	**B**
Root: Shoot	0.328	0.033	**A**	0.304	0.041	**AB**	0.287	0.021	**B**

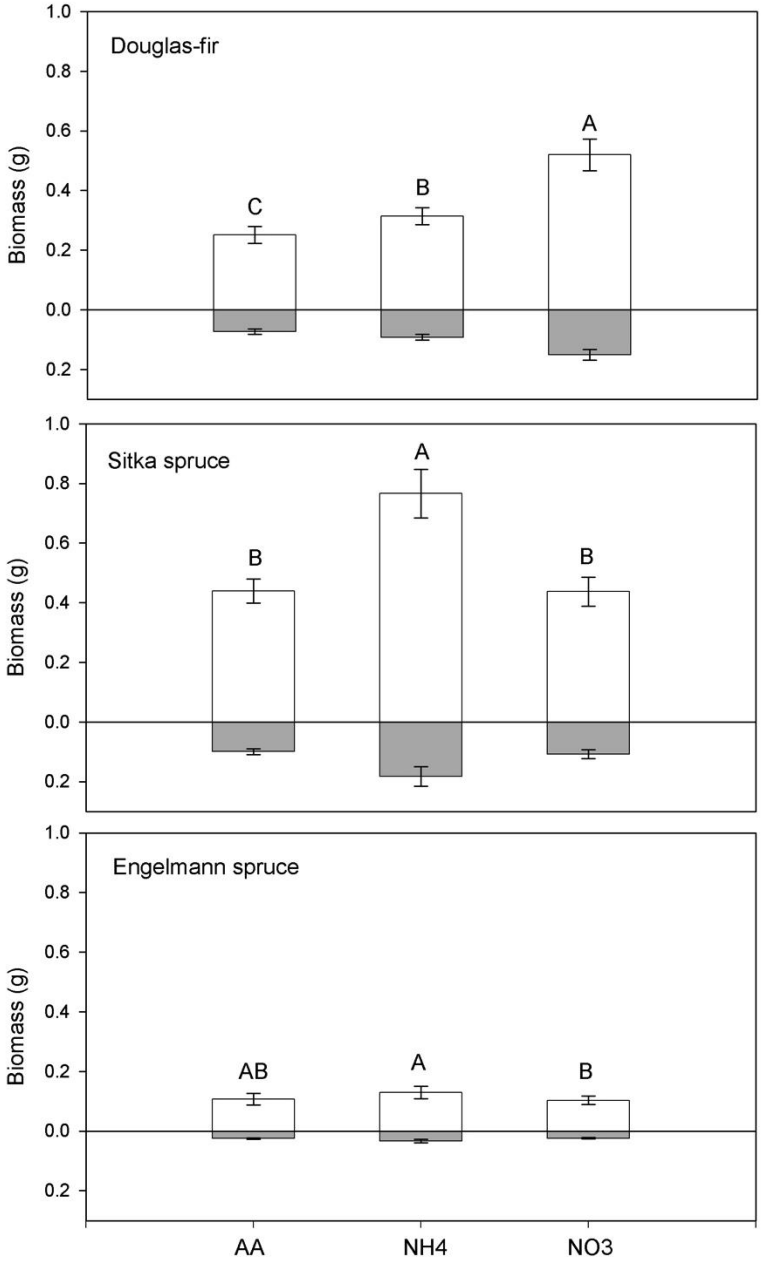

Figure 3.6. Total biomass of conifer species in three N-form treatments. Plant dry weight (g) was measured in two seedlots of three species (Douglas-fir, Engelmann Spruce, and Sitka Spruce), given various nitrogen treatments (AA, NH_4^+ or NO_3^-) incubated at three temperatures (10, 16 or 20 °C) for four months. Temperature treatments were combined for this figure. Bars represent means values of root (grey bars) and shoot (white bars) dry weight, and error bars represent standard error (n = 45-51).

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(c) Effect of Temperature

There was a significant effect of temperature for each species for shoot height, root collar diameter, dry weight of conifer shoots, dry weight of conifer roots, and total conifer weight (Table 3.10). Averaged over the three N-form treatments, Douglas-fir and Sitka spruce showed increased growth with temperature, whereas the height and RCD of Engelmann spruce increased between 10 and 16 °C but decreased from 16 to 20 °C (Table 3.11).

Table 3.10. Anova table for values for the temperature treatment term for analyses of major growth parameters including degrees of freedom (df), F values and significance values at $\alpha \leq 0.05$ (p) for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. Temperature effects were examined by species.

Douglas-fir	**df**	**F**	**p**
Root Collar Diameter (mm)	2	26.25	<0.0001
Shoot Height (cm)	2	54.45	<0.0001
Root Dry Weight (g)	2	50.94	<0.0001
Shoot Dry Weight (g)	2	46.69	<0.0001
Total Weight (g)	2	50.19	<0.0001
Root: Shoot	2	47.54	<0.0001
Sitka spruce	**df**	**F**	**p**
Root Collar Diameter (mm)	2	105.89	<0.0001
Shoot Height (cm)	2	48.43	<0.0001
Root Dry Weight (g)	2	35.98	<0.0001
Shoot Dry Weight (g)	2	74.92	<0.0001
Total Weight (g)	2	65.61	<0.0001
Root: Shoot	2	64.47	<0.0001
Engelmann spruce	**df**	**F**	**p**
Root Collar Diameter (mm)	2	5.08	0.0076
Shoot Height (cm)	2	2.26	0.1091
Root Dry Weight (g)	2	2.52	0.0844
Shoot Dry Weight (g)	2	0.87	0.4224
Total Weight (g)	2	1.35	0.2621
Root: Shoot	2	0.84	0.4335

Table 3.11. Mean ($\bar{x}$) and standard error (SE) of growth parameters for three temperature treatments, averaged over three N-form treatments, where a significant effect of temperature treatment was found. Post hoc test (Tukey) results are shown where a difference in lettering indicates a significant difference in means.

Douglas-fir		**10**			**16**			**20**	
GROWTH PARAMETER	$\bar{x}$	**SE**	**Tukey**	$\bar{x}$	**SE**	**Tukey**	$\bar{x}$	**SE**	**Tukey**
Root Collar Diameter (mm)	1.139	0.055	**A**	1.64	0.081	**B**	1.739	0.067	**B**
Shoot Height (cm)	9.824	0.608	**A**	15.53	0.867	**B**	20.492	1.149	**C**
Root Dry Weight (g)	0.042	0.004	**A**	0.097	0.009	**B**	0.164	0.015	**C**
Shoot Dry Weight (g)	0.137	0.012	**A**	0.389	0.036	**B**	0.516	0.042	**C**
Total Weight (g)	0.180	0.015	**A**	0.485	0.044	**B**	0.68	0.057	**C**
Root: Shoot	0.326	0.02	**A**	0.272	0.040	**B**	0.322	0.043	**A**
Sitka spruce									
GROWTH PARAMETER									
Root Collar Diameter (mm)	1.024	0.058	**A**	1.759	0.069	**B**	2.136	0.055	**C**
Shoot Height (cm)	11.607	0.873	**A**	17.451	0.764	**B**	20.019	0.681	**C**
Root Dry Weight (g)	0.041	0.006	**A**	0.102	0.011	**B**	0.241	0.028	**C**
Shoot Dry Weight (g)	0.171	0.020	**A**	0.626	0.055	**B**	0.832	0.057	**C**
Total Weight (g)	0.210	0.025	**A**	0.729	0.065	**B**	1.058	0.081	**C**
Root: Shoot	0.346	0.021	**A**	0.171	0.051	**B**	0.307	0.059	**A**
Engelmann spruce									
GROWTH PARAMETER									
Root Collar Diameter (mm)	0.820	0.043	**A**	1.047	0.051	**B**	0.982	0.061	**A**
Shoot Height (cm)	8.099	0.589	**.**	8.691	0.459	**.**	7.421	0.450	**.**
Root Dry Weight (g)	0.021	0.002	**.**	0.030	0.006	**.**	0.038	0.006	**.**
Shoot Dry Weight (g)	0.107	0.021	**.**	0.117	0.012	**.**	0.145	0.027	**.**
Total Weight (g)	0.129	0.022	**.**	0.146	0.015	**.**	0.143	0.031	**.**
Root: Shoot	0.280	0.021	**.**	0.274	0.013	**.**	0.361	0.028	**.**

3.3.3 Principal Component Analysis

Axis/component 1 accounted for 67% of the variability in the data, and axis/component 2 accounted for 9%. 24% of the variability in the data is unaccounted for by these figures. When data are grouped by species and N treatment, medium colors (Engelmann spruce) coalesce on the x-axis (Figure 3.7). When data are grouped by temperature and species there is coalescence of blues (Engelmann spruce), and like-shades of color (temperatures) align themselves along the x-axis (Figure 3.8). When data are grouped by temperature and N treatment there is coalescence of like-shades of color (temperatures) along the x-axis (Figure 3.9). These groupings indicate that temperature and species have a larger effect on growth parameters measured than N form supplied.

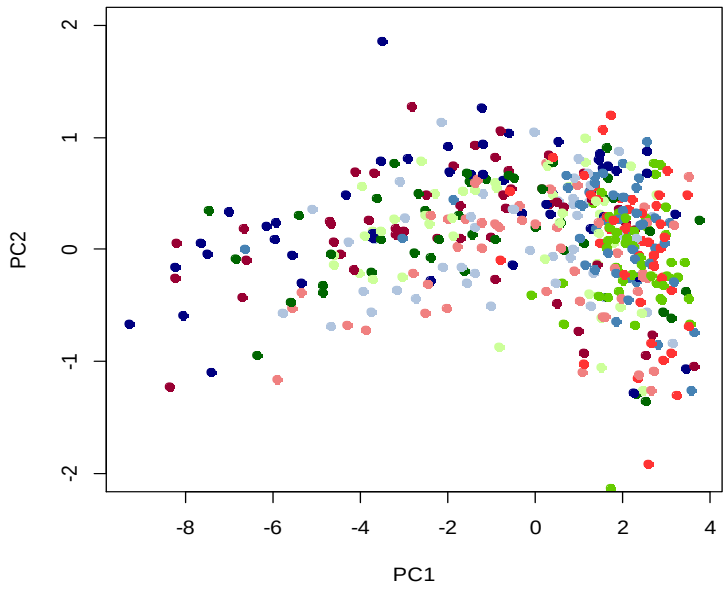

Figure 3.7. PCA coloured by species and N treatment. Axis/component 1 accounted for 67% of the variability in the data, and axis/component 2 accounted for 9%. Light colors represent Douglas-fir, medium represents Engelmann spruce, and dark colors represent Sitka Spruce. Reds indicate amino acid treatment blue indicates ammonium, and green represents nitrate. Axis 1 accounts for 84% of the variation in data. Colours are as follows: light red ( ), light blue ( ), light green ( ), medium red ( ), medium blue ( ), medium green ( ), dark red ( ), dark blue ( ), and dark green ( ).

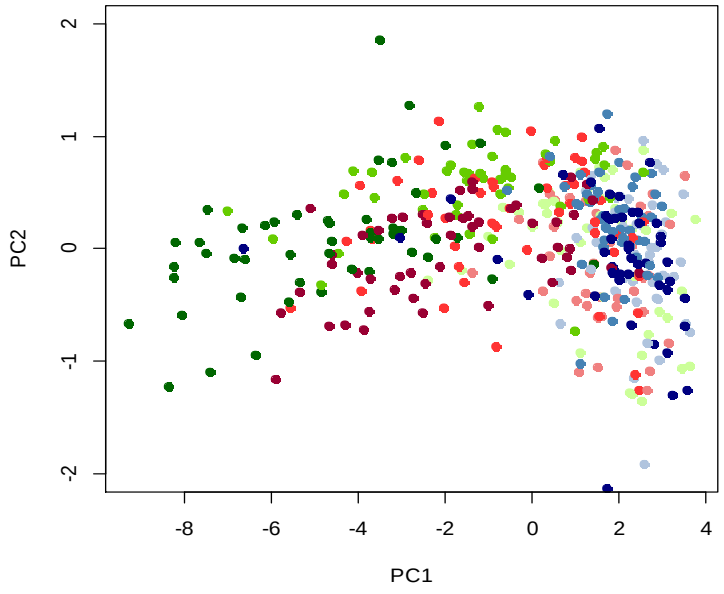

Figure 3.8. PCA coloured by temperature and species. Axis/component 1 accounted for 67% of the variability in the data, and axis/component 2 accounted for 9%. Light colors represent 10 °C, medium represent 16 °C, and dark colors represent 20 °C. Reds indicate Douglas-fir, blue indicates Engelmann spruce, and green represents Sitka spruce. Axis 1 accounts for 84% of the variation in data. Colours are as follows: light red ( ), light blue ( ), light green ( ), medium red ( ), medium blue ( ), medium green ( ), dark red ( ), dark blue ( ), and dark green ( ).

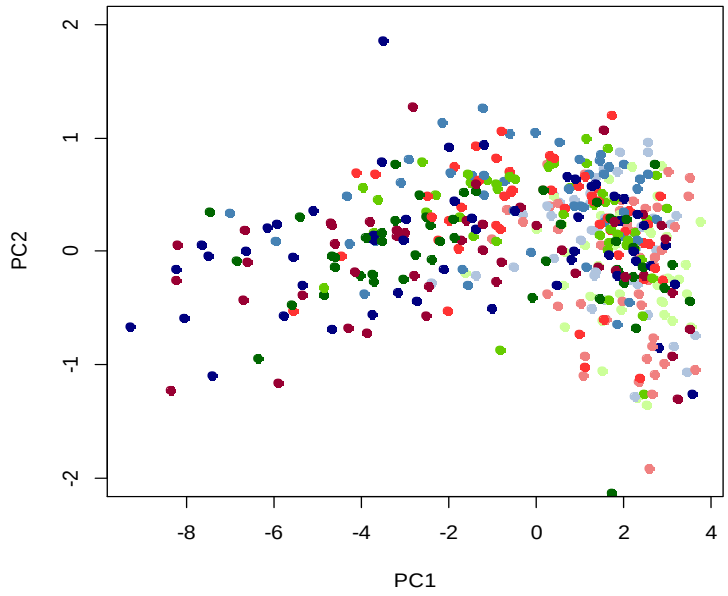

Figure 3.9. PCA coloured by temperature and N treatment. Axis/component 1 accounted for 67% of the variability in the data, and axis/component 2 accounted for 9%. Light colors represent 10 °C, medium represent 16 °C, and dark colors represent 20 °C. Reds indicate amino acid treatment, blue indicates ammonium, and green represents nitrate. Axis 1 accounts for 84% of the variation in data. Colours are as follows: light red ( ), light blue ( ), light green ( ), medium red ( ), medium blue ( ), medium green ( ), dark red ( ), dark blue ( ), and dark green ( ).

3.3.4 ^{15}N Uptake and Total N

Average ^{15}N (atom %) and total N concentration of conifers was significantly affected by temperature, treatment, and species, and there was a significant three-way interaction between these factors ($p<0.05$; Table 3.12); thus, data were reanalyzed by species.

Table 3.12. Anova table for ^{15}N (atom %) of plant, and total N concentration of plants, including degrees of freedom (DF), F values and p values for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. Temperature (Temp), N treatment (NTrtmt), Species (Spp), and seedlot (SDlot) within species effects were examined.

	^{15}N (atom %)			N conc (%)		
	df	F	p	df	F	p
Temp	2	36.44	<0.0001	2	34.63	<0.0001
NTrtmt	2	15.55	<0.0001	2	6.62	0.0017
Temp*NTrtmt	4	10.15	<0.0001	4	15.22	<0.0001
Spp	2	7.14	0.0011	2	63.55	<0.0001
Temp*Spp	4	1.26	0.2876	4	6.99	<0.0001
NTrtmt*Spp	4	3.21	0.0142	4	6.22	0.0001
SDlot(Spp)	7	0.73	0.6487	7	2.11	0.0452
Temp*NTrtmt*Spp	8	2.14	0.0350	8	5.36	<0.0001

Data Analyzed by Species

*(a) N Treatment * Temperature*

When data was analyzed by species, a significant interaction between N treatment and temperature was found for N concentration of Douglas-fir seedlings, and for atom % ^{15}N and N concentration of Sitka spruce and Engelmann spruce seedlings (Table 3.13). Total N concentration of Douglas-fir was highest when supplied with NH_4^+ at 10 °C, but highest when supplied with NO_3^- at 16 and 20 °C (Figure 3.11).

Sitka spruce uptake of ^{15}N-NO_3- was low at 10 °C, but relative uptake of NO_3^- increased with temperature (Figure 3.10). ^{15}N-NH_4^+ and ^{15}N-arginine uptake were relatively high in all cases (Figure 3.10). Total N concentration of Sitka spruce was always highest when supplied with NH_4^+ (Figure 3.11).

Engelmann spruce uptake of ^{15}N-NO_3^- was very low at 10 °C, but relative uptake of NO_3- increased greatly with temperature (Figure 3.10). ^{15}N-NH_4^+ uptake was high at all temperatures, and ^{15}N-arginine uptake decreased with temperature (Figure 3.10). N concentration of Engelmann spruce was highest when supplied with amino acids at 10 °C, NO_3- at 16 °C and NH_4^+ at 20 °C (Figure 3.11).

Table 3.13. Anova table of values for the N-form x temperature treatment interaction term for analyses of ^{15}N uptake (atom %) and N concentration of three conifer species including degrees of freedom (df), F values and significance values at $\alpha \leq 0.05$ (p) for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. ^{15}N was applied to conifers 24 hour before harvest.

Douglas-fir	**df**	**F**	**p**
%15N	4	2.24	0.0793
N Conc	4	3.53	0.0135
Sitka spruce	**df**	**F**	**p**
%15N	4	3.97	0.0077
N Conc	4	2.25	0.0789
Engelmann spruce	**df**	**F**	**p**
%15N	4	8.03	<0.0001
N Conc	4	17.26	<0.0001

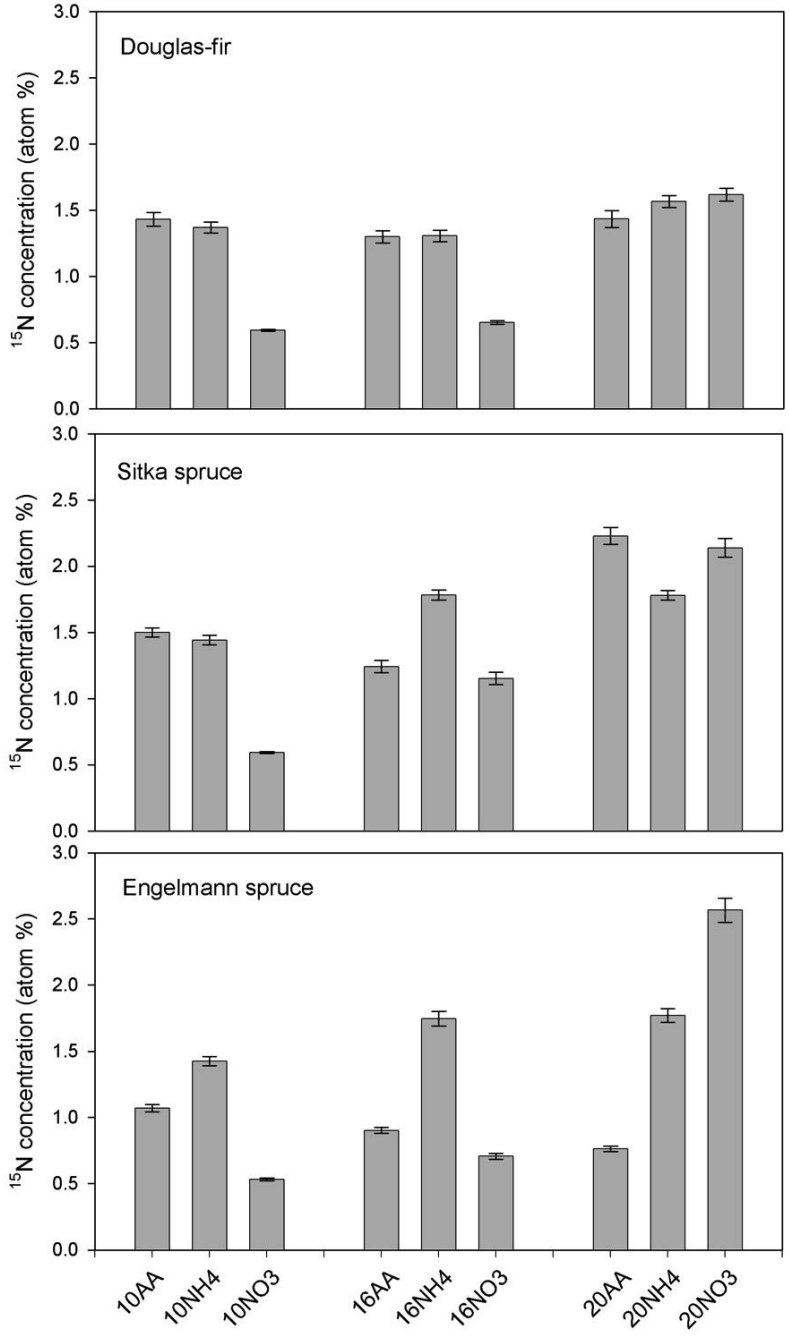

Figure 3.10. Average ^{15}N concentration (atom %) of conifers after 24 hours of uptake Douglas-fir, Engelmann spruce, and Sitka spruce seedlings were supplied with ^{15}N labeled arginine (AA), NH_4^+, or NO_3^- as a nutrient source, while being incubated at either 10, 16, or 20 C. Plants were harvested 24 h after application of ^{15}N.

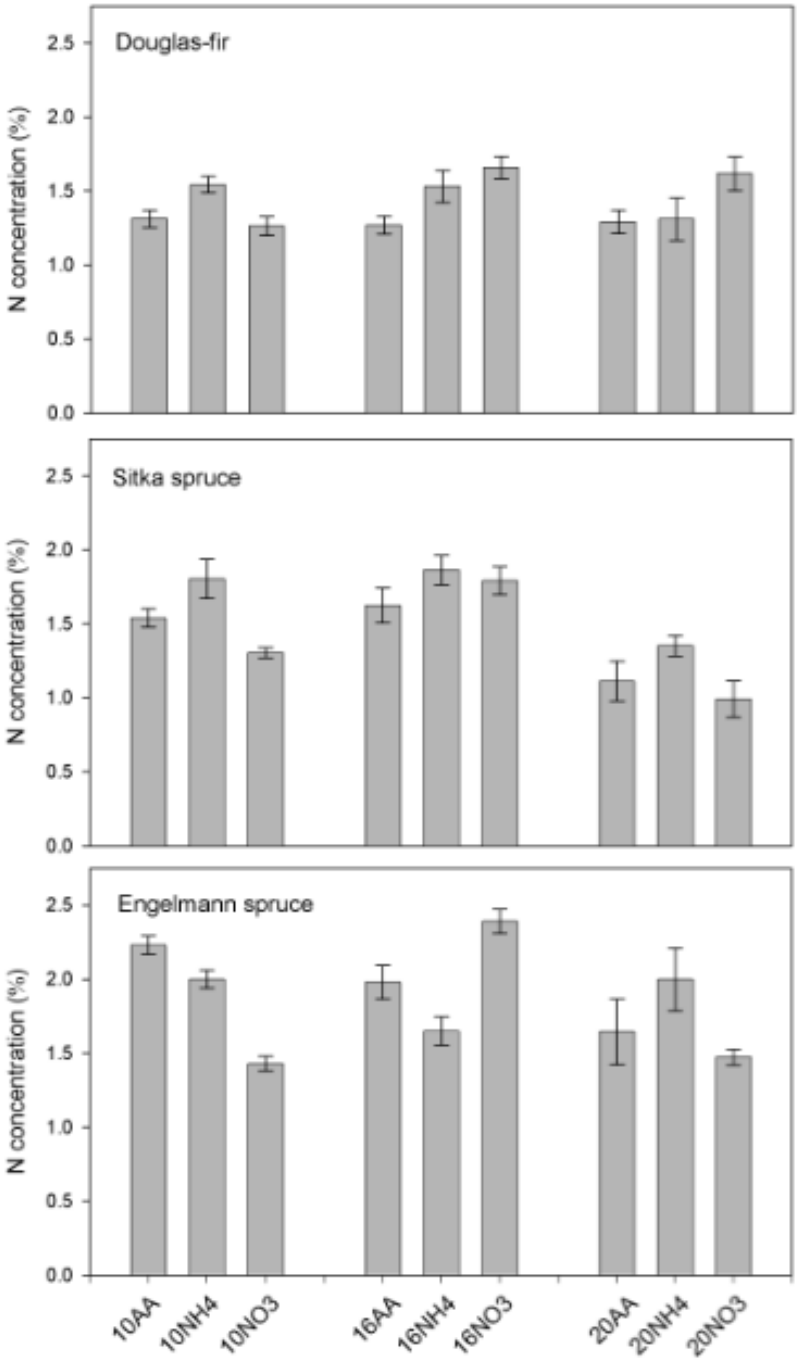

Figure 3.11. Total N concentration of conifers. Douglas-fir, Engelmann spruce, and Sitka spruce seedlings were supplied with arginine and alanine (AA), NH_4^+, or NO_3^- as a nutrient source, while being incubated at either 10, 16, or 20 °C. Plants were harvested 16 weeks after first application.

(b) N Treatment

When data was analyzed by species, N treatment significantly affected atom % ^{15}N and N concentration of Douglas-fir and Sitka spruce, and atom % ^{15}N of Engelmann spruce seedlings (Table 3.14). On average, Douglas-fir exhibited highest uptake of ^{15}N-arginine or ^{15}N-NH_4^+ (Figure 3.12), but N concentration of Douglas-fir was highest when supplied with NH_4^+ or NO_3^- (Figure 3.13). Sitka spruce also exhibited high uptake of ^{15}N-arginine or ^{15}N-NH_4^+ (Figure 3.12), and N concentration of Sitka spruce was highest when supplied with NH_4^+, but this was not significantly different from NO_3^- supply (Figure 3.13). Engelmann spruce exhibited very high uptake of ^{15}N-NH_4^+ (Figure 3.12), but N concentration of Engelmann spruce did not differ significantly among N forms (Figure 3.13).

Table 3.14. Anova table of values for the N-form treatment term for analyses of atom % ^{15}N uptake and N concentration of conifers including degrees of freedom (df), F values and and significance values at $\alpha \leq 0.05$ (p) for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. ^{15}N was applied to conifers 24 hour before harvest. The effect of N treatment was examined by species.

Douglas-fir	**df**	**F**	**p**
%15N	2	5.88	0.0053
N Conc	2	5.99	0.0049
Sitka spruce	**df**	**F**	**p**
%15N	2	4.23	0.0207
N Conc	2	8.7	0.0006
Engelmann spruce	**df**	**F**	**p**
%15N	2	9.49	0.0004
N Conc	2	0.05	0.9492

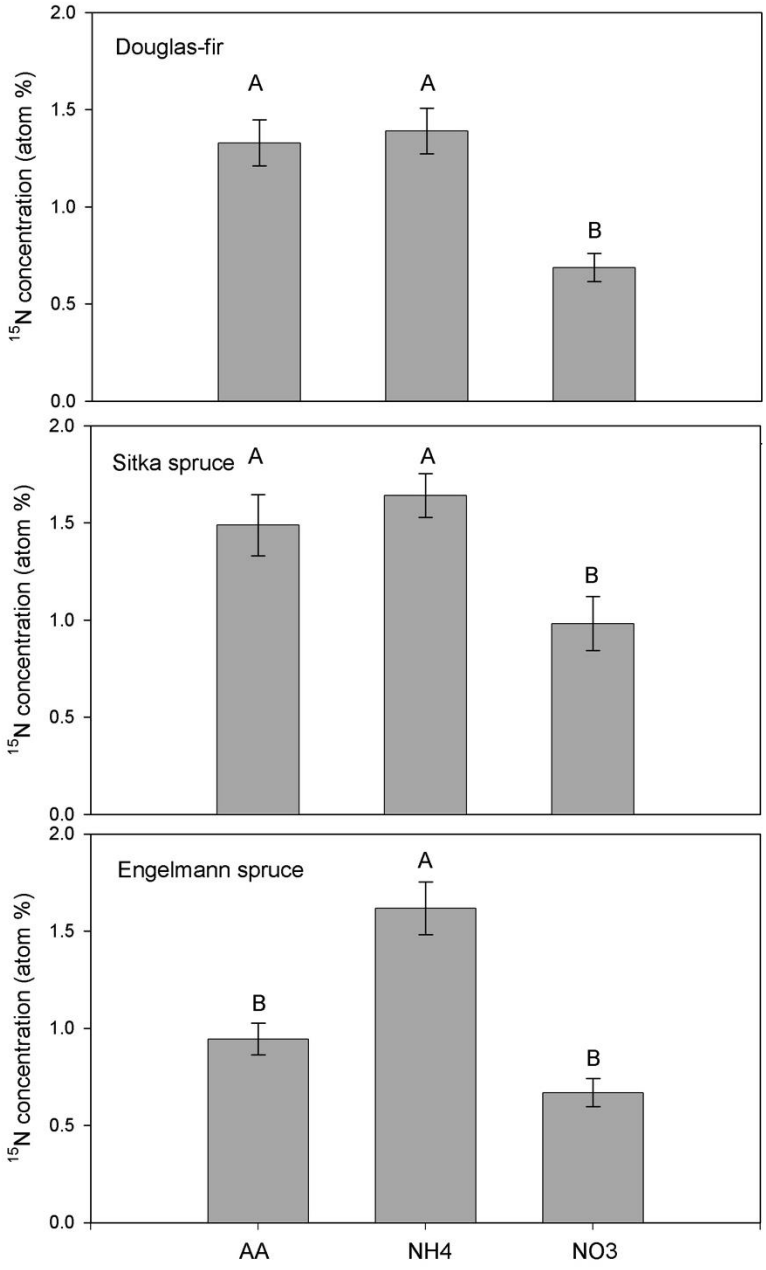

Figure 3.12. Average ^{15}N (atom %) of conifers after 24 hours of uptake Douglas-fir, Engelmann spruce, and Sitka spruce seedlings were supplied with ^{15}N labeled arginine (AA), NH_4^+, or NO_3^- as a nutrient source. Plants were harvested 24 h after application of ^{15}N.

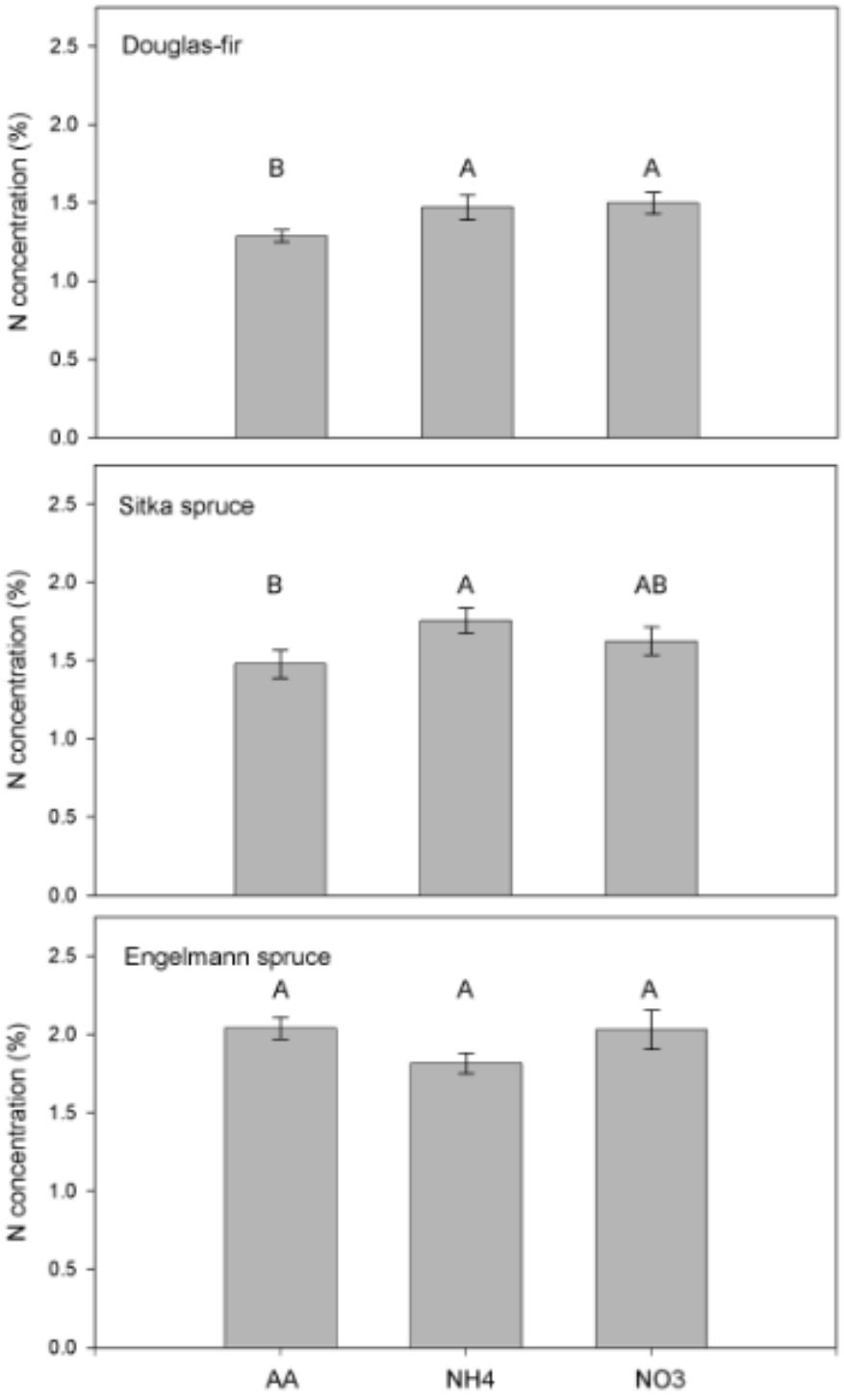

Figure 3.13. Total N concentration of conifers. Douglas-fir (DF), Engelmann spruce (ES), and Sitka spruce (SS) seedlings were supplied with arginine and alanine (AA), NH_4^+, or NO_3^- as a nutrient source. Plants were harvested 16 weeks after first application.

(c) Temperature (data by species)

When data was analyzed by species, a significant effect of temperature on atom % ^{15}N and N concentration of Sitka spruce, and Engelmann spruce and atom % ^{15}N of Douglas-fir was found (Table 3.15). On average, uptake of ^{15}N increased with temperature in all species (Table 3.16). However, N concentration of Sitka spruce and Engelmann spruce was greatest at 16 °C, on average (Table 3.16).

Table 3.15. Anova table of values for the temperature treatment term for analyses of ^{15}N uptake (atom %) and N concentration of conifers including degrees of freedom (df), F values and significance values at $\alpha \leq 0.05$ (p) for two seedlots of Douglas-fir, Engelmann spruce, and Sitka spruce. Seedlings were grown at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids. ^{15}N was applied to conifers 24 hour before harvest. The effect of temperature was examined by species.

Douglas-fir	**df**	**F**	**p**
%15N	2	6.26	0.0039
N Conc	2	1.78	0.1809
Sitka spruce	**df**	**F**	**p**
%15N	2	15.9	<0.0001
N Conc	2	36.49	<0.0001
Engelmann spruce	**df**	**F**	**p**
%15N	2	15.28	<0.0001
N Conc	2	8.04	0.0011

Table 3.16. Mean ($\bar{x}$) and standard error (SE) of ^{15}N uptake (atom %) and total N concentration (%) where a significant effect of N treatment was found. Post hoc test (Tukey) results are shown where a difference in lettering indicates a significant difference in means.

Douglas-fir	**10**			**16**			**20**		
	$\bar{x}$	**SE**	**Tukey**	$\bar{x}$	**SE**	**Tukey**	$\bar{x}$	**SE**	**Tukey**
%15N	1.101	0.129	**A**	1.077	0.118	**A**	1.532	0.134	**B**
N conc	1.940	0.006	**.**	2.114	0.100	**.**	1.996	0.098	**.**
Sitka spruce									
%15N	1.177	0.124	**A**	1.363	0.141	**B**	2.040	0.135	**C**
N conc	2.206	0.092	**A**	2.513	0.114	**B**	1.631	0.096	**C**
Engelmann spruce									
%15N	1.033	0.116	**A**	1.093	0.142	**A**	1.929	0.240	**B**
N conc	2.717	0.116	**A**	2.883	0.133	**A**	2.421	0.133	**B**

3.3.5 N in the Growing Medium

Soil data confirms that N was applied often enough to maintain the abundance of the appropriate N form in soils. The N form supplied was consistently the N form found in the greatest amount in soils (Figure 3.14, Figure 3.15, Figure 3.16; $p<0.001$; Table 3.17); however, all N forms measured were present in all soils. There was a significant effect of temperature on the concentration of nitrate of N in soils (Table 3.17).

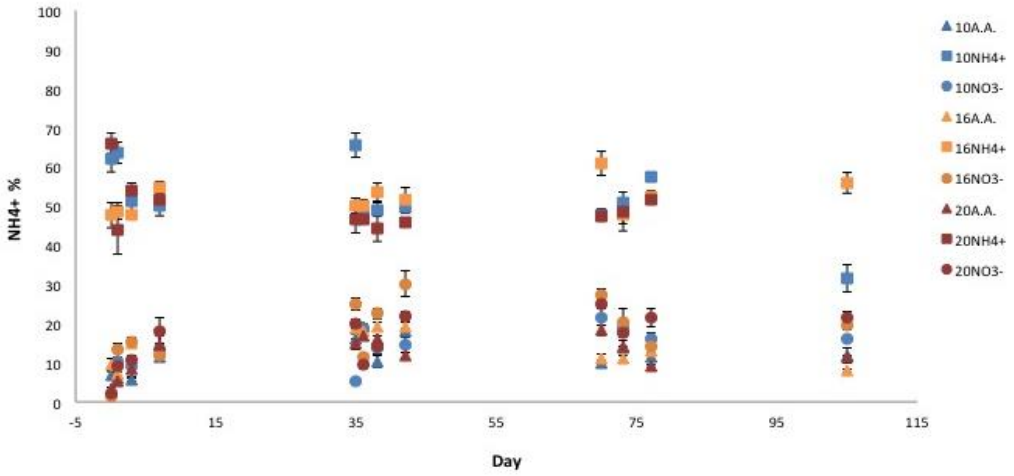

Figure 3.14. Percent of ammonium (NH_4^+) as a proportion of total soluble N in growing medium. Three N forms: NH_4^+, NO_3^-, or arginine and alanine (AA) were applied weekly to soils incubated at three temperatures (10, 16 and 20 ^oC) and NH_4^+ in soils was measured 4 hours, 1, 3 and 7 days after application on weeks 1, 5, and 9. NH_4^+ was also measured at the end of the 16-week incubation.

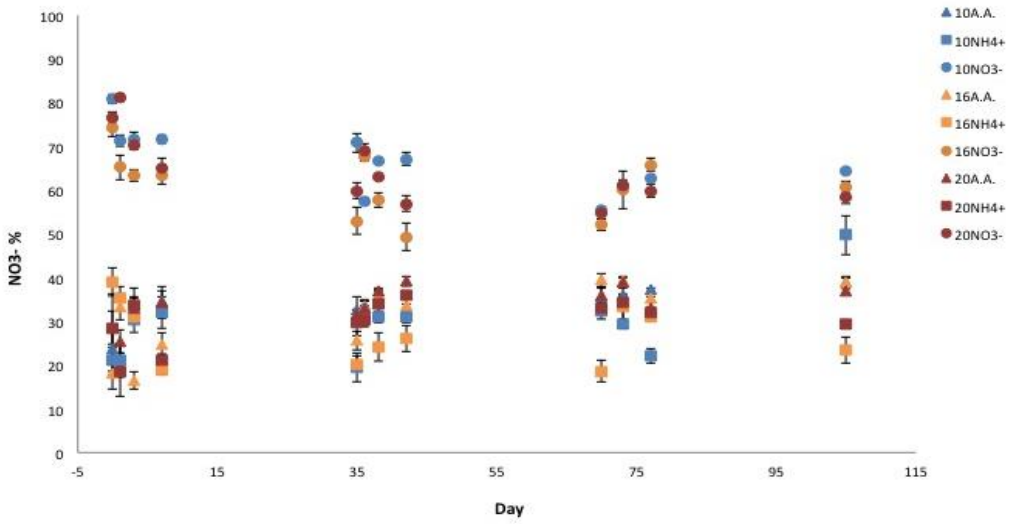

Figure 3.15. Percent of nitrate (NO_3^-) as a proportion of total soluble N in growing medium. Three N forms: NH_4^+, NO_3^-, or arginine and alanine (AA) were applied weekly to soils incubated at three temperatures (10, 16 and 20 °C) and NO_3^- in soils was measured 4 hours, 1, 3 and 7 days after application on weeks 1, 5, and 9. NO_3^- was also measured at the end of the 16-week incubation.

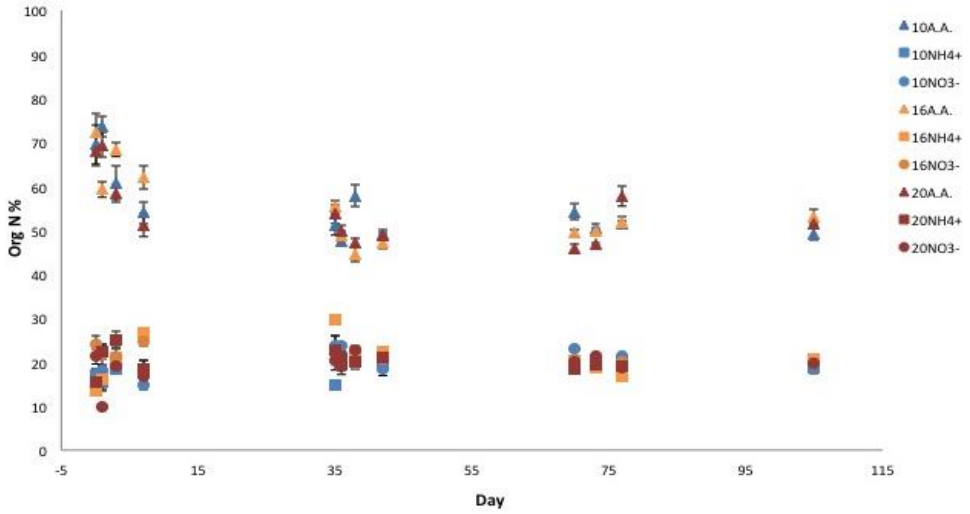

Figure 3.16. Percent of soluble organic N (Org N) as a proportion of total soluble N in growing medium. Three N forms: NH_4^+, NO_3^-, or arginine and alanine (AA) were applied weekly to soils incubated at three temperatures (10, 16 and 20 ^oC) and Org N in soils was measured 4 hours, 1, 3 and 7 days after application on weeks 1, 5, and 9. Org N was also measured at the end of the 16-week incubation.

Table 3.17. Anova table for NH_4^+, NO_3^-, and organic N concentration of soils, including degrees of freedom (df), F values and p values. Soils were incubated at either: 10, 16, or 20 °C and given N treatments of NH_4^+, NO_3^- or amino acids once a week over the course of 16 weeks. Temperature (Temp), N treatment (NTrtmt), week (Wk), and day within week effects were examined.

	NH_4^+			**NO_3^-**			**Org N**		
	df	F	p value	df	F	p value	df	F	p value
Temp	2	5.879	0.094	2	8.374	<0.001	2	0.846	0.431
NTrtmt	2	831.710	<0.001	2	522.152	<0.001	2	1130.175	<0.001
Temp* NTrtmt	4	2.411	0.668	4	1.973	0.402	4	1.400	0.233
Wk	3	15.339	<0.001	3	1.038	<0.001	3	5.427	0.001
Temp*Wk	6	1.535	0.600	6	1.925	0.023	6	0.741	0.616
NTrtmt *Wk	6	10.478	0.001	6	11.564	0.011	6	15.942	<0.001
Day(Wk)	8	1.910	0.058	8	1.580	0.128	8	1.740	0.088
Temp*Day(Wk)	16	0.860	0.620	16	1.290	0.200	16	1.630	0.060
NTrtmt *Day(Wk)	16	0.530	0.929	16	1.100	0.348	16	1.210	0.255
Temp* NTrtmt *wk	12	1.733	0.057	12	1.527	0.111	12	1.100	0.358

3.4 Discussion

My objective was to determine what preference (if any), shown by either greater N uptake or enhanced growth, three key conifer species had for soluble N forms, and if these preferences were altered by temperature. I did see differences in ^{15}N, N concentration, and growth parameters when different N forms were supplied; however *in-situ* root N uptake data was much less consistent. Furthermore, there was a change in N form preference as temperatures were increased. ^{15}N uptake and N concentrations of conifers generally supported growth data.

3.4.1 N-form Preference of Species

Species preferences for certain N forms were apparent. As van den Driessche (1978) found, Douglas-fir growth was greatest when supplied with NO_3^- under most soil conditions (especially acidic pH=4). Hawkins *et al.,* (2008) also found that Douglas-fir seedlings had higher root influx of NO_3^- than NH_4^+ when bathed in a solution containing both forms. I too saw that NO_3^- was a preferred N source for Douglas-fir, causing increases in RCD, shoot height, shoot/root weight, and total dry weight, relative to other N forms (Figure 3.6; Table 3.9). Kronzucker *et al.* (2003) determined that Douglas-fir has an uncontrolled cytosolic uptake of NH_4^+, which may lead to toxicity as NH_4^+ is thought to be poorly metabolized in this species. As NH_4^+ is a necessary intermediate in the N metabolism pathway for assimilation, inhibited growth with NH_4^+ applications has been a controversial subject (Gerendas *et al.*, 1997; Britto & Kronzucker, 2002). ^{15}N concentration of NH_4^+ was very high in Douglas-fir (Figure 3.12), which may indicate uncontrolled uptake. However, I would expect very low total N in harvested plants when supplied with NH_4^+ due to poor metabolism; as, plant N concentration in this study was similar among all three N forms for Douglas-fir (Figure 3.13), total N content of conifers would mirror biomass trends. Thus, our data supports the idea that NH_4^+ is toxic to Douglas-fir where uptake did not lead to assimilation.

Sitka spruce appears to be like other spruces and showed a preference for NH_4^+ (Ingestad & Molin, 1960; Marschner *et al.*, 1991; Lumme, 1994; Farrar *et al.*, 1995; Kronzucker *et al.*, 1997), as application of NH_4^+ increased RCD, shoot height, root and shoot weight, and total dry weight/biomass (Figure 3.6; Table 3.9), as well as ^{15}N uptake

(Figure 3.12). Nelson & Shelby (1974) also found Sitka spruce shoot height was greatest when grown with NH_4^+. Lumme (1994) and Gessler *et al.* (1998) determined that Norway spruce takes up exclusively NH_4^+ from soils, and Farrar (1995) reported that white spruce was never found on disturbed sites known for high NO_3^- content. Poor growth with NO_3^- in white spruce may be a result of poor uptake, metabolism, and cellular storage of the N form (Kronzucker *et al.*, 1997). In contrast, Sitka spruce in this study did show some influx of NO_3^- in solution (Figure 3.4) and uptake of ^{15}N-NO_3^- (Figure 3.12). The preference for NH_4^+ in Sitka spruce is thus likely not due to insufficient uptake capacity of the NO_3^- ion, but perhaps due to poor metabolism/assimilation.

Engelmann spruce seedlings exhibited greater growth with AA and NH_4^+ compared to NO_3^- for many growth parameters (Figure, 3.6; Table 3.9). Engelmann spruce is similar to white spruce, showing a preference towards NH_4^+ (Farrar, 1995, Figure 3.10); however, I also saw a similar preference for amino acids, a N form commonly found in cool areas.

Kronzucker *et al.* (1997) previously argued that evolution has caused trees to become adapted to the N characteristics of their native environments. The preferences I observed are likely due to adaptation. Douglas-fir grows in dry, warm soils and on disturbed sites where NO_3^- is relatively more abundant (Kronzucker *et al*., 1989; Prescott, 1997; Kronzucker, 2007). NO_3^- availability is relatively high in the interior soils of B.C. where our seed was collected (Hope *et al.*, 2003). Sitka spruce grows naturally in moist cooler soils where there is abundant NH_4^+. NH_4^+ concentrations have been reported to be about 400 times higher than NO_3^- concentrations in a Sitka spruce forest in Oregon (Stark & Hart, 1997), and more than ten times higher in coniferous spruce forests of the Rocky Mountains (Prescott *et al*. 1992). The preference for amino acids and NH_4^+ in Engelmann spruce may indicate an adaptation to cold areas. Organic N increases in relative availability when temperatures drop, reducing mineralization and nitrification rates (Atkin *et al.*, 2000; Kranabetter, 2007; Warren, 2009). As the alpine climate of Engelmann spruce habitat is much cooler than that of the other two species, perhaps this conifer is adapted to N forms found in cool areas. Other cold soil conifers such as Jack pine have also shown a preference for NH_4^+ (Lavoie *et al.*, 1997).

Soil temperature elicits a differential effect on the uptake of NH_4^+ and NO_3^-. Most tree species take up more NH_4^+, and this uptake increases when temperatures drop below 10 °C (Bassisirad, 2000). Plants that experience higher temperatures show a preference (increased uptake) towards NO_3^- that mirrors soil N availability (Bassisirad, 2000). However, soils are dynamic, undergo seasonal changes, and not all soils from one geographic area show a consistent pattern of N availability. For example Prescott *et al.* (2000) found similar concentrations of NO_3^- and NH_4^+ in the coniferous forests of coastal B.C., from where my Sitka spruce seed was obtained, whereas other spruce forests show relatively higher NH_4^+ concentrations (Prescott *et al.*, 1992; Hart, 1997).

Different N form utilization may lead to N-niche differentiation, which could influence plant community structure (McKane *et al.*, 2002). For example, perhaps the preference of NH_4^+ by Sitka spruce may allow it to grow alongside other species, as Sitka spruce is commonly found alongside western hemlock, western redcedar, and yellow-cedar that may take up other available N forms from soils. However, Houlton (2007) and Harrison (2007) found no evidence of niche partitioning when looking at multiple plant species from the same ecosystem.

3.4.2 N-form Preference Affected by Temperature

Whether a plant species can adjust to new environmental conditions will determine its ability to acclimate to climate change. Many growth parameters showed a change in species' N-form preference with a change in root temperature (Figure 3.5, Table 3.7), although the effect of species on preference was much stronger than the effect of temperature, as illustrated by our PCA (Figure 3.11). For Douglas-fir, the preference (in terms of growth) for NO_3^- was amplified with temperature (Figure 3.5, Table 3.7). In contrast, ^{15}N-NO_3^- uptake was lowest of the three N source treatments at low temperatures but equalled $^{15}NH_4^+$ uptake at 20 °C (Figure 3.10). Perhaps at the end of this experiment, when ^{15}N was applied, Douglas-fir seedlings were saturated with N and thus did not show uptake that correlated with previous growth.

Engelmann spruce grew best with both amino acids and NH_4^+ at low temperature, which is likely a result of the species being adapted to these N forms prevalent at cool temperatures. As temperatures increased, the preference (in terms of

growth) did not significantly change (Figure 3.5, Table 3.7), but the relative uptake of $^{15}NO_3^-$-N increased while the uptake of ^{15}N- arginine decreased with temperature (Figure 3.10). These changes in preference indicate that the uptake mechanisms for N are flexible.

Lastly, Sitka spruce also showed a change in preference with temperature. Although NH_4^+ produced the best growth of this species at all test temperatures, similar to other species; the uptake of ^{15}N-NO_3^- and growth with NO_3^- increased with temperature (Figure 3.10), while growth (in terms of shoot height and root collar diameter) with a.a. did not increase with temperature in this species (data not shown). Despite enhanced growth with NH_4^+, the uptake of ^{15}N-NH_4^+ was similar to other N forms, indicating that likely metabolism/assimilation of the different forms also changes with temperature (Figure 3.5, Figure 3.10). The uptake of NH_4^+ by Sitka spruce has previously been correlated with seasonal temperature, being higher in warmer months (Gessler *et al.*, 1998), supporting our findings.

Generally, for the three conifers, there was flexibility in preferences, and preference shifted more towards nitrate and away from organic N as temperatures increased (Figure 3.17). Plants have shown flexibility in the capacity to grow with different N forms, usually switching preference to the most abundant form in soils (Houlton, 2007). Clarkson & Warner (1979) determined that at root temperatures below 14 °C, the absorption of NH_4^+ by ryegrass plants was much greater than NO_3^-. The difference in uptake at low temperatures may be due to differences in transition temperatures of cellular compartments as uptake of NO_3^- and NH_4^+ may occur in different parts of a cell, differing in lipid concentrations (Clarkson & Warner, 1979; Miller & Cramer, 2004). Furthermore, Warren (2009) found that, when ^{15}N-labelled N forms were injected into soil, the uptake of glycine was favoured at very low temperatures and NO_3^- by warm temperatures in *Eucalyptus pauciflora*. This suggests that the trend of increased NO_3^- uptake with warmer temperature is consistent across plant species.

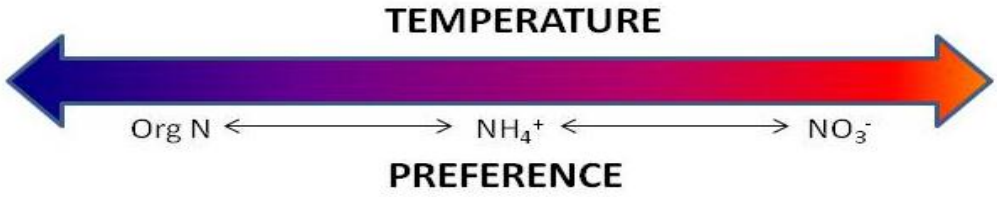

Figure 3.17. How temperature affected the relative preference of N forms by conifers. Regardless of adapted preference, conifers of this study shifted relative preference as inferred from growth and ^{15}N uptake towards the left side of the N form spectrum when incubated at cool temperatures and towards the right side when incubated at warm temperatures.

Generally, growth of conifers reflected the predicted availability of certain N-forms in relation to temperature. At lower temperatures, relatively more organic N and NH_4^+ should be available in soils (Atkin *et al*., 2000; Kranabetter *et al.*, 2007; Warren, 2009); whereas at higher temperatures, mineralization and nitrification will increase, increasing the relative amount of nitrate in soils. Despite the fact that each conifer species tended to have a N form preference, I observed that at high temperatures, the relative preference for ^{15}N-NO_3^- increased whereas the uptake of ^{15}N-AA did not change or decreased with temoerature (Figure 3.10, and Figure 3.17). Perhaps conifers have adapted to take up certain N forms at certain temperatures based on availability (as previously discussed). Or perhaps the uptake or assimilation mechanisms for a certain N form are optimal at different temperatures. For example, as we saw efflux of NH_4^+ increased at high temperatures, NH_4^+ would produce relatively less growth compared to other N sources (Figure 3.3).

3.4.3 Short-term Preferences (in-situ uptake)

MIFE data for root fluxes was much less consistent than growth data, indicating that short-term N uptake may be a poorer indicator of N form preference than longer-term growth data. It is difficult to make conclusions based on this data as there were few trends, and trends contradicted each other at times. Although there were few patterns in NH_4^+ uptake, there was a general trend of higher NH_4^+ efflux with temperature.

H^+ fluxes are used as an indicator in MIFE experiments as high efflux can indicate stress to plant roots (Hinsinger *et al.*, 2003). H^+ flux increased with temperature, but not above observed levels (Hawkins *et al*., 2008), and plant growth also

generally increased with temperature; thus, it is unlikely that stress was a major factor causing H^+ efflux from roots (Figure 3.5).

Proton-coupled ATPases drive the uptake of nutrients from the soil into the root, and are also involved in the loading of these solutes into the xylem of the plant. Protons may be co-transported with anions such as NO_3^- through symports into root cells; or protons may be co-transported with cations such as NH_4^+ (Wang *et al.*, 1994). There was constant proton efflux by root of my study species, and I saw significantly higher proton pumping in Douglas-fir at 20 °C indicating the potential for greater nutrient uptake (Figure 3.2). At higher temperatures, plant-metabolic processes occur at higher rates due to enhanced enzyme activity, more rapid diffusion, and more fluid membranes, among other factors (Reece *et al.*, 2011). Thus, H^+-ATPase activity would likely increase at higher temperatures. Furthermore, with increased metabolism and growth, I might expect greater activity of proton pumps and nutrient transporters due to higher nutrient requirements leading to amplified activity of H^+-ATPases. Ullrich & Novacky (1990) determined that the induction of NO_3^- uptake causes cytosolic alkalinization, which is due to the increased H^+-ATPase activity of the membrane in *Limnobium*. Furthermore, the conversion of NO_3^- to NH_4^+ (an intermediate step in the incorporation of NO_3^- -N into plant tissues) also produces protons that are pumped out of cells during assimilation. Roots are also likely growing more rapidly at warmer temperatures, and as cell wall acidification occurs with growth, this may increase proton efflux at warmer temperatures.

Below 20 °C, root permeability decreases and water viscosity increases, inhibiting nutrient/water uptake and consequently plant growth (Lopushinsky & Kaufmann, 1984). Previous studies have shown a correlation with plant productivity and growing temperature in Engelmann spruce, Sitka spruce, and Douglas-fir (Tabush, 1986; Delucia, 1987; Balisky & Burton, 1997). This increase in plant performance with warming is likely due to increased metabolic rates for growth, supported by increased H^+-ATPase activity (previously discussed) allowing the uptake of NO_3^-. Conifer NO_3^- uptake supported this idea as at warmer temperatures (16 °C and 20 °C) I saw more uptake than at 10 °C (Figure 3.4). Other studies have also found increased N uptake at higher temperatures (Clarkson & Warner, 1979; Garnett & Smethurst, 1999).

The highest (although not significant) NO_3^- uptake occurred unexpectedly at 16 °C not 20 °C for Douglas-fir and Engelmann spruce, suggesting that there may be other processes that temperature is affecting, causing a negative or inhibitory feedback effect on NO_3^- uptake. At increased temperatures, the membrane permeability of plant cells may increase. This was shown in poplar seedlings (Lee Kalcits, personal communication). This increase in membrane fluidity may reduce net ion uptake by plant roots as many ions can passively diffuse out of root cells, which might explain why I did not see a linear increase of NO_3^- uptake with temperature.

Plant adaptations may partially determine the optimal root-temperature for N uptake of each species (Pregitzer *et al.*, 2000). For example, if Engelmann spruce is cold- adapted and is rarely exposed to high temperatures, perhaps the ability to maintain optimal membrane fluidity in warm conditions is poor. In contrast, Sitka spruce may be adapted to mild climates and may be able to prevent nutrient leakage at warmer temperatures, but has low NO_3^- uptake in cool temperatures. Although interior Douglas-fir should also, theoretically, be adapted to warmer temperatures, McKay & Milner (2000) describe Douglas-fir as a sensitive species that is highly prone to root electrolyte leakage when handled/disturbed. Thus, although root growth potential of most western conifers is at a maximum about 20 °C, higher root temperatures in Douglas-fir have been shown to inhibit root and shoot growth (Lopushinsky & Max, 1990).

Although the concentration of N available to the roots was kept constant, I saw differences in the uptake of NH_4^+ and NO_3^- measured with microelectrodes. Generally, NH_4^+ influx only occurred at 10 °C, and efflux was exhibited at higher temperatures. This efflux may be due to two factors: membrane fluidity or root saturation. At higher temperatures, there may have been more nutrient leakage out of roots with higher membrane fluidity (previously discussed). Membrane permeability may increase with warming and cytoplasmic NH_4^+ control may decrease with higher temperatures resulting in efflux (Kronzucker *et al*., 2003). Britto & Kronzucker (2006) suggested higher efflux leads to high respiratory costs. However, high-energy costs and leakage in conifers given the NH_4^+ treatment does not explain growth data (growth increased with temperature when conifers were supplied with NH_4^+). Alber *et al.*, (2012) and von Wittgenstein (2013) have also observed NH_4^+ efflux, but efflux was thought to be due to

N saturation. Lee Kalcits (personal communication) found in poplar that NH_4^+ efflux is higher than NO_3^- efflux This is because trees grown in high amounts of ammonium undergo futile cycling, where in an effort to regulate cellular NH_4^+ levels, plants lose control of their ability to hold onto NH_4^+ (Kronzucker *et al.*, 2003). Alber *et al.* (2012) found that at 1500 μM N, efflux of NO_3^- and NH_4^+ occurred in white spruce; however, at low concentrations (50 μM), influx occurred. Saturation of N usually would occur more easily at low temperatures due to low growth rates; however, as I only measured flux at one position, NH_4^+ may be taken up at a different position while undergoing efflux at the measured position on the root. Furthermore, as I measured flux in a NH_4NO_3 solution, perhaps seedlings were obtaining enough N from the NO_3^- and excreting the excess NH_4^+. The pattern of NH_4^+ uptake was not consistent between species or seedlots, and this may be due to stress of the uptake system causing roots to behave erratically; however, the overall ammonium (and nitrate) fluxes were not very large so perhaps the variability I saw was due to slow plant growth and a consequently low N demand.

3.4.4 Adaptation or Acclimation

A goal of this study was to determine if preference in N forms by conifers was a result of adaptation (long-term, heritable physiological change) or acclimation (non-heritable physiological change occurring over the life of an individual) (Hopkins, 1999). As species showed preference consistently among growth parameters towards the same N form (Figure 3.6, Table 3.9), and preference of N was consistent with previous findings related to species and ecosystem N availability, it is likely that adaptation is the major factor causing a baseline preference in my conifers. It was difficult to match up short-term flux data and long-term growth data to determine if acclimation was also a factor; however, I can say that some acclimation does occur in these species, as preference (in terms of increased growth and ^{15}N-NO_3^- uptake), changed with the environmental test variable of temperature (Figure 3.5). Relatively few studies have shown N adaptation in conifers; however, I believe this is the first study with evidence of acclimation in terms of preference.

3.4.5 Other Findings

(a) Temperature Affects Growth

Increased plant growth with increased temperature is a trend that is well documented. In this study, generally, most growth parameters increased with temperature (Table 3.11). Bassisirad (2000) determined that the effect of soil warming on plant N uptake (and thus growth) was greater in species from warm fluctuating habitats, rather than from cold climates due to a difference in fatty acid composition and the increased operation of low affinity transport system (LATS) uptake. Thus, in conifers adapted to cold climates, temperatures may become too high for a plant to function. At temperatures outside a plant's optimal range, proteins such as key enzymes of metabolic processes may start to denature (Reece *et al.*, 2011). This is a possibility for Engelmann spruce. Shoot height increased from 10 to 16 °C but decreased once temperatures reached 20 °C (Table 3.11). Growth trends mirrored total N concentration (Table 3.16) indicating that temperatures beyond 16 °C may be past the plant's optimal range.

(b) Root: Shoot Ratio

Many root structure parameters were not significantly affected by N treatments. Major root parameters (root dry weight, surface area etc.) followed the same trends as overall growth data, and we saw a decrease in root:shoot with warming up to 16 °C, but an increase when Douglas fir and Sitka spruce were further warmed to 20 °C (Table 3.11). Tingey *et al.*, (2003) found increased temperatures led to decreased root:shoot ratio, perhaps to facilitate more CO_2 uptake as roots theoretically should take up nutrients faster and more efficiently. Furthermore, a review by Mokany *et al.*, (2006) found that root:shoot ratios were negatively correlated with shoot biomass, mean annual temperature, and forest stand height, again supporting my warming results from 10 °C to 16 °C. But past 16 °C perhaps as N applications provided conifers with abundant N for uptake, root growth increased with temperature, so uptake could facilitate increases in growth. Williams (1988) reviewed many studies that maintained shoot temperature while increasing root temperature, and found that root:shoot of conifers decreased with

temperature, but only up to their optimal temperature for growth; thus, perhaps this increase of root:shoot at 20 °C was because the plant was at too high a temperature for optimal uptake. As temperature control was affected by monitoring temperature in the centre of styroblocks, it is also possible that root temperatures in many styroblock cavities were too low for effective root growth. Evidence of this was observed in occasional freezing of edge cavities in some styroblocks held at 10 °C.

Studies on the role of N source on biomass allocation are rare, and previous studies rarely used organic N in determining the influence of N form on biomass allocation. Sitka spruce showed a higher root:shoot when plants were supplied with NO_3^-, but Engelmann spruce and Douglas fir showed a higher root:shoot with AA (Table 3.9). Heiskanen (2005) found that N form did not influence root:shoot of Norway spruce when testing NH_4^+ and NO_3^-, only. Evans *et al.* (1996), found that plants supplied with NO_3^- possessed a larger leaf area while plants supplied with NH_4^+ allocated growth towards the roots. Bauer & Bernston (2001) determined that root:shoot was lower with NH_4^+ than NO_3^- as overall biomass increased with NH_4^+. Cambui *et al*. (2011) determined that organic N increased root: shoot of conifers, and root: shoot was actually lowest for NO_3^-. This was thought to be because roots need to grow to exploit less-mobile organic N forms, whereas NO_3^- is highly mobile in soils, therefore plants can allocate more biomass to shoots. Furthermore as roots take up organic N, it would be less energetically expensive to incorporate N into proteins there, rather than break down organic N into inorganic N for transport, supporting our findings for Douglas fir and Englemann spruce. However, the difference we saw in Sitka spruce from these other studies indicates that far more studies on the effect of N form application on the allocation of biomass in conifers needs to be pursued.

3.4.6 Implications

(a) Best N form for Growth

I did not see large differences in growth with N form (Figure 3.5); however, NH_4^+ caused significantly larger RCD, shoot height, and root/shoot dry weights (Table 3.7). This was followed by NO_3^- and organic N, respectively. Currently urea is the most

commonly used N source for forest fertilization in B.C. due to low costs and high N content (Forest fertilization guidebook for B.C., 1995). Although I did not include urea as a N form, it is readily hydrolyzed into NH_4^+ in the presence of water. Ammonium was a good N source that increased growth of the two spruce species of study; however, Douglas-fir seedlings grew best with NO_3^-; thus, perhaps forestry practices should consider other N forms in cooler or warmer areas, or in seedling nurseries differing in species composition to increase productivity.

(b) Climate Change

Global climate change will likely be associated with increased soil temperatures due to increases in the number of hot days and increases in global mean air temperature (Atkin *et al.*, 2000). With global temperatures predicted to increase anywhere up to 6.5 °C in the next 100 years, it is possible that conifer growth rates will increase and the uptake of soil nutrients will also increase to meet the growth demands. This scenario assumes that soil moisture is not limiting, which it may well be in many regions. If conifer nitrate uptake increases with warming as suggested by this study, soils may be depleted of N over time as more nutrients will be stored in the biomass. Increased rates of N mineralization in soils at warmer temperatures may not match the greater growth demands of trees in forests, and growth may be limited by the amount of soil nutrients available. If growth is N-limited, the theoretical increased uptake of nitrate that was shown in all three species with warming may not occur in the long-term.

Identifying the adaptation of a conifer to N is an important first step to determine changes in distribution of temperate tree species under climate-influenced changes in the N cycle. Changes in N uptake can affect competitive abilities of conifers as soils contain differing amounts of N forms. Furthermore, as tree lines move north, conifers may find themselves in soils to which they are not adapted; thus, it is important to know if conifers are able to acclimate to new N forms. . Given the results of Chapter 1, that different soils respond differently to temperature, specific soil N changes with changing climates must be know before conclusions can be drawn about future relative competitive abilities among tree species.

3.4.7 Future Studies

Although I saw no significant differences in the effect of temperature on rates of ammonium vs. nitrate uptake between seedling species, seedlings may not perform the same way as full-sized conifers. Brix (1981) found that NH_4^+ is a good fertilizer for spruce during the first years of growth, but as trees mature, organic N fertilizers resulted in better growth. Seedling nutrient uptake per weight is usually relatively high compared to mature trees as most of the nutrient uptake is directed towards growth. In mature trees, a greater proportion of annual nutrient demand is met by internal retranslocation. Thus, perhaps seedling nutrient demand is relatively high and differential N form preferences by older trees are not evident. Future studies should be aimed at determining if the same patterns of preference exist in older trees, and if there is a difference in preference with temperature.

N uptake in conifers is highly affected by mycorrhizal associations. Fungal associations increase surface area for root nutrient uptake. When conifers were harvested, it was noted that mycorrhizal associations were visible on all conifers. Although I did not measure uptake by mycelium, the preferential growth of conifers with N form may actually be preferential uptake by mycorrhizae rather than the conifer. It is very difficult to differentiate between plant and mycorrhizal uptake of N, but similar studies examining conifer preferences with and without mycorrhizal associations could be done to determine which organism is causing the preference.

The effect of root temperature on *in-situ* NH_4^+ uptake was not able to be conclusively determined. Perhaps a larger sample size, lower N concentrations in bathing solutions (to prevent saturation), and less handling is needed to conclude the general trend of NH_4^+ uptake with temperature.

3.5 Summary and Conclusions

As research on conifer N form preferences lacks experimental manipulation of climate variables, our understanding of how conifer N preference responds to these changes, and what causes N form preferences is poor. I set out to determine the N form

preferences of three conifer species from contrasting environments (interior—Douglas-fir, coastal—Sitka spruce, and montane—Engelmann spruce), and to determine how conifer N preference and uptake rates respond to increases in soil temperature.

Warming increased N uptake by conifers. Nitrogen form preferences of conifers were seen with evidence in the form of increased RCD, shoot height, shoot and root weight, N content and total dry weight. Douglas-fir showed preference towards NO_3^-, Sitka spruce preferred NH_4^+, and Engelmann spruce showed equal preference for organic N and NH_4^+. Preference of N form did change with temperature when looking at shoot height. Generally, preferences were for reduced and organic N forms at cool temperatures and more oxidized N forms (NO_3^-) at warm temperatures. There is evidence for both adaptation and acclimation as the cause of N form preference, but adaptations to climactic factors are likely a larger factor. Differences in N uptake can affect competitive abilities of conifers as soils are usually limiting for N and contain differing proportions of N forms.

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Chapter 4: Soil Conifer Interactions

As soil N cycling may change with climate warming, so too will the N availability to conifers. As N is an important nutrient for conifer growth and is often limiting, this has implications on forest health and conifer species success. Preferences of N forms have been exhibited by conifers, and these preferences may stay the same or be altered as soils warm. If I consider the long-term possibility of tree lines moving north or to higher elevations, conifers may migrate to soils differing in N composition. The preference of conifers may then match or differ from soil N availability. In this study, I determined how N cycling in soils changed with temperature, changing the relative availability of N to conifers. I also wanted to determine what conifer N preferences were, and how these preferences were affected by warming.

4.1 Summary of Results

I saw changes in the forest soils from Jordan River with warming. Furthermore, soils from different elevations within that forest differed in their response to warming. Soils from a low elevation increased in availability of every N form, although organic N and NH_4^+ increased the most. Soils from a high elevation increased in NH_4^+ with warming, and the potential for nitrification was not detectable in these soils; indicating that a soils history is very important in its cycling abilities, and due to the large amount of factors that affect soil N cycling (pH, litter, microbial communities etc.), it is very difficult to make generalizations about changes to N forms with soil warming. However, it is likely that overall availability of N to conifers will increase, and the relative amounts of N forms will change with global warming.

I saw preferences by species (generally: Douglas-fir preferred NO_3^-, Sitka spruce preferred NH_4^+, and Engelmann spruce preferred both organic N and NH_4^+ as a N source). Furthermore, although I saw evidence of acclimation to N form, most preferences were maintained despite increases in temperature. If soils N form availabilities are likely to change, so too will the relative competitive abilities of

conifers. As climate change progresses, tree species also may migrate north to cooler climates, this will also affect the competitive abilities of conifers.

4.2 Conclusions

If I consider these conifer species on my soil of study, I can infer that Sitka spruce may have a competitive advantage in the future, due to higher amounts of available NH_4^+. Furthermore, Douglas-fir may do poorly, especially if migrating to higher elevations due to relatively less NO_3^- availability but more NO_3^- uptake/assimilation. Engelmann spruce, may prefer the available N forms, but this species shows stunted growth with high temperature, indicating that although N preference is not as affected by temperature, N uptake or other physiological processes are.

Other soils have shown differing trends in terms of N form availability. In previous studies, cooler soils tended to contain more NH_4^+ than NO_3^- (Bloom, 1981; von Wiren *et al.*, 1997). Furthermore, warm soils contained relatively more inorganic N forms (Miller & Cramer, 2004; Cookson *et al*., 2007; Andresen, 2010), while soluble organic N is thought to increase as temperatures decline, as mineralization of amino acids into NH_4^+ and NO_3^- by microbial species is inhibited (Atkin, 1996). These previous studies contradict our results, and if these trends are of the norm, it is likely that Douglas fir will be the most competitive species in a warmer climate due to high uptake of NO_3^- coupled with a higher availability of NO_3^-. Engelmann spruce, will have to endure less of its preferred N forms. However, as all species uptake of NO_3^- increased somewhat with temperature, it is possible that all forest species will adapt to newly available N forms (NO_3^-) leaving competitive advantages in regards to N, undisturbed.

4.3 Future Research

Research that makes a link between plant and soil processes that affect N cycling in the ecosystem is lacking. It is important to determine N form preferences and whether these preferences are flexible to changes in temperature, as this will determine conifer competitive capabilities in a changing climate. This study touches on this link, however, larger scale soil studies determining how various soils in BC respond to warming should

be done to predict how conifers might react in this ecosystem. Furthermore, as mycorrhizae are an important link between N in soils and plants, mycorrhizal fungi, and their role in the effect of soil warming on N form uptake by conifers should be investigated.

I saw a difference in two soils from different elevations; however, as my sample size was small (I only tested one site at each elevation) I can only conclude that different soil communities compose these groups and I do not definitively know if that difference in climate was the cause of differences in soil cycling. Furthermore, there were many possibilities as to why I saw changes in my pools of N. Future studies should be aimed at tracking what specific processes of the N cycle are changing with temperature and why.

Climate change affects both soil temperature and moisture. Although moisture is unlikely to limit plant growth on the B.C. coast, it is possible that moisture will limit growth in the interior, and in other parts of Canada. Moisture may also be significant in affecting conifer N preference, and the effects of moisture on N cycling should be studied.

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