THE NITROGEN NUTRITION OF WESTERN REDCEDAR, WESTERN HEMLOCK AND SALAL: SPECIES-SPECIFIC MECHANISMS FOR ACCESSING NITROGEN IN CEDAR-HEMLOCK FORESTS ON NORTHERN VANCOUVER ISLAND

By

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B.Sc. University of British Columbia, 1996

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN

THE FACULTY OF GRADUATE STUDIES
Department of Forestry

We accept this thesis as conforming to the required standard

University of British Columbia
June 2001
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ABSTRACT

Old-growth cedar-hemlock (CH) forests on northern Vancouver Island are characterized by thick forest floors with large total nitrogen (N) contents and low extractable NO$_3^-$ and NH$_4^+$ concentrations. Western redcedar (*Thuja plicata* Donn.), western hemlock (*Tsuga heterophylla* (Raf.)Sarge), and salal (*Gaultheria shallon* Pursh) growing in these forests are N-limited but show different productivities. Salal forms a vigorous understorey and cedar grows better than hemlock. The co-occurrence of cedar, hemlock and salal in N-poor CH forests and their respective productivities may be the result of the three species: 1. utilizing different organic and inorganic N forms. 2. having different rooting distributions allowing them to access spatially separated N pools. In this thesis, I attempted to evaluate the potential importance of these two mechanisms for providing N to cedar, hemlock and salal in these forests. In two pot trials, the abilities of the three species to take up and/or access different organic and inorganic N compounds were examined to determine species-specific differences in N form use. Cedar, hemlock and salal were all able to take up $^{15}$N & $^{13}$C-labelled glutamic acid intact and showed similar abilities to access $^{15}$N-labelled NO$_3^-$, NH$_4^+$, glutamic acid, protein and protein-tannin compounds. Nitrate and NH$_4^+$ accounted for the largest proportions of N absorbed by the three species and were the most available sources of N during a 20-day period. To examine if differences in access to spatially separated N pools is possible, the vertical fine root distributions of cedar, hemlock and salal were measured in three old-growth CH forests. Salal and hemlock had the highest root densities in the upper forest floor horizons. In contrast, cedar showed a more even distribution of fine roots in the forest floor horizons and the upper 10 cm of mineral soil. These correlated patterns suggest that salal and hemlock are forced to share a common pool of resources because of overlapping depletion zones around the roots, whereas cedar probably has access to a greater proportion of N present in the lower forest floor and mineral soil horizons. It appears that accessing spatially separated N pools may be more important than differing organic and inorganic N uptake patterns in CH forests, and may, in part, explain the different productivities of cedar, hemlock and salal in these systems.
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ACKNOWLEDGEMENTS

During the five years of my thesis, many people were of great assistance, guidance and support. Firstly, I would like to thank my supervisor, Cindy Prescott, for her inspiration, insightful comments and frankness, and my committee members, John Barker, Anthony Glass and Hamish Kimmins for their guidance, good humour and tolerance. Numerous people were of technical assistance including: Rob Guy, Brian Ellis, Les Lavkulich, Shannon Berch, Brian Holl, Roger Brownsy and Bill Ramey who provided expertise from their respective backgrounds; Leandra Blevins, Val LeMay, Tony Kozak who provided assistance with the statistical analyses; David Harris, Carol Ritland, Kirsten Hannam, Dina Schwertfeger, Karen Ferguson and Carol Dyck who assisted with chemical analyses; and Caroline Preston and Bob Copeman who willingly provided materials and supplies. Your assistance and advice were much appreciated. In addition, I would like to express my gratitude to many of my fellow students and technicians who spent many an hour washing roots, watering plants, gathering forest floor, and transplanting seedlings. Thanks to Colin Mahoney, Ben Andrew, Karen Bothwell, Doug Rowell, John Lavery, Emily Pritchard, Ben Gilbert, Candis Staley, Yuka Ota, Christina Cockle, Angela Plautz and Sarah Harper for your help. Also, thanks to Forest Renewal British Columbia (FRBC) who funded the research projects. Lastly, and definitely not least, I would like to express a very special thanks to my mother Myrna Bennett and friend Phil Cross. Without their unwavering support, confidence and countless hours, you probably would not be reading these words today.
Chapter 1

General Introduction and Background

Introduction

Cedar-hemlock (CH) forests account for approximately 50,000 ha of the managed forest land in Western Forest Product Ltd. Tree Farm License 6 (J. Barker pers. comm.). These forests are located within the very wet maritime Coastal Western Hemlock (CWHvml) biogeoclimatic sub-zone (Green and Klinka 1994) and receive an average annual precipitation of 1700 mm, 65% of which falls between October and February. The mean annual temperature is 7.9 °C with an average daily range from 2.4 °C (January) to 13 °C (August) (Prescott and Weetman 1994).

Most CH forests are in the old-growth condition and are uneven aged with some trees over 1000 years old. The overstory vegetation in CH forests is predominantly composed of cedar and hemlock, with average basal areas of 84.3 and 21.6 m²·ha⁻¹, respectively (Keenan 1993). Cedar occupies the majority of the main canopy layer, while hemlock predominates in the co-dominant, intermediate and suppressed canopy layers. Pacific silver fir (*Abies amabilis*) is also present, but is a minor component with an average basal area of 2.5 m²·ha⁻¹. The understory is largely dominated by a vigorous cover of the ericaceous shrub salal, which has an average above-ground biomass of 4.31 Mg·ha⁻¹ in CH forests (Keenan 1993). Deer fern (*Blechnum spicant*) and *Vaccinium* spp., false azalea (*Menziesia ferruginea*), bunch berry (*Cornus canadensis*) and salmonberry (*Rubus spectabilis*) are also present, and the moss layer is composed mainly of *Hylocomium splendens*, *Kindbergia oregana* and *Rhytidiadelphus loreus* (deMontigny 1992).

Thick forest floors typify old-growth CH forests. The two main humus forms found in these forests are humimors with well-developed H horizons and lignomors characterized by the presence of decomposing wood (Prescott and Weetman 1994). The depths of these forest floors have been reported to range from 0-60 cm, have an average weight of 642.5 Mg·ha⁻¹ and contain up to 1.845 Mg/ha of N (Keenan 1993). Duric Humo-Ferric podzols underlay the forest floors (Prescott and Weetman 1994). The soil parent materials are mainly sandy loam glacial tills with smaller areas of glacial fluvial, fluvial terrace or finer-textured saprolites (Lewis 1985).
Despite the large tree biomass and thick understory of salal in old-growth CH stands, these forests show low productivity. Cedar and hemlock typically display mean annual increments (MAI) of 5.25 and 1.5 m$^3$·ha$^{-1}$·yr$^{-1}$, respectively, in the first ten years of growth on cleared and burned CH sites (L. Blevins pers. comm.). Within 5–7 years of clear-cutting and burning, the initial vigorous growth of young cedar and hemlock regenerating on CH forest sites slows, and the trees develop foliar chlorosis, indicating nutrient deficiency (Prescott and Weetman 1994). Fertilizer trials have established that N is the main nutrient limiting growth in CH forests (Weetman et al. 1989). Based on net N mineralization measurements, Weetman et al. (1990) estimated that only 20–30 kg N/ha is available annually to the plants growing on eight-year-old regenerating CH sites. Therefore, although old-growth CH forests have a large tree biomass, thick understories of salal and high total N capitals, it is evident that N available for plants is low and limits growth in these forests.

Although N apparently limits productivity in CH forests, cedar, hemlock and salal differ substantially in their growth following clear-cutting and harvesting, and also show different responses to fertilization (Prescott et al. 1993a; Prescott and Weetman 1994; Prescott and Brown 1998). Of the two conifers, the growth of cedar is greater, but the species is less responsive to N and P fertilization than hemlock. Thus cedar is the preferred species for planting in unfertilized CH sites. Hemlock, in contrast, shows greater growth stagnation during regeneration, and has a lower MAI, but responds more to fertilization. Salal, unlike the two conifers, vigorously re-sprouts on CH forest cutovers from rhizomes remaining in the forest floor and occupies more than 50% of the available ground space within 8 years of harvesting (Messier and Kimmins 1991). Although it has been noted to respond positively to N and P fertilization of CH sites (G. Weetman pers. comm.) and to the application of N and S in other areas (Prescott et al. 1993a), several studies have shown that salal growth is negatively affected by N applied repeatedly and at a high rate (Prescott et al. 1993a). At rates of 400–500 kg N·ha$^{-1}$ and 1540 kg N·ha$^{-1}$ salal was almost completely eliminated while the volume growth of Douglas-fir (Pseudotsuga menziesii) was unaffected or stimulated.

The co-occurrence of cedar, hemlock and salal in N-poor CH forests, the different productivities of these three species, and their distinct responses to fertilization prompt the questions: How are the three species able to grow together in these N-poor systems? Why do they show different productivities and responses to N and P fertilization in CH forests? One possible explanation to
these growth patterns is that cedar, hemlock and salal access different N sources and potentially different amounts of N.

Within the last ten years, several researchers have examined plants growing together in N-limited systems and have suggested that individual species in such systems may have or develop mechanisms for accessing N that is relatively unavailable to other species on site (Veresoglou and Fitter 1984; Fitter 1986; McKane et al. 1990; Schulze et al. 1994; Nadelhoffer et al. 1996; Schmidt and Stewart 1997; Chang and Handley 2000). Two such mechanisms include species-specific access to N in different:

- forms
- forest floor and soil horizons

First, plants may differ in their abilities to utilize organic and inorganic N compounds. This may occur by direct intact uptake of different N compounds through mycorrhizal roots or by supporting the activities of microbial communities in the rhizosphere that supply N to the plant from different N sources. Secondly, due to different rooting distributions, access to N may be spatially separated and plants may utilize N in different forest floor and soil horizons. Both mechanisms would allow multiple plant species limited by the same resource to grow in association (Tilman 1988). Furthermore, by utilizing different N forms or spatially separating access to N, the plants may be able to get different amounts of N and would therefore vary in their productivity levels. Such species-specific mechanisms for accessing N may be important in CH forests and enable salal, cedar and hemlock to co-exist on these N-poor sites. These mechanisms may also explain the different productivities of the three species in CH forests. Therefore, in this thesis I evaluated the abilities of cedar, hemlock and salal to access different sources of N in these forests. More specifically the questions posed were:

- Do cedar, hemlock and salal differ in their abilities to take up and access organic and inorganic N?
- Do the three species spatially separate N acquisition by accessing N in different forest floor and soil horizons?
- How do these species-specific differences relate to the relative productivities of cedar, hemlock and salal on CH forests?
Nitrogen typically accounts for between 0.01 and 3.5% of the total elemental composition of agricultural and forest surface soils\(^1\) (Sowden et al. 1977; Campbell 1978; Abuarghub and Read 1988; Stevenson 1994), and in most soils, more than 90% of this N is in organic forms (Greenfield 1972; Parsons and Tinsley 1975; Anderson and Vaughan 1985; Warman and Isnor 1991; Stevenson 1994; Kelley and Stevenson 1995). The remaining N is in a mineral form, mostly as \(\text{NO}_3^-\) and \(\text{NH}_4^+\). Most of the N entering the soil system originates as compounds contained within litter or senescing tissues, but once these nitrogenous compounds are added to the soil they go through multiple decomposition, transformation and chemical fixation pathways to produce N compositions that are unique to soil. Many of the resulting organic N compounds are difficult to identify and require chemical extraction and/or acid hydrolysis prior to analysis (Stevenson 1994; Kelly and Stevenson 1995). Soil organic N is therefore classified into groups based on the analysis technique, and these groups include amino acid, protein/peptide, amino sugar, nucleic acid, ammonia, non-hydrolysable, and hydrolysable unknown N (Table 1.1) (Anderson and Vaughan 1985; Schnitzer 1991; Stevenson 1994; Kelley and Stevenson 1995). In this section, I review what is currently known about the organic N groups and their relative abundances in soils, to gain an understanding of the N potentially available for plant uptake.

**Amino Acids**

Amino acids typically account for 20-50% of the total organic N in surface soil horizons and normally comprise the majority of the identifiable organic N fraction (Greenfield 1972; Verma et al. 1975; Sowden et al. 1977; Campbell 1978; Anderson and Vaughan 1985; Abuarghub and Read 1988). Their presence in soils is largely due to the breakdown of proteins from plant, microbial and animal origin (Senwo and Tabatabai 1998). Up to 30 different amino acid types have been isolated from soil samples including the 20 amino acids found in plant cells, bacterial non-protein D-amino acids, and non-protein ornithine, diaminopilemic acid and amino-N-butyric acid molecules (Kowalenko 1978; Stevenson 1994).

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\(^1\) soil refers to both organic and mineral horizons in unless otherwise specified.
Within the soil, amino acids exist in "free", polymerized and complexed forms. "Free" refers to amino acids present in monomeric form and found within the soil solution and soil micropores. Free amino acids typically represent less than 0.002% of the total soil N (Stevenson 1994). However, in colder systems such as boreal forests, free amino acids can account for 0.3% of total N (Nasholm et al. 1998), and may be present in higher concentrations than mineral N forms (Abuarghub and Read 1988; Kielland 1995; Nasholm et al. 1998). In most other systems, however, NH$_4^+$ and/or NO$_3^-$ concentrations are higher (Kowalenko 1978).

Most of the amino acids contained in soils are tightly bound to mineral and organic colloids, and can only be recovered from soils by acid hydrolysis (3 or 6 M HCl for 16 – 24 hours) (Kowalenko 1978; Stevenson 1982; Anderson and Vaughan 1985; Schnitzer 1991). Commonly, 23–45% of total soil N is hydrolysable amino acid-N (Sowden et al. 1977; Kowalenko 1978; Abuarghub and Read 1988; Stevenson 1994). These complexed amino acids are either bound singularly or as a member of a polymer. However, it is not possible to determine their original chemical form in soils or the compounds of which they were constituents, because the acid hydrolysis treatment cleaves most peptide bonds.

Table 1.1. The fractions of soil N following acid hydrolysis (modified from Stevenson 1994).

<table>
<thead>
<tr>
<th>N fraction</th>
<th>Definition of fraction</th>
<th>% of soil N</th>
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<tr>
<td>Amino acid-N</td>
<td>free and complexed amino acids</td>
<td>30-45</td>
</tr>
<tr>
<td>Amino sugar-N</td>
<td>complexed amino sugars</td>
<td>5-10</td>
</tr>
<tr>
<td>NH$_3$-N</td>
<td>ammonia recovered from hydrolysates following distillation with MgO</td>
<td>20-35</td>
</tr>
<tr>
<td>Hydrolysable unknown-N</td>
<td>hydrolysable N not accounted for by NH$_3$, amino acids and amino sugars (contains nucleic acid derivatives).</td>
<td>10-20</td>
</tr>
<tr>
<td>Acid insoluble-N</td>
<td>nitrogen remaining in residue following acid hydrolysis</td>
<td>20-35</td>
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Proteins and Peptides

The importance of intact proteins (including peptides and polypeptides) within soil organic matter is still largely unknown (Stevenson 1982). However, it is likely that proteins account for at least a proportion of the organic N in most soils. As suggested by Warman and Isnor (1991) and Schulten and Schnitzer (1998), most of the amino acids in soils are probably in the form of proteins and peptides closely associated with other organic or mineral materials. However, due to the limitations of analysis techniques the extraction of intact proteins is difficult and results only suggest the presence of proteinaceous compounds (Stevenson 1994). Most of the methods to measure proteins in soils include the use of proteases, phenol and formic acid extractions, and cold HCl hydrolysis (Verma et al. 1975; Stevenson 1982; Kelley and Stevenson 1995) and these techniques appear to provide only crude estimates of soil proteins. In addition, as discussed above, the acid hydrolysis technique cleaves most peptide bonds, and therefore, any proteins within the soil are only accounted for as extracted amino acid monomers in the hydrolysates. As a result, little information regarding different proteins and their respective concentrations in soils has been published.

Amino Sugars

Next to amino acids, amino sugars or hexosamines represent one of the most abundant identifiable organic N compounds found in soils, and amino sugar – N can account for 3–10% of total soil N (Sowden et al. 1977; Stevenson 1994). However, unlike amino acids, amino sugars have not been found in a free state in soil solution (Kowalenko 1978; Schulten and Schnitzer 1998) and require acid hydrolysis for 6–9 hours before being detected in soils (Anderson and Vaughan 1985; Stevenson 1994). The common theory is that amino sugars are readily degraded or complexed with clay and organic colloids following appearance in the soil. D-glucosamine, D-galactosamine, muramic acid, D-mannosamine and D-fucosamine (Anderson and Vaughan 1985; Stevenson 1994) are the main amino sugars found in soil hydrolysates, but D-glucosamine and D-galactosamine are often the most dominant forms (Stevenson 1994). Of these two, D-glucosamine concentrations are usually greater, especially in soils dominated by fungi (Anderson and Vaughan 1985). Fungal cell walls are made with chitin, a polymer of N-acetyl glucosamine (Cochrane 1958; Parsons and Tinsley 1975; Anderson and Vaughan 1985; Stevenson 1994).
**Nucleic Acids**

The nucleic acid bases purine and pyrimidine are present in most soils but the N contained within these compounds typically accounts for less than 1% of the total soil N (Stevenson 1994; Schnitzer 1991; Schulten and Schnitzer 1998). However, average values of 3.1% can be found in agricultural soils (Schulten and Schnitzer 1998) and concentrations up to 7.4% have been reported in podzolic soils (Cortez and Schnitzer 1979). Nucleic acid bases are typically recovered in the hydrolysable unknown N fraction following hydrolysis, and can account for 1-3% of this fraction (Schnitzer 1991). Therefore, despite the relatively large inputs of DNA and RNA into soils, nucleic acid bases are not found in large concentrations and furthermore, are not detected in a free state within soil solutions. Similar to amino sugars, nucleic acids are probably rapidly decomposed and incorporated and/or adsorbed to organic and mineral soil materials. Specialized extraction and identification techniques are required to detect nucleic acids within soils and most of the extractions are conducted following acid hydrolysis (Anderson and Vaughan 1985).

**Ammonia**

Approximately 20-35% of the N in surface soils is in ammonia (NH$_3$)-N isolated from hydrolysate solutions through distillation with magnesium oxide (MgO) (Stevenson 1994; Anderson and Vaughan 1985). This NH$_3$-N fraction is produced during acid hydrolysis and is largely an artifact of the hydrolysis procedure. Only 50% of this N can be identified in origin (Stevenson 1994; Kelley and Stevenson 1995), but it is known that many of the NH$_3$ molecules originate from several sources including the deamination of nitrogenous molecules, amino acid and amino sugar degradation and the release of fixed NH$_4^+$ (Stevenson 1982; Kelley and Stevenson 1995; Greenfield 1972). Of the amino acids, tryptophan, serine, tyrosine, threonine and methionine amino acids are particularly susceptible to deamination during hydrolysis. Similarly, N-acetyl glucosamine molecules can also be degraded and up to 30% of these amino sugar molecules are added to the NH$_3$-N fraction. Therefore this NH$_3$-N fraction is mainly composed of amine groups or ammonium that are easily cleaved or released from soil compounds or minerals.

**Hydrolysable Unknown and Non-hydrolysable N**

Despite the proportion of soil organic N accounted for by amino acids, amino sugars, nucleic acids, and NH$_3$, 30-60% of the total soil N typically cannot be attributed to identifiable sources
(Sowden et al. 1977; Stevenson 1994; Kelley and Stevenson 1995). Following acid hydrolysis two main, predominantly unidentified groups of soil N are produced and these fractions are hydrolysed unknown N (HU-N) and acid insoluble/non-hydrolysable N (NH-N).

The HU-N fraction is the soil N released through acid hydrolysis that is not accounted for by the identifiable amino acid-, amino sugar- and NH$_3$- N fractions. Typically, HU-N accounts for approximately 10-20% of the total soil N (Anderson and Vaughan 1985; Greenfield 1972; Stevenson 1994; Kelley and Stevenson 1995). It has been postulated that 25-50% of the HU-N fraction is composed of hydrolyzed derivatives of non $\alpha$-amino N compounds including arginine, tryptophan, lysine, histidine and proline (Kelley and Stevenson 1995) and 1-3% is purine and pyrimidine bases (Schnitzer 1991). However, usually less than half of the N contained within this fraction can be identified.

The NH-N fraction is contained in the residue remaining after acid hydrolysis and typically represents 20-35% of the total soil N (Stevenson 1994; Anderson and Vaughan 1985; Kelley and Stevenson 1995). The identities of the nitrogenous compounds in this acid insoluble fraction are largely unknown, but may include structural components, N-phenyl amino acids (amino acids bound to aromatic rings), heterocyclic ring members (-NH- in indole or pyrrole rings and -N= in pyridine rings), and structural “bridge” constituents linking together two quinone molecules (Stevenson 1982; Schnitzer 1991; Kelley and Stevenson 1995). Pyrolysis of soil HU-N has determined the presence of at least 81 different heterocyclic N containing compounds within this fraction (Schulten and Schnitzer 1998). It is believed that a large portion of these ringed compounds originate as pyrrole rings from plant chlorophylls and cytochromes and purine and pyrimidine nucleic acid bases (Mengel 1996).

As is apparent from the above discussion, our current understanding of the composition of N compounds in soils is very poor and is largely bound by limitations in analysis techniques. At best, only 50% of organic N compounds can be partially characterized (Warman and Isnor 1991; Stevenson 1994). Organic soil N compounds are very heterogeneous and are the products of many processes including biotic and abiotic transformation, complexation, polymerization and fixation processes. As of yet, we do not have the ability to isolate and identify most of the N compounds or complexes formed through these soil processes. However, the assemblage of these compounds comprises the pool of N experienced by plant roots. Therefore, to improve our
understanding of N forms potentially available for plant uptake and their relative importance in plant nutrition, a characterization of N forms present in the soil is necessary.

**Inorganic and Organic N Uptake By Plants**

Nitrogen is abundant in the lithosphere and atmosphere and these two spheres comprise a summed weight of $1.95 \times 10^{20}$ kg of N (Porter 1975). However, despite its apparent abundance the N in the atmosphere and lithosphere pools is relatively inaccessible to biota, and N limits the growth of much terrestrial vegetation (Kimmins 1986; Vitousek and Howarth 1991; Chapin et al. 1993; Killam 1994). For most plants, the majority of N absorbed by plants comes from the soil and this N in the soil has accumulated because of biotic N fixation and deposition. As outlined in the previous section a large proportion of this N is in organic forms with typically less than 10% as NO$_3^-$ and NH$_4^+$. Therefore, plant roots experience a large variety of N compounds. Nitrogen limits the growth of most vegetation and it is necessary to understand what N compounds can be taken up by plants. The abilities of plants to take up organic and inorganic N compounds may be different and these differences may facilitate co-occurrence of species and reduce competition for the same N sources. To better understand the potential importance of organic and inorganic N uptake by plants, I summarize what is currently known about species-specific organic and inorganic N plant nutrition.

**Inorganic Nitrogen Nutrition**

Nitrate and NH$_4^+$ are the main forms of inorganic N absorbed and utilized by plants (Marschner 1995; Haynes and Goh 1978; Fredeen and Field 1992), and a number of trials have demonstrated that plants differ in their abilities to take up these two mineral N forms. For example, species growing in acidic soils and soils with low redox potentials often show a greater ability to take up NH$_4^+$, while calcicole plants present in more basic soils tend to utilize NO$_3^-$ preferentially (Marschner 1995). In a pot trial, Krajina et al. (1973) compared the growth of Douglas-fir, lodgepole pine (*Pinus contorta*), western hemlock and western redcedar seedlings supplied with different ratios of NO$_3^-$:NH$_4^+$ and found that the four species differed in their responses to the N treatments. Douglas-fir and cedar accumulated the largest biomass on NO$_3^-$, while lodgepole pine and hemlock grew best on NH$_4^+$ and a mixture of NO$_3^-$ and NH$_4^+$, respectively. Similar conclusions were reached by van den Driessche (1971) in his examination of the growth of Sitka
spruce (*Picea sitchensis*), Douglas-fir, white spruce (*Picea glauca*), and western hemlock. The results of these trials suggest that when grown in the same environment and substrate, plant species differ in their NO$_3^-$ and NH$_4^+$ uptake patterns. However, when N uptake is studied under a variety of conditions, the same plant species may show different uptake patterns.

Marschner et al. (1991) showed that four-year-old non-mycorrhizal Norway spruce (*Picea abies*) seedlings transferred to hydroponic solutions absorbed NH$_4^+$ ions up to eight times faster than NO$_3^-$ molecules. Furthermore, NO$_3^-$ uptake was initially delayed until NH$_4^+$ concentrations were reduced to levels below 100µM, suggesting that higher NH$_4^+$ levels inhibited the uptake of NO$_3^-$. This inhibitory effect of NH$_4^+$ on NO$_3^-$ uptake has been noted by other researchers (Vessey et al. 1990; Marschner et al. 1991; Fredeen and Field 1992). However, Marschner et al. (1991) found very different results in a 60-year-old spruce stand growing at Mauzenberg in Germany. Roots taken from the field and placed in a 10:1 NO$_3^-$:NH$_4^+$ treatment solution (ratio determined by the extractable NO$_3^-$ and NH$_4^+$ concentrations in the forest soil) absorbed NO$_3^-$ at a higher rate than NH$_4^+$. Therefore, despite laboratory results identifying NH$_4^+$ as the preferred N source, NO$_3^-$ may comprise the main form of N taken up by Norway spruce in some field situations.

Dissimilar results have also been reported in trials examining inorganic N uptake by white spruce (van den Driessche 1971; Kronzucker et al. 1997), western hemlock (van den Driessche 1971; Krajina et al. 1973; Knoepp et al. 1993), lodgepole pine (Krajina et al. 1973; Bigg and Daniel 1978), and Douglas-fir (van den Driessche 1971; Krajina et al. 1973; van den Driessche and Dangerfield 1975; van den Driessche 1978; Bigg and Daniel 1978). Kronzucker et al. (1997) examined NO$_3^-$ and NH$_4^+$ uptake by non-mycorrhizal white spruce seedlings in a hydroponic culture. When the two molecules were supplied separately, NH$_4^+$ uptake was up to 20 times that of NO$_3^-$. From these results, Kronzucker et al. (1997) concluded that white spruce is poorly adapted to utilize NO$_3^-$. However, in the study by van den Driessche (1971), the largest dry weights of spruce seedlings occurred when equal concentrations of NO$_3^-$ and NH$_4^+$ were supplied. Several studies have also compared the growth of western hemlock on NO$_3^-$ and NH$_4^+$ and had different results (van den Driessche 1971, Krajina et al. 1973 and Knoepp et al. 1993). In the first two studies, seedlings were grown in sand and sand/peat mixtures and produced the largest biomasses on mixed N sources. In contrast, in a hydroponics study, Knoepp et al. (1993) determined that net NH$_4^+$ uptake was twice that of NO$_3^-$ during a 24-hour period, and over twelve days, NH$_4^+$ was depleted more quickly in a mixed NO$_3^-$ and NH$_4^+$ solution.
These dissimilar patterns of N uptake suggest that the absorption of NO$_3^-$ and NH$_4^+$ is not fixed by species and can be strongly influenced by environmental and thus trial conditions. Studies have examined N concentration, light levels, temperature, pH, rooting substrate and mycorrhizae and have shown that all can influence NO$_3^-$ and NH$_4^+$ uptake. Solution N concentrations can affect NO$_3^-$ and NH$_4^+$ uptake patterns even when ratios of the two N ions are maintained. Marschner (1995) made reference to a study that showed that maize grew best on NO$_3^-$-dominated N sources at high N levels but not at the lowest N concentrations. This influence of total N concentrations on NO$_3^-$ and NH$_4^+$ uptake patterns has been noted by other researchers and the main conclusion has been that species-specific preferential N uptake patterns change according to both total N concentrations and the proportions of NO$_3^-$ and NH$_4^+$ (Stock and Lewis, 1984; Vessey et al., 1990; Marschner et al. 1991; Marschner 1995).

Differences in the patterns of NO$_3^-$ and NH$_4^+$ uptake by the same plant species have also been found in response to available light levels. Lavoie et al. (1992) showed that Jack pine (Pinus banksiana) seedlings exposed to full sunlight grew best on NH$_4^+$, but when seedlings were grown in shade, the biomass of plants fed NO$_3^-$ and NH$_4^+$ were similar. However, a study of tropical Piper species found that NO$_3^-$ uptake was depressed under low light conditions (Fredeen and Field 1992), and this phenomenon has been noted by other researchers (Knoepp et al. 1993). These results suggest that light may influence NO$_3^-$ and NH$_4^+$ uptake patterns and that individual plant species may be affected differently.

Soil temperatures and rhizosphere pH are two other variables that can also affect uptake of NO$_3^-$ and NH$_4^+$ by plants. Low soil temperatures often inhibit the uptake of NO$_3^-$ by plant roots (Haynes and Goh 1978; Vessey et al. 1990; Marschner 1991), so species growing in colder soils may absorb relatively more NH$_4^+$. Under warmer soil conditions, plants of the same species may absorb larger amounts of NO$_3^-$-N. Frota and Tucker (1972) showed this in their study of lettuce (Lactuca sativa). At 8 °C, NH$_4^+$ was preferentially taken up, whereas when temperatures were raised above 23 °C, NO$_3^-$ was the dominant form of N absorbed. Soil pH can also effect NO$_3^-$ and NH$_4^+$ uptake (Haynes and Goh 1978; Stock and Lewis, 1984; Vessey et al. 1990). At soil pH of 4-5.5, Sitka spruce and Douglas-fir accumulated higher biomass on NO$_3^-$-enriched substrates, but under more basic conditions, both species grew better on NH$_4^+$ (van den Driessche 1971; van den Driessche 1978).
Rooting substrate can also significantly influence NO$_3^-$ and NH$_4^+$ uptake rates. Bigg and Daniel (1978) showed that when lodgepole pine seedlings were grown in perlite and sand substrates, the seedlings produced the highest dry weights on mixed NO$_3^-$ and NH$_4^+$ and 100% NH$_4^+$ treatments, respectively. Similarly, Engelmann spruce (Picea engelmanni) accumulated more biomass on NH$_4^+$ when grown in sand. Nitrate and NH$_4^+$ have very different mobilities in soils; NO$_3^-$ diffusion can be ten times faster than that of NH$_4^+$ (Paul and Clark 1996). Also, because of different ionic charges, NH$_4^+$ is often bound to cation exchange sites in soils. Therefore, soil or substrate type can substantially influence availabilities and uptake rates of NO$_3^-$ and NH$_4^+$. This has particular implications in N uptake trials that use hydroponic systems where the mobilities of NO$_3^-$ and NH$_4^+$ are virtually equal.

An additional test condition, which can influence N uptake rates, is the presence or absence of mycorrhizal fungi in the root systems. Mycorrhizae can significantly influence the abilities of plants to take up and access nutrients (Finlay et al. 1992; Carlile and Watkinson 1994), and mycorrhizal fungi may also affect plant growth on NO$_3^-$-N versus NH$_4^+$-N sources. When cultured independently of a host plant, mycorrhizal fungi can differ in their abilities to grow on the two N sources (Finlay et al. 1992). Therefore, although very few trials have compared inorganic N uptake by mycorrhizal and non-mycorrhizal plants, it appears that the presence of mycorrhizal fungi may influence NO$_3^-$ versus NH$_4^+$ uptake patterns (Wallenda and Read 1999). Trials examining organic N nutrition (discussed further below) also demonstrate that mycorrhizae significantly alter the relative uptake patterns of organic N compounds.

It is evident that a variety of environmental, soil and physiological conditions affect NO$_3^-$ and NH$_4^+$ uptake patterns within a species. This plasticity of uptake makes the comparison of trial results difficult (Adams and Attiwill 1982) and points to the necessity of recognizing trial conditions when interpreting results. Furthermore, when researchers want to accurately estimate inorganic N uptake patterns of plants growing in natural systems, it is necessary to reproduce many of the conditions found in the system of interest. Nutrient concentrations, temperature, soil type, pH and mycorrhizal colonization are several variables which can influence NO$_3^-$ and NH$_4^+$ uptake and should be considered in trial design.
Organic Nitrogen Nutrition

Until recently, it was commonly believed that inorganic N was the only form of N available for plant uptake in natural systems (Schimel and Chapin 1996). According to this assumption, plants rely on the microbial community to mineralize organic N, and have to compete with both microbes and other vegetation for inorganic N in the soil. However, in some ecosystems, plant growth and N uptake exceed the amount of N mineralized (net) annually, and this was interpreted as an indication that inorganic N was not the only form of N available to plants (Chapin et al. 1993; Raab et al. 1996; Schimel and Chapin 1996). In other ecosystems, there is no measurable net N mineralization during the growing season, and the majority of the inorganic N is immobilized (Raab et al. 1996; Schimel and Chapin 1996). Furthermore, in arctic and alpine ecosystems with low microbial activity (Kielland 1994), free amino acid concentrations can be 4–8 times higher than concentrations of mineral N (Chapin et al. 1993). Therefore, it has been hypothesized that some forms of organic N are potentially available for plant uptake and may account for the growth that cannot be explained solely by inorganic N.

Several studies have focussed on the abilities of arctic and alpine plant species, that are typically non-mycorrhizal (here after referred to as “naturally non-mycorrhizal”), to take up organic forms of N. This research is of considerable importance because it determines the physiological capacity of plants to absorb organic N molecules without the assistance of a fungal symbiont. Results from studies conducted in these cold systems strongly suggest that some of these plants have the ability to take up amino acids and, in some cases, may be able to produce larger biomasses on amino acids than on NH$_4^+$. Chapin et al. (1993) compared the growth of *Eriophorum vaginatum* and *Hordeum vulgare* germinants on amino acids, NO$_3^-$ and NH$_4^+$. They found that *E. vaginatum* accumulated the largest biomass on the amino acid treatment and that *H. vulgare*, a species adapted to mineral soil with lower free amino acid concentrations, grew significantly better on NO$_3^-$ and NH$_4^+$. In an investigation of the alpine sedge, *Kobresia myosuroides*, plant dry weights were 2.1 times larger on glycine-enriched treatments relative to NH$_4$NO$_3$ solutions (Raab et al. 1996). Kielland (1994) showed comparable results in his examination of the organic N nutrition patterns of ten different arctic plant species. The roots of most of the naturally non-mycorrhizal species absorbed glycine at a faster rate than NH$_4^+$, and for some species, glycine was estimated to account for up to 76.2% of the N taken up annually. Amino acids therefore appear to be important sources of N to many of the naturally non-mycorrhizal plants growing in arctic and alpine systems.
Mycorrhizal fungi are important in the nutrition of most terrestrial vegetation. Over 80% of all dicotyledenous plants, 79% of all monocots, and all gymnosperms share mycorrhizal associations with fungi (Wilcox 1991). Therefore, mycorrhizal fungi should be included when examining plant uptake of N compounds, especially organic N because some forms cannot be taken up without the assistance of the fungal symbiont (Bajwa and Read 1985; Abuzinadah and Read 1986b; Abuzinadah et al. 1986; Turnbull et al. 1996).

The abilities of mycorrhizal plants to take up simple organic N compounds including amino sugars and amino acids have been examined by several researchers, mostly under aseptic laboratory conditions (Stribley and Read 1980; Bajwa and Read 1986; Kerley and Read 1995; Cliquet et al. 1997). Kerley and Read (1995) investigated the uptake of amino sugars by non-mycorrhizal and ericoid mycorrhizal plants. They showed that *Vaccinium macrocarpon* and *Calluna vulgaris* colonized with *Hymenoscyphus ericae* took up greater amounts of N-acetyl glucosamine than the non-mycorrhizal equivalents. Similarly, Stribley and Read (1980) grew *V. macrocarpon* in mycorrhizal and non-mycorrhizal states on NH₄⁺ and five different amino acid N sources. They found that the colonized plants outgrew non-mycorrhizal plants on all the treatments and that of the mycorrhizal plants, the ones growing on glycine, alanine and glutamine, produced the largest shoot biomasses. In addition, after 60 days, the shoot weights of the colonized plants growing on the three amino acids were greater than those in the NH₄⁺ treatments. In a similar trial with *Lolium perenne* colonized with the vesicular arbuscular mycorrhizae (VA) *Glomus fasciculatum*, Cliquet et al. (1997) found that the presence of the fungus in the roots systems significantly increased the growth and total N contents of *L. perenne* in the serine and aspartic acid treatments relative to the non-mycorrhizal plants. Uptake of amino acids by mycorrhizal plants growing in situ has also been investigated. Nasholm et al. (1998) examined glycine uptake by mycorrhizal Scotch pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), *Vaccinium myrtillus* and *Deschampsia flexuosa* growing in a boreal forest and found that at least 42% of the absorbed amino acid was taken up intact by all the plants. Thus, the plants were able to compete with microbes for glycine under natural conditions. These trials outlined above also indicate that all three mycorrhizal types, VA, ecto and ericoid, can take up amino acids.

Because of the ability of mycorrhizal plants to take up amino acids, researchers started investigating the uptake of more complex organic N compounds such as chitin and proteins.
under controlled laboratory conditions (Bajwa and Read 1985; Abuzinadah and Read 1986b; Abuzinadah et al. 1986; Abuzinadah and Read 1989a; Abuzinadah and Read 1989b; Finlay et al. 1992; Kerley and Read 1995). Kerley and Read (1995) established that mycorrhizal *V. macrocarpon* and *C. vulgaris* accumulated significantly greater biomass on chitin than did the non-mycorrhizal plants over a 60-day period. Similarly, Abuzinadah et al. (1986) found that lodgepole pine inoculated with *Paxillus involutus* had higher average N content and biomass relative to the non-mycorrhizal plants when grown on the protein bovine serum albumin (BSA) for 120 days, and took up more N when grown with BSA than with NH$_4^+$.

However, mycorrhizal fungi differ in their capacities to utilize organic compounds, especially the more complex forms. For example, Finlay et al. (1992) showed that lodgepole pine colonized by a pink, unidentified ectomycorrhizal fungi took up similar amounts of N when grown on NH$_4^+$ and BSA. However, when the conifer seedlings were infected with *Telephora terrestris*, the plants in the BSA treatment had significantly lower N contents, equivalent to the amount of N found in the non-mycorrhizal seedlings. In a study of peptide uptake by birch (*Betula pendula*) inoculated with three different fungi, Abuzinadah and Read (1989a) found that seedlings colonized with *P. involutus* had lower growth and N contents. Differences in the abilities of mycorrhizal fungal species to access complex organic N have led to the classification of fungi as either "early" or "late" successional stage (Finlay et al. 1992). The late-stage fungi have greater ability to produce necessary exo-enzymes and take up complex organic N compounds (Bajwa et al. 1985; Abuzinadah and Read 1989c; Leake and Read 1990). Conversely, the early-stage fungi have poor abilities to use complex organic N compounds and are mainly restricted to mineral and simple organic N sources such as amino acids and amino sugars. This theory has not been rigorously tested, but it is evident that mycorrhizal fungi do differ in their capacities to utilize complex organic N compounds and therefore fungal species composition should be considered when evaluating organic N uptake by mycorrhizal plants in natural systems.

Mycorrhizal type is also thought to affect the ability of plants to access organic N compounds. The long-standing hypothesis is that only ericoid and to a limited degree ectomycorrhizae are able to utilize organic N compounds (Read 1991; Michelsen et al. 1998), and the results of several studies support this hypothesis. In addition to the aforementioned trials examining amino acid, amino sugar, chitin and protein uptake by mycorrhizal plants, Bending and Read (1996a) examined the abilities of ericoid and ectomycorrhizal fungi to access protein N bound to tannins.
Only three of the eighteen examined ectomycorrhizal fungi could grow on the N source while all the tested ericoid fungi had the capacity. Although the abilities of the fungi to take up and transfer the N to a host plant were not examined, the results suggest that ericoid mycorrhizae have a greater capacity to use more complex organic N compounds. In contrast to ericoid and ectomycorrhizae, VA mycorrhizae are believed to have limited abilities to utilize organic N compounds. However, there has been very little work examining VA mycorrhizal plants or fungi and therefore this assumption is largely untested. As outlined above, previous studies demonstrated that VA mycorrhizal plants are able to absorb amino acids, and therefore VA colonized plant may also be able to utilize a variety of organic N compounds.

Many mycorrhizal and some naturally non-mycorrhizal plants are able to take up amino acids and many mycorrhizal plants can utilize both simple and complex organic N compounds. However, these abilities appear to vary by species. Our understanding of the importance of organic N in the nutrition of plants growing in natural systems is still poor. The availabilities of many of the complex organic N compounds present in soils have not yet been measured and the capacities of plants to utilize complex organic N compounds while growing in natural systems is still unknown. More work should compare the capacities of VA, ecto and ericoid mycorrhizal plants to take up complex organic N compounds. Furthermore, because the capacities of mycorrhizal fungi to enzymatically degrade complex organic N compounds is species-specific, research should include the range of fungal species found on a plant root system in order to accurately access organic N uptake in the natural system of interest.

**Fine root production and distribution**

Fine roots (< 5 mm) typically only account for about 1% of the total biomass of forests, but are very important in plant growth and nutrition (Grier et al. 1981). Most nutrient and water uptake occurs across the fine root surface, and therefore, these absorptive organs play a critical role in forest productivity. Plants commonly allocate a large amount of photosynthate to fine root biomass, and as much as two-thirds of annual net primary productivity (NPP) may be partitioned to the growth and maintenance of these organs (Grier et al. 1981). The annual investment in fine roots is comparable to or exceeds that allocated to leaf and needle biomass, indicating the relative importance of fine roots in plant survival and growth (Persson 1978; Vogt 1991).
Therefore, factors that influence root development will also probably affect the plant's productivity and success. Furthermore, like overlapping leaf areas and variable light interception in mixed forests, fine roots and interactions between the fine roots of different species would also be expected to influence community composition and plant productivities. To better understand the growth of plants in a forest community, it is therefore necessary to evaluate this below-ground component of the forest. What affects the production of fine roots? What influences fine root distributions? In this section I address both questions, reviewing what is currently known about the factors influencing fine root production and distribution patterns.

Factors influencing fine root production
A large amount of research has been conducted to determine the main factors affecting fine root production and turnover, particularly the effects of site quality, stand development/stage and season. There has been considerable debate over the relationship between fine root productivity and site quality. Fine root production may be highest on sites with low resource availability to increase access to nutrients, and the results of many studies comparing weights of live and dead roots on different sites support this hypothesis (Keyes and Grier 1981; Vogt et al. 1983; Landsberg and Glower 1997). Vogt et al. (1985) also demonstrated that repeated fertilization decreased total fine root biomass, further supporting the idea that fine root production is greater on nutrient poor sites. However, several studies using alternative estimation techniques have produced contrary results (Aber et al. 1985; Nadelhoffer et al. 1985). Nadelhoffer et al. (1985) assessed thirteen deciduous and evergreen forests in southern Wisconsin and found an inverse relationship between fine root production and average fine root biomass. These results are consistent with the hypothesis posed by Grime (1977) and Chapin (1980) that, although standing biomass is often higher on poor sites, fine root production and turnover is greater on sites with higher nutrient availability. According to this hypothesis, on poor sites, larger fine root biomasses result from the maintenance of long-lived root systems. However, despite the number of trials examining the relationship between fine root productivity and site resource availability, neither hypothesis has been conclusively disproven and the influence of site quality on fine root production is still poorly understood (Aber et al. 1985). Fine root production in natural systems is very difficult to measure. The life spans of individual roots are difficult to follow and therefore most of the fine root production estimates are based on formulas with non-validated assumptions. An accurate understanding of the effects of site quality on fine root production will
therefore not be gained until measurement techniques are improved and comprehensively applied to natural systems.

Fine root production in forests has been shown to change with stand age (Vogt et al. 1980; Grier et al. 1981; Kozlowski et al 1991). In general, total fine root biomass increases with stand age and levels-off after crown closure (Vogt et al. 1981; Vogt et al. 1987). With the maturation of the stand and the development of the overlaying forest floor, the fine root biomass often expands into the organic matter (Grier et al. 1981; Vogt et al. 1981). Frequently, 30-60% of the fine roots can be found in these developed surface horizons (Ehrenfeld et al. 1992). Vogt et al. (1983), however, showed that the plateauing of fine root biomass with crown closure is not consistent and may differ with site productivity. In an examination of Douglas-fir chronosequences with different productivities, they found that following crown closure, fine root biomass levels were maintained on the poor sites but declined on the sites with higher nutrient availability.

Fine root biomass can also change significantly during the year. Fine root production is not linked temporally to above-ground growth (Persson 1983; Persson 1978) and is commonly thought to be bimodal, with greatest production occurring in the spring and in the fall (Vogt et al. 1981; Grossnickle 2000). The results from several studies have supported this hypothesis (Ford and Deans 1977; Grier et al. 1981; Cote et al. 1998), but some research has indicated that fine root production is unimodal with peaks in the spring (Keyes and Grier 1981; Burton et al. 2000), fall (Vogt et al. 1981) or summer (Gholz et al. 1986; Burton et al. 2000). In other studies, fine root biomass did not change significantly during the year (Keyes and Grier 1981; Nadelhoffer et al. 1985; Gholz et al. 1986) or showed high within-year variability (Persson 1978). These inconsistencies suggest that annual root growth is not strictly determined by the time of year, and many authors have concluded that fine root production is more influenced by environmental conditions (Persson 1983; Veresoglou and Fitter 1984; Fogel 1991; Fredericksen and Zedaker 1995; Landsberg and Gower 1997; Cote et al. 1998).

Factors influencing fine root distributions

The spatial distribution patterns of fine roots differ between plant species and in response to soil and environmental conditions. Fine roots are often concentrated in the upper 20 cm of soil (Herman 1977; Vogt et al. 1981; Strong and La Roi 1983b), however fine feeder roots are also found at depths of 100 cm (Joslin and Henderson 1987; Jonsson et al. 1988). A variety of factors
including plant genetics, soil physical characteristics, soil water, nutrient availability and competition can influence fine root distributions (Herman 1977; Vogt et al. 1981; Fogel 1983; Persson 1983; Gale and Grigal 1987; Hayes and Seastedt 1987; Mou et al. 1995; Parker and Van Lear 1996; Bhatti et al. 1998).

During the initial stages of development, fine root growth is largely under genetic control (Herman 1977; Fogel 1983). Thereafter, some species exhibit greater phenotypic plasticity and alter fine root distributions in response to external factors, while other species appear to be more rigid in root allocation patterns (Pulling 1918; Presston 1942). For example, *Quercus robur* is deep-rooted and its fine root distribution did not change between sites with different soil moisture characteristics (Thomas and Hartmann 1998). In contrast, Presston (1942) concluded that root distribution of lodgepole pine seedlings (1–15 years) was plastic and determined by soil conditions.

Soil physical characteristics also influence fine root distribution patterns. Fine root growth is often negatively correlated with bulk density and soil texture/clay content (Heilman 1981; Breda et al. 1995; Parker and Van Lear 1996). Root are generally unable to penetrate soils with bulk densities greater than 1.5–1.8 g·cm⁻³ (Heilman 1981; Tworkoski et al. 1983), and this has been attributed to both physical impermeability and poor aeration. Root growth and elongation can only occur in soils when root pressure exceeds mechanical impedance (Bennie 1991). Therefore, in soils with high bulk densities and reduced pore space, roots may not be able to penetrate through the soil, and will only grow through pores larger than the minimum fine root diameter (Waring and Schlesinger 1985). Alternatively, when soils are impenetrable, root growth is confined to cracks and macropores established by worms and decayed roots (Passioura 1991).

In addition to the physical barrier posed by soils with high bulk densities, poor aeration due to reduced porosity and infiltration can also impede the growth of fine roots (Kozlowski et al. 1991; Marschner 1995). Root growth has been noted to decline substantially when soil oxygen (O₂) content drops below 10% (Boynton et al. 1938; Leyton and Rousseau 1958; Saglio et al. 1984) and these conditions can occur in compact soils. With high bulk densities and reduced porosity, gas diffusion is impaired and O₂ is not replenished at an adequate rate. Ethylene and carbon dioxide (CO₂) produced by the roots and soil microbes also accumulate near the root surface due to the poor diffusion (Marschner 1995; Lambers et al. 1998) and both compounds can inhibit
root growth at elevated concentrations (Geisler 1968 from Marschner 1995; Lambers et al. 1998).

Moisture and soil water availability also affect the distribution of fine roots (Rytter and Hansson 1996; Xu et al. 1997; Bhatti et al. 1998) with both low and high soil water contents negatively influencing fine roots. Dry conditions can stop root growth and cause dieback (Eissenstat and Yanai 1997), with tree root growth being inhibited at soil water potentials around -0.6 MPa (Larson 1980). For example, Rytter and Hansson (1996), in their measurement of Salix viminalis fine root dynamics over two years, found a reduction in the fine root number and an increase in the mean root depth in the second year, after a particularly dry summer. Similar results have been reported for agricultural and grass species (see Eissenstat and Yanai 1997). These results suggest that low soil water potentials in the surface soils can reduce root growth and surface horizon occupation. Fluctuations in moisture content are most extreme in the topsoils. An over-abundance of water can also alter fine root distribution patterns. For flood-intolerant species, rooting depth is often correlated with water table depth (Lieffers and Rothwell 1986; Lieffers and Rothwell 1987). In a comparison of sites with varying degrees of seasonal waterlogging, Xu et al. (1997) found that Norway spruce and grand fir (Abies grandis) confined fine roots to the upper 30 cm on heavily waterlogged sites. Similar responses have been noted for black spruce, Tamarack larch (Larix laricina) and mountain alder (Alnus rugosa) on wet peat soils (Bhatti et al. 1998; Lieffers and Rothwell, 1986). Lieffers and Rothwell (1987) found that the roots of larch and black spruce were concentrated in hummocks, while on drier sites, fine roots grew to a 60 cm depth.

A main mechanism responsible for reduced root growth in waterlogged soils is the replacement of O₂ with water in soil pores. The high respiratory demands of fine roots and soil microbes for O₂ causes a rapid depletion of dissolved O₂ (Marschner 1995; Waring and Running 1998) and in water-logged soils O₂ is not replenished quickly enough due to poor diffusion of gases through water. Gas diffusion through air is about 10,000 times faster than through water (Kozlowski et al. 1991; Marschner 1995). When respiratory requirements are not met, roots are unable to take up nutrients and may die within two days if maintained under anoxic conditions (Marschner 1995). In addition, ethylene and CO₂ can accumulate around the roots and inhibit root growth. Furthermore, low molecular weight (MW) organic solutes such as phenolics and short-chain
fatty acids often build-up during decomposition in poorly aerated soils (Marschner 1995). These compounds are known to inhibit root growth.

Some species are more flood-tolerant and possess metabolic and physiological traits that enable the survival and growth of roots in anaerobic soil environments (Kozlowski et al. 1991). These traits involve the internal transport of \( \text{O}_2 \) through structures such as lenticels and aerenchyma cells. For example, Philipson and Coutts (1978) found that lodgepole pine seedlings were able to effectively conduct \( \text{O}_2 \) over a 30 cm distance through the stem into submerged roots, and lenticels and aerenchyma cells were thought to be responsible for the \( \text{O}_2 \) transport. Plants develop lenticels on stem and root surfaces (Kozlowski 1991). These formations are permeable and facilitate gas exchange (Philipson and Coutts 1978; Kozlowski et al. 1991). In addition, they are connected to a continuous intercellular pathway in the cortex and phloem, thus allowing the transport of \( \text{O}_2 \) to the roots. In water-logged soils, lenticels also allow for the excretion of toxins produced by the flooded roots and the release of excess \( \text{O}_2 \) to oxidize potentially harmful compounds that develop in the water-saturated rhizosphere (Lambers et al. 1998). Flood-tolerant plants can also produce aerenchyma cells in the root cortex. These cells are formed by the collapse or separation of cell walls (Kozlowski et al. 1991; Marschner 1995) and increase the proportion of air-filled spaces in stems and roots, resulting in greater \( \text{O}_2 \) transport to the roots. Although aerenchyma are formed independently of soil moisture conditions, in many plants adapted to water-logged conditions, \( \text{O}_2 \) deficiency can also stimulate their formation (Taiz and Zeiger 1991).

Plant species display considerable variability in flood tolerance and it has been suggested that the degree of tolerance is related to the number of metabolic and physiological adaptations to anaerobic conditions (Hook and Brown 1973). The species best able to grow in waterlogged soils possess the greatest number of adaptations. Fine root distribution patterns would therefore be expected to vary in water-saturated soils. Flood-intolerant species would be restricted to surface soil horizons, and species adapted to anaerobic conditions could be more deeply rooted on the same sites.

Fine root distribution patterns can also be influenced by nutrient availability. Under field and greenhouse conditions, fine roots proliferate in areas with high nutrient concentrations (Fitter 1976; Coutts and Philipson 1977; Eisenstat and Caldwell 1988; see Robinson 1994; Marschner
1995; Eckhard et al. 1997; Hodge et al. 1999). For example, grass root densities increased 10-15 times in soil patches enriched with Miracle-gro fertilizer (Eissenstat and Caldwell 1988), and lodgepole pine seedlings with divided root systems had nearly twice as much fine root biomass in nutrient rich solutions than in solutions with lower nutrient concentrations (Coutts and Philipson 1977). It has also been suggested that the larger amounts and densities of fine roots in the surface layers of soil or forest floors are due to higher soil nutrient concentrations (Ford and Deans 1977). Studies that have examined the effects of nutrients on fine root proliferation have demonstrated that specific nutrients can cause different responses in plant roots (Cuevas and Medina 1982, Friend et al. 1990; Marschner 1995). According to Marschner (1995), root growth, morphology and distribution patterns are affected most by N and less-so by P and magnesium (Mg$^{2+}$), while other nutrients have little influence on fine root dynamics. However, Cuevas and Median (1982) showed root proliferation in soils after the addition of calcium (Ca$^{2+}$) and Mg$^{2+}$ to systems in the central Amazon. In contrast, NH$_4^+$ additions did not increase fine root production. Using a spatially separated system, Friend et al. (1990) showed that only N-stressed Douglas-fir had significantly greater root growth in N-enriched substrates. Seedlings that were not N-limited did not exhibit this response, and grew similar amounts of roots in the low and high N substrates. These results suggest that only limiting nutrients will stimulate a root responses in nutrient-rich patches. Because N limits the growth of vegetation in many terrestrial ecosystems (Killam 1994; Chapin et al. 1993; Vitousek and Howarth 1991), increased fine root density in patches with higher N concentrations is probably common.

Root competition is also thought to influence fine root distributions (Herman 1977; Caldwell et al. 1996; Mikola et al. 1966), and can occur by way of two main mechanisms (Schoener 1983). Exploitative competition takes place when two species utilize a common pool of resources (Grubb 1994), such as water or nutrients, and occurs when depletion zones surrounding adjacent active roots of different individuals overlap (Fitter 1976; Mou et al. 1995; Robinson 1991). Interference competition involves harming another species by the production and excretion of toxins or allelochemicals (Schoener 1983). Both can result in a reduction in population size and individual growth or fitness, and may influence fine root distribution patterns. Several studies examining plants growing in mixed and monoculture states have shown that rooting patterns can be altered when more than one species grows in the same area (Harris 1967; McKay and Malcolm 1988; D'Antonio and Mahall 1991; Fredericksen and Zedaker 1995). For example, Fredericksen and Zedaker (1995) found that the fine roots of three-year-old Acer rubrum
monocultures were concentrated in the upper 10 cm of the soil. However, when planted with loblolly pine (Pinus taeda), 80% of the fine roots were in the 20-30 cm depth. Similarly, trenching experiments that isolate the roots of an individual from the roots of surrounding vegetation have shown that both fine root biomass and distribution patterns can change following isolation, suggesting that the presence of fine roots from other plants influences the fine root allocation patterns of the isolated individual (Cook and Ratcliff 1984; Christy 1986). Many researchers have found differences in the vertical fine root distribution patterns of plant species growing together (Mikola et al. 1966; McQueen 1968; Persson 1983; Strong and La Roi 1983a; Gholz et al. 1986; Jonsson et al. 1988), and some have linked these differences to the relative plant productivities. In their examination of the vertical arrangement of roots systems of Larrea tridentata, Franseria deltoidea and Opuntia fulgida, Yeaton et al. (1977) found that the roots of L. tridentata overlapped with those of the other two species. However, the F. deltoidea and O. fulgida root systems were vertically separated. Above-ground plant size was reduced when L. tridentata was growing closer to either O. fulgida or F. deltoidea. In contrast, when O. fulgida and F. deltoidea were growing in close proximity, plant biomass did not appear to be affected, suggesting that the vertical separation of their root systems reduced competition.

Exploitative and interference competition may influence fine root distributions, but the exact mechanisms responsible are still poorly understood. In addition, very few trials have separated the influences of the two forms of competitive interactions and in those that have, the results are usually just suggestive. For example, Callaway et al. (1991) examined the influence of blue oak (Quercus douglasii) on the growth of Bromus diand in the understory and found that in situations where the roots of the grass had greater overlap with the roots of blue oak, the productivities of the grass was reduced. Tests indicated that exploitative competition for nutrients and water probably was not responsible for the growth reduction, and they attributed the reduced productivity of the grass to antagonistic interactions or allelopathy. However, this hypothesis was not supported by significant results.

In summary, fine roots are critical to the survival and productivity of plants in terrestrial systems, and therefore are starting to receive more research attention. Studies have established that fine root productivity and distributions can be affected by a variety of factors including site quality, stand age, season, nutrient availability, soil physical characteristics, soil water, competition and
 Individual plant species are affected differently by these external factors, and these differences may ultimately determine where and with whom plants grow.

**Hypotheses**

From the previous review, it is apparent that plants differ in their uptake of NO$_3^-$ and NH$_4^+$ and probably also have different abilities to take up organic N compounds. It is therefore possible that cedar, hemlock and salal growing in CH forests access different N forms. Greenhouse pot trials have shown that cedar grew best on NO$_3^-$ (Krajina et al. 1973) while hemlock was found to take up greater amounts of NH$_4^+$ (Knoepp et al. 1993; van den Driessche 1971) but produced greater biomass on mixed NH$_4^+$-N and NO$_3^-$-N sources (Krajina et al. 1973; van den Driessche 1971). Both ericoid and ectomycorrhizal plants can take up a variety of organic N compounds (Abuzinadah and Read 1986b; Bajwa and Read 1985; Kerley and Read 1995), but the ericaceous plants and/or ericoid mycorrhizae often display a greater ability (Bending and Read 1996a; Griffiths and Caldwell 1991). Based on these findings, in this thesis, I hypothesized that salal would take up more organic N, cedar would take up more NO$_3^-$-N, and hemlock would take up more NH$_4^+$-N.

Vertical distributions of plant fine roots often vary among species. For example, slash pine (*Pinus elliottii*) concentrates fine root biomass in the surface soil horizons (Gholz et al. 1986), while *Eucalyptus camaldulensis* has a more even fine root distribution down to 100 cm in the soil profile (Jonsson et al. 1988). In a mixed-species system, spatial separation of fine root distributions may allow plants to access N from different forest floor and soil horizons. Such a mechanism that facilitates access to different N sources may occur in the cedar, hemlock and salal growing in CH forests. Therefore, in this thesis I tested the hypotheses that 1) cedar, hemlock and salal have different vertical fine root distribution patterns, and 2) the fine root distributions of the three species are correlated with different N forms present in the forest floor or soil surrounding the roots.
Objectives

Four main trials were conducted to address the following objectives:

- design a controlled test system to evaluate the abilities of mycorrhizal cedar, hemlock and salal seedlings germinated and grown in CH forest floor to take up different forms of organic and inorganic N.
- test the abilities of cedar, hemlock and salal growing in CH forest floor to take up amino acids intact\(^2\).
- compare the abilities of cedar, hemlock and salal growing in CH forest floor to utilize different forms of organic and inorganic N.
- determine the potential importance of rhizosphere microbial communities in supplying N to cedar, hemlock and salal.
- compare the vertical fine root distribution patterns of cedar, hemlock and salal live fine roots in old-growth CH forests and to determine if the measured root patterns are correlated with the distributions of different N forms within the forest floor/soil profile.

\(^2\) intact uptake refers to the detection of excess \(^{15}\)N and \(^{13}\)C in the plants suggesting that the compound was assimilated in the form applied
Chapter 2

A controlled test system to evaluate inorganic and organic N uptake by mycorrhizal cedar, hemlock and salal seedlings

Introduction

The ability of plants to take up organic forms of N has received considerable attention recently. Many laboratory studies have used plants inoculated with single mycorrhizal fungal species to determine the physiological ability of some species to use complex organic N compounds such as proteins (Abuzinadah and Read 1986b; Abuzinadah et al. 1986; Finlay et al. 1992) and chitin (Kerley and Read 1995). Other studies have been conducted in the field and examined the uptake of amino acids under more natural conditions (Schimel and Chapin 1996; Nasholm et al. 1998). However, no trials to date have attempted to pair the two approaches to evaluate the use of more complex forms of organic N in situ. The roots of a single plant growing in a natural system is typically colonized by many different fungal species and fungi differ in their abilities to access complex N compounds (Abuzinadah et al. 1986; Abuzinadah and Read 1989a; Abuzinadah and Read 1989b; Finlay et al. 1992). Therefore, it is necessary to consider the variety of fungal species found in a system when evaluating N uptake, especially of more complex organic N compounds.

In this study, a controlled test system was designed to examine inorganic and organic N uptake by mycorrhizal plants inoculated with fungi found in CH forest floors. To achieve this, cedar, hemlock and salal test seedlings were germinated and grown in CH forest floor prior to trial establishment to ensure that their roots were colonized by mycorrhizal fungi found in these forests. The main objective of the study was to use the test system to determine the abilities of mycorrhizal cedar, hemlock and salal to take up NO$_3^-$, NH$_4^+$ and different organic N compounds. Three main trials were conducted and involved the transplanting of plants into sterile growth containers supplied with nutrient solutions enriched with different N compounds.
Materials and Methods

Test Seedling Preparation

In May 1997, samples from F and upper H forest floor horizons (Green et al. 1993) were collected from five old-growth CH forests. Microsite depressions and locations with decomposing wood were avoided, and surface litter and moss layers were brushed off prior to removing the samples. The collected forest floor was then brought back to U.B.C., composit ed and run through a chipper-shredder to thoroughly homogenize the forest floor substrate and break-up root mats. Square (0.00105 m$^3$) plastic pots were filled with the homogenized substrate and cedar, hemlock or salal seeds were sown in the pots. Three to five cedar or hemlock$^3$ seeds were inserted 1 cm into the forest floors and approximately twenty salal seeds were sprinkled on the forest floor surface in the respective pots to provide approximately 500 pots of each species.

After planting, the pots were transferred to the horticulture greenhouse at U.B.C. and were watered as necessary. The pots remained in the greenhouse from May 26 to September 5, 1997 and were exposed to 24-hour temperatures ranging from 10-34 °C. On September 5, the pots were then transferred to the South Campus Forest Nursery Facility to over-winter. They remained at this facility until they were used in the trials during the following year.

Preliminary Uptake Trial

The Preliminary Uptake Trial (PUT) was initiated on April 16-17, 1998 to test the experiment system prior to conducting the subsequent Amino Acid and Complex N Uptake Trials. The objectives of the PUT were to determine optimal treatment N concentrations and water contents, and to examine seedling survival, carbon dioxide (CO$_2$) content and the transformation of treatment compounds in the test containers.

The seedlings from the South Campus Forest Nursery Facility were removed from the pots, forest floor loosely adhering to the roots was brushed off and the root systems were thoroughly rinsed with tap water to remove all visible particles of forest floor. The seedlings were then rinsed with autoclaved deionized water and two seedlings of the same species were transplanted into sterilized sand contained within modified Magenta tissue culture containers (Fig. 2.1). The

$^3$ The hemlock seed required stratification prior to sowing. The stratification process involved soaking the seed for 24 hours followed by refrigeration at 2 °C for 28 days.
sand consisted of a 50/50 mixture of Forestry Industrial and Industrial Fine granite sands purchased from Target Products Ltd., and the mixture was thoroughly rinsed with tap water to remove all silt particles prior to sterilization. The Magenta containers were modified by painting the outsides black to reduce light transmission to the roots and the lids were cut in half to facilitate transplanting. In addition, eight 0.5 cm holes were melted into the lids with a soldering iron. The seedling stems were placed in the two middle holes such that the roots were enclosed in the sterile container while the shoots were exposed to the open air. The remaining six holes were covered with silicon septa (Mandel Scientific) to allow for the sterile injection of water during the trial. To prepare the Magenta containers for the trial, the containers were filled with 550 g of the sand mixture, placed on stainless steel cookie trays, and enclosed in aluminum foil. The trays of containers were then dry-sterilized for 16-24 hours at 70 °C followed by 16-24 hours at 105 °C (L. Robillo pers. comm.).

Figure 2.1 The modified Magenta treatment containers used in the uptake trials.
After transplanting the cedar, hemlock or salal seedlings, 55, 85 or 115 ml of autoclaved 1/10 strength modified Johnson’s nutrient solution (Epstein 1972) was evenly dispersed over the sand surface using a 60 ml plastic syringe. The Johnson’s solution was modified by replacing the mineral N component with $^{15}$N-labelled glycine (98.53 atom% $^{15}$N - Cambridge Isotope Laboratories) added at concentrations of 0, 50 and 100 μM to provide three different N treatments. The nutrient solutions were adjusted to pH 4.5 with potassium hydroxide (KOH) and sulphuric acid ($\text{H}_2\text{SO}_4$) to provide a pH similar to those found in CH forest floors (Prescott and Weetman, 1994). The treatment solutions were then autoclaved at 121 °C for 35 minutes.

After adding the treatment solutions, the lids were placed on the Magenta containers and the junctions between the two halves were sealed with Dow Corning 832 silicone sealant and the spaces around the stems were filled with anhydrous lanolin (Sigma-Aldrich). The silicone sealant was sterile due to the methanol carrier and the lanolin was sterilized with gamma irradiation for a total of 2.5 megarads prior to use in the trial. All other equipment used during the transplants and the sealing of the Magenta containers was autoclaved or ethanol-sterilized and the transplanting and sealing procedures were conducted under aseptic conditions in a laminar flow hood. A total of 135 (3 species x 3 amounts of solution x 3 N concentrations x 5 replicates) experiment containers were prepared for the PUT.

After completing the transplants, all the experimental containers were randomly arranged on a bench in the horticulture greenhouse at U.B.C. The containers were weighed to provide starting weights and the plants were watered whenever the moisture contents were reduced through evapotranspiration by 5%, to bring the containers back to their respective starting weights. The watering procedure involved the injection of autoclaved deionized water through the six holes in the lids of the Magenta containers. A stainless steel (1.5 inch 22 gauge) needle was inserted through the silicon septa and equal amounts of water were dispensed in each hole from the attached 60 ml plastic syringe. The water was filter-sterilized with 0.2 μm Gelman Supor Acrodiscs syringe filters attached between the syringes and needles, by wiping the septa surfaces and the needles with 70% ethanol, and by clearing the water within the syringe needle before watering subsequent Magenta containers. The containers were weighed every second day and were watered when necessary. The plants were grown under the experimental conditions for 30 days and were exposed to daily (24-hour) temperature ranges of 21.0-28.5 °C.
On May 18, 1998 prior to the completion of the PUT, the CO₂ content of the head-spaces in the 135 experiment containers were measured to determine if anoxic conditions had developed in the containers over the 30-day trial period. From each container, 1 ml of air was withdrawn from the headspace and injected into an infra-red gas analyzer (IRGA). Ten 1 ml air samples were also analyzed to produce CO₂ standards. Non-elevated concentrations of CO₂ in the container headspaces were assumed to indicate that the roots experienced typical CO₂ concentrations and that root activity was not affected by reduced O₂ availability.

The PUT was completed on May 19, 1998 and the seedlings were harvested May 19-21. After being removed from the containers, the seedling roots were submerged in 100 mM non-labelled glycine to remove the labelled ¹⁵N and the whole seedlings were then rinsed thoroughly with deionized water. Seedling condition (alive or dead) was noted and all live seedlings were dried to constant weight. Most of the seedlings were dried at 70 °C. However, due to a problem with the drying oven, 37 seedling pairs were dried at 105 °C. The seedlings dried at the higher temperature were not used in the analyses due to the potential impact of the high temperature on the N content. Therefore, only 2-5 of the 5 experimental units within each treatment were analyzed. These test seedlings dried at 70 °C were weighed, combined (the two seedlings in each Magenta container) and ground to homogeneous powder by a Wiley mill followed by a Fritsch ball mill. Sub-samples were then sent to the Stable Isotope Facility at the University of Saskatchewan to determine total N and ¹⁵N concentrations.

To estimate glycine transformation in the PUT, the nutrient solutions in the Magenta containers were extracted and analyzed for the presence of NO₃⁻ and NH₄⁺. After removing the seedlings, the sand in each container was emptied into a 500 ml plastic tub and a total of 150 ml of deionized water was used to rinse the Magenta container and was added to the sand to dilute and extract the remaining nutrient solution. Deionized water was used as the extractant to prevent the biasing of the NH₄⁺ measurements with the cleaving of the amine group from the glycine (L. Lavkulich pers. comm.). The plastic tubs were then shaken by hand 20 times to thoroughly mix the solution and water (T. Ballard pers. comm.), and the extracts were decanted into filter funnels lined with 12.5 mm Whatman #42 filter paper for gravity filtration. The filtered samples were then analyzed for NO₃⁻ and NH₄⁺ with a Lachat autoanalyzer in the Soil Science Laboratory at U.B.C.
After reviewing the results from the extractions, an additional sub-trial was conducted to determine the amounts of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) present in the granite sand rinsed in tap water. Three replicates of 550 g of sand extracted with 200 ml of either 0 \( \mu \text{M} \) N nutrient solution or deionized water were gravity filtered as described above. These filtrates were also analyzed in the Soil Science Laboratory at U.B.C.

To confirm that the cedar, hemlock and salal seedlings were mycorrhizal, the presence or absence of mycorrhizal fungi on roots of the three species was assessed prior to the Amino Acid Uptake Trial. Twenty seedlings of each species were randomly selected and removed from the CH forest floor pots and all loosely adhering forest floor was removed from the root systems. The roots were then carefully rinsed in tap water, separated from the shoots, and stored in FAA (formalin-acetic acid-ethanol) until they were examined. Hemlock root systems were inspected under a dissecting microscope for the presence of stunted mycorrhizal lateral root tips, and salal root fragments were immersed in lactic acid and examined under a compound microscope for the presence of fungal tissues in the cortical cells. The cedar roots required clearing and staining prior to examination. According to the methods of Kormanik and McGraw (1984), the roots were cleared in an autoclave (30 minutes at 15 PSI) in 10% KOH (w/v), bleached in an \( \text{H}_2\text{O}_2 \) solution (10% \( \text{H}_2\text{O}_2, \text{NH}_4\text{OH} \) and distilled water) for 30-90 minutes and then stained with a trypan blue solution (lactic acid:glycerol:distilled water at a 1:1:1 ratio with 0.1% trypan blue). To set the stain, the roots were autoclaved for 15 minutes at 15 PSI in the staining solution. The samples were then transferred to a de-staining/storage solution of lactic acid:glycerol:distilled water (1:1:1). Root fragments were then immersed in lactic acid and examined under a compound microscope for the presence of fungal structures in the cedar root cortical cells.

**Amino Acid Uptake Trial**

The Amino Acid Uptake Trial (AAUT) was established on July 15, 1998 and used the same Magenta container system with modified methods. The objective of this trial was to compare the relative uptake rates of \( \text{NO}_3^- \), \( \text{NH}_4^+ \) and three amino acids (glycine, aspartic acid and glutamic acid) by cedar, hemlock and salal, and \( ^{15}\text{N} \)-labelled treatment compounds were used.

Because high levels of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) were detected in the tap water rinsed granite sand (see **Results** – pg. 43), perlite was used as the growth substrate in the AAUT. Pre-trial tests involving

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4 Stable Isotope Facility at Department of Soil Science, 51 Campus Drive, Saskatoon, Saskatchewan.
the extraction of 400 ml of perlite with 400 ml of deionized water showed that the amounts of inorganic N in the perlite were much lower than those in the granite sand. On average, only 0.0056 mg of NO\textsubscript{3}\textsuperscript{-}-N and 0.0189 mg of NH\textsubscript{4}\textsuperscript{+}-N were added to each Magenta container with the perlite, which was only 0.15% and 24% of the NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} added to the containers with the sand, respectively. In addition, extractions with 2 M KCl did not increase the amount of NO\textsubscript{3}\textsuperscript{-} or NH\textsubscript{4}\textsuperscript{+} detected in the perlite. Therefore, containers were filled with approximately 47 g of perlite, enclosed in aluminum foil and dry-sterilized using the same procedures as in the PUT.

The results of the PUT indicated that the highest N concentration and moderate moisture content provided the greatest overall survival and N uptake for the three species. However, the porosity of the perlite substrate used in the AAUT required that 110 ml of nutrient solution be added. This volume of solution produced moisture conditions similar to those in the 85 ml treatment used in the PUT. Therefore, in the AAUT, 110 ml of 1/10 strength modified Johnson’s nutrient solution enriched with 100 μM N as \textsuperscript{15}N-labelled Ca(NO\textsubscript{3})\textsubscript{2}, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, glycine, aspartic acid, glutamic acid or control (no N) was evenly distributed over the perlite surface. The labelled compounds were purchased from Cambridge Isotope Labs Inc. and the \textsuperscript{15}N isotopic enrichments of each is presented in Table 2.1. The treatment solutions were filter-sterilized with Duroapore PVDF 0.2 μm filter paper and were stored in autoclaved bottles until trial establishment. Autoclave-sterilization was not used in the AAUT to prevent potential transformation of the N treatments with the high pressures and temperatures used in the sterilization process (B. Ellis pers. comm.). Five replicates of each treatment without plants were also established during the transplants to detect mineralization of organic N by microbes not associated with plant roots (foreign microbes). In total, 120 Magenta containers were established in the Amino Acid Uptake (4 plants (3 plant species + no plant controls) x 6 treatments x 5 or 7 replicates (5 for cedar and hemlock, 7 for salal)). The larger number of salal replicates was established because salal survival was lower in sub-trials. During trial establishment, five cleaned and rinsed seedlings of cedar, hemlock and salal were also put aside and dried at 70 °C to provide estimates of pre-treatment N concentrations.
After application of the treatment solutions, the containers were sealed under sterile conditions with the silicon sealant and sterilized anhydrous lanolin, as described for the PUT (pg. 29). The containers were then randomly arranged in a growing room in the horticulture greenhouse and watered as in the PUT (pg. 29). Seedlings were exposed to daily (24-hour) temperature ranges of 17-23 °C and a 16-hour photoperiod of 34-48 μeinsteins·m⁻² for the 40-day trial period.

On August 24th, the seedlings were removed from the perlite, seedling status (alive or dead) was noted and all live plants were rinsed thoroughly in deionized water and dried to constant weights at 70 °C. The seedlings were then ground to a homogeneous powder, as described in the PUT (pg. 30). Plant samples were sent to the Stable Isotope Facility in Saskatchewan for analysis of total N and ¹⁵N enrichment.

To determine if some of the amino acids were mineralized by foreign microbes during the AAUT, NO₃⁻ and NH₄⁺ concentrations were measured in the treatment containers without plants. The perlite in each container was emptied into a 500 ml plastic tub and 300 ml of deionized water was first used to rinse the container and was then added to the tub to extract NO₃⁻ or NH₄⁺ present in the perlite. The extractions proceeded as described for the PUT (pg. 30) and the extracts were analyzed for NO₃⁻-N and NH₄⁺-N at the Soil Science Laboratory at U.B.C.

### Table 2.1. The ¹⁵N atom% enrichments of the N treatment compounds used in the Amino Acid Uptake Trial.

<table>
<thead>
<tr>
<th>Treatment Compound</th>
<th>¹⁵N enrichment (atom%)</th>
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<tbody>
<tr>
<td>Ca(NO₃)₂</td>
<td>98.5</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>98.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>98.53</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>98.3</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>98.5</td>
</tr>
</tbody>
</table>
**Complex N Uptake Trial**

The Complex N Uptake Trial (CNUT) was established on October 13, 1998 and used the Magenta container system with modified methods. The objectives of this trial were to examine the relative uptake rates of NO$_3^-$, NH$_4^+$ and four organic N compounds (glutamic acid, BSA, acetyl glucosamine and chitin) by the three plant species. As discussed earlier, complex organic N compounds could not be labelled, so N uptake was measured through estimates of changes in seedling N content during the trial.

Because of the late start date, test seedlings had to be moved from the South Campus Forest Nursery Facility to the horticulture greenhouse in August, prior to trial establishment. After being transferred, these seedlings were exposed to an extended 16-hour photoperiod to prevent bud set in the salal and hemlock. Only cedar and hemlock were used in the CNUT. Salal had high rates of mortality in the AAUT and four sub-trials conducted between July and October could not establish the cause(s) of the mortality. Different growth substrates, nutrient concentrations, leaf pruning, leaf misting, test seedling preparation and replanting into CH forest floor were all examined, but none improved salal survival. It was concluded that the salal plants were too large to tolerate the transplanting stress and/or test conditions.

To estimate starting N contents, seedling fresh weights were recorded prior to the transplants, and the trial lasted for 106 days to allow sufficient N uptake. The Johnson’s solution and N treatment concentrations were therefore increased to 2 mM to ensure an adequate N supply for the duration of the trial. Twenty cleaned and rinsed cedar and hemlock seedlings were put aside to provide estimates of starting N contents and were dried to constant weight at 70 °C.

Cedar and hemlock seedlings were transplanted into dry-sterilized Magenta containers filled with Target Products silica sand. The silica sand was a 50/50 mixture of coarse-grained Unimum and fine-grained 16/30 Filter sand, and was thoroughly rinsed with tap water followed by distilled water prior to being sterilized. Silica sand was used because results of sub-trials suggested that the survival of hemlock was greater when the seedlings were transplanted into a sand substrate. The silica sand mixture was chosen because low amounts of water-extractable N were added to the Magenta containers with the sand. In an extraction sub-trial, it was estimated that the sand added 0.0339 mg NO$_3^-$-N and 0.0014 mg NH$_4^+$-N to each container. In this sub-trial, 50 ml of deionized water was added to 50 g and 70 g of the Filter and Unimum sands, respectively. The
mixtures were shaken for 1 hour and gravity-filtered as described for the PUT (pg. 30). Concentrations of NO₃⁻-N and NH₄⁺-N in the extracts were analyzed at the Soil Science Laboratory at U.B.C. A similar sub-trial was conducted with 2 M KCl, but the salt extractant did not increase the estimates of NO₃⁻ or NH₄⁺ present in the silica sand.

Following transplanting of seedlings into each container, 70 ml of 1/5 strength modified Johnson’s solution enriched with 2 mM N as Ca(NO₃)₂, (NH₄)₂SO₄, glutamic acid, BSA, acetyl glucosamine, chitin (no N nutrient solution added) or control (no N nutrient solution) was evenly dispersed over the sand surface. The treatment compounds were purchased from Fisher Scientific and Sigma-Aldrich, and the nutrient solutions were filter-sterilized, as described for the AAUT (pg. 32). However, due to the low solubility of chitin in water, the chitin treatments were thoroughly mixed in the silica sand in the respective treatment containers and were dry-sterilized along with the sand. In an earlier sub-trial using methods of Tsuji et al. (1969), it was determined that <0.3% of the chitin was degraded into glucosamine during the dry sterilization process. Following the transplants, 70 ml of the control (no N) nutrient solution was added to each of the containers in the chitin treatment. Five replicates of each treatment without plants were also established to provide controls to estimate the amount of organic N mineralized by foreign microbes during the trial. In total, 133 Magenta containers were established in the CNUT ((2 species x 7 treatments x 7 replicates) +(7 treatments x 5 replicates of no-plant containers)).

Following each transplant, the Magenta containers were sealed with the silicon sealant and lanolin, as in the PUT, and the containers were randomly arranged in the growing room in the horticulture greenhouse. While in the growing room, the containers were aseptically watered, and were exposed to daily (24-hour) temperature ranges of 19-27.5 °C and a 16-hour photoperiod of 34-48 μeinsteins•m⁻².

The CNUT was completed on January 27, 1999. The seedlings were removed from the containers, seedling status (alive or dead) was noted, and the seedlings were rinsed thoroughly with distilled water, patted dry and weighed. Hemlock mortality was very high during the CNUT, and therefore, hemlock seedlings were not measured. The cedar seedlings were dried to constant weight at 70 °C, combined (the two seedlings in each Magenta container) and ground in a Wiley Ball Mill. Sub-samples of the plant tissues were then analyzed for total N.
concentrations using total Kjeldahl nitrogen (TKN) digestions outlined by Kalra and Maynard (1991). Briefly, the ground plant tissues were digested with H₂SO₄ at 370 °C for two hours to release bound NH₄⁺, and the NH₄⁺ in the digestates were determined colorimetrically with an Alpkem FLOW Solution IV segmented-flow autoanalyzer. The TKN analyses were conducted at Soilcon Laboratories Ltd.

To determine if any of the organic N compounds were mineralized by foreign microbes during the CNUT, NO₃⁻ and NH₄⁺ concentrations in the treatment containers were measured. The sand in each of the containers without plants was emptied into a 500 ml plastic tub and extracted with 150 ml of deionized water. The gravity-filtered extracts were analyzed for NO₃⁻-N and NH₄⁺-N concentrations at Soilcon Laboratories Ltd.⁵

A trial was conducted to examine the recoverability of NO₃⁻ and NH₄⁺ added to perlite and silica sand and to determine the cause of the low NH₄⁺ contents in the NH₄⁺ treatment containers without plants in both uptake trials. In the trial, 50 ml of 100 µM or 1 mM NH₄NO₃ was added to 5 g of perlite, and 50 ml of 1 mM NH₄NO₃ was added to 50 g and 70 g of Unimum and 16/30 filter sands, respectively. The mixtures were shaken for 1 hour and then gravity-filtered as in the PUT (pg. 30). The extracts were analyzed for NO₃⁻-N and NH₄⁺-N concentrations at the Soil Science Laboratory at U.B.C.

Calculations
In the Preliminary and Amino Acid Uptake Trials, N assimilated by the cedar, hemlock and salal seedlings was calculated from the atom% ¹⁵N measurements using equation 1.

\[ F = T(A_S - A_B)/A_F \]

where \( F \) is the total weight of the N derived from the treatment; \( T \) is the total weight of N in the plant sample (\( F \) and \( T \) are in the same units); \( A_S \) is the atom% excess ¹⁵N in the plant sample; \( A_B \) is the atom% excess ¹⁵N in the control plant samples; and \( A_F \) is the atom% excess ¹⁵N in the treatment applied (Table 2.1). Atom% excess is defined as atom% ¹⁵N in the sample minus 0.3663 atom% (the natural abundance of ¹⁵N) (Powlson and Barraclough 1993).

⁵ Soilcon Laboratories Ltd. at 275-11780 River Road, Richmond, B.C.
From the values calculated in equation 1, total N assimilated was calculated for each plant and was expressed as a percentage of the N applied using equation 2.

\[
\text{\% N assimilated} = \frac{\text{total mg N assimilated}}{\text{mg N applied}} \times 100
\]

In the AAUT, when only one live seedling was present in a container at the time of harvest, the %N assimilated estimates were doubled to account for the lost seedling. It was assumed that N uptake by the two seedlings was not limited by competition or N availability.

In the CNUT, total N uptake by the cedar plants was determined by the changes in N content during the trial. Starting N contents were estimated using the starting fresh weights and regression equations relating seedling fresh weights to dry weights and seedling dry weights to N contents. Fresh weight to dry weight regression equations were generated from the seedling weights at the end of the trial (Fig. 2.2), and seedling dry weight to N content regressions consisted of a compilation of three independent sets of data from N analyses of cedar test seedlings (twenty seedlings set aside during the establishment of the CNUT, ten seedlings from a sub-trial, and five seedlings set aside during the establishment of the AAUT) (Fig. 2.3). The seedlings in the first two sets were composited into pairs to provide enough sample for TKN analysis and the third set was analyzed by combustion with a combined elemental analyzer at the Stable Isotope Facility. The three analyses gave similar results so they were pooled together to generate the regressions for dry weight to N content of cedar seedlings. The N contents had to be square-root transformed to meet the assumptions of regression analysis.

The final N contents of the seedlings in the CNUT were determined from the seedling dry weights and TKN analyses, and total N uptake of each seedling was calculated as the difference between the N contents at the start and end of the trial. Seedlings from containers with fewer than two live seedlings were not included in the analyses.

**Statistical Analyses**

The PUT was conducted to determine the efficacy of the test system and what moisture and nutrient concentrations to use in the subsequent uptake trials. Therefore, no statistical analyses were conducted on the results from this trial.
To determine differences in the %N assimilated by the cedar, hemlock and salal seedlings in the AAUT, treatment means were compared with analysis of variance (ANOVA) using the general linear model procedure (GLM). The ANOVAs were conducted by species as completely randomized designs and were followed by pairwise t-test comparisons of the least square means. The alpha level (0.05) was adjusted for the number of comparisons using Bonferroni’s adjustment (Neter et al. 1996).

An ANOVA (GLM procedure) was also used to compare measures of N uptake (total N assimilated and seedling N concentrations) by cedar in the CNUT. The seedlings set aside during the trial establishment were included to determine if the seedling N concentrations changed during the trial. The analysis was followed by pair-wise t-test comparisons with adjusted alpha levels, as previously outlined. In addition, changes in seedling fresh weights and N contents during the CNUT were tested using pairwise t-tests to determine if either variable increased significantly over the 106-day trial period. SAS was used for all statistical analyses (SAS Institute 1993).

To determine if significant amounts of the organic N compounds were mineralized in the AAUT and CNUT, the NO₃⁻, NH₄⁺ and total inorganic N contents in the treatment containers were compared with zero using t-tests.
Figure 2.2. The relationship between fresh and dry weights of cedar seedlings.

Figure 2.3. The relationship between dry weights and N contents of cedar seedlings.
Results and Discussion

Preliminary Uptake Trial (PUT)
The glycine-N assimilated by cedar, hemlock and salal in the PUT is presented in Figure 2.4. In general, the largest amounts of N were taken up by plants growing in the containers treated with 100 μM N nutrient solutions, although hemlock showed the greatest average uptake in the containers receiving 85 ml of 50 μM N. These results indicating generally higher total uptake in the 100 μM N treatments were not surprising. Nitrogen uptake is typically related to the amount of available N present at the root surface. The movement of a given amino acid or ion to a root surface is a function of its concentration, diffusion coefficient and the buffering capacity of the soil (Kielland 1994; Chalot and Brun 1998). Therefore, with the glycine-N present in a higher concentration, a larger amount of the treatment N would reach the root surface and be available for plant uptake.

The volumes of solution applied to the containers also affected N uptake in the PUT (Fig. 2.4). Glycine-N uptake by salal appeared to be positively affected by the volume of nutrient solution, with the amounts of N assimilated increasing with volume added. Similarly, N uptake by cedar and hemlock was greater when 85 ml of solution was applied relative to the application of 55 ml. This greater uptake could be the result of the increased amount of total N applied and/or the greater amount of moisture. The increased moisture would have allowed for greater movement of the glycine-N by both diffusion and mass flow. However, when 115 ml of nutrient solution was applied, cedar and hemlock responded negatively. The uptake of glycine-N by cedar declined and 80% of the hemlock seedlings died in the 115 ml – 100 μM N treatment (Table 2.2). Despite the reduced N uptake by cedar, there was no mortality of cedar seedlings in any treatment. Salal also showed no mortality. These results suggest that hemlock seedlings were not able to tolerate the higher moisture conditions in containers that received 115 ml. Hemlock is typically most abundant in soils with moderate moisture regimes while cedar is commonly found in wetter soil conditions (Klinka and Worrall 1989).

Measurements of inorganic N in the sand substrates at the end of the PUT were used to estimate glycine transformation in the treatment containers during the trial (Fig. 2.5). There were no clear patterns of mineralization of the amino acid among the different N treatments (applied at different volumes). In several cases, the 0 μM N treatments had larger amounts of inorganic N
Figure 2.4. Glycine-N assimilated by cedar, hemlock and salal seedlings in the Preliminary Uptake Trial. Asterisks indicate the absent values due to seedling death and/or seedlings not included in the samples dried at 70 °C (n=2-5, mean + S.E.M.).

Table 2.2. Cedar, hemlock and salal mortality in the Preliminary Uptake Trial. The values indicate the total number of seedlings that died out of a possible 10.
than did the 100 µM N treatments. In addition, the amount of inorganic N found in the sands that received glycine sometimes exceeded the amount of N applied with the treatment. In the 50 µM N – 55 ml treatments, the inorganic N in the sand accounted for 106-110% of the total N applied. These results suggest that there was a considerable amount of background N present in the granite sand, despite the thorough rinsing. The extraction analyses of sand samples confirm this (Fig. 2.6). An average of 3.86 mg of NO₃⁻-N and 0.08 mg of NH₄⁺-N was contained in the sand added to each container. It was therefore difficult to determine how much, if any of the glycine-N was mineralized in the containers.

Because anaerobic conditions in soils or rooting substrates can significantly influence nutrient uptake by plant roots (Marschner 1995), it was necessary to confirm that the oxygen (O₂) levels in the containers were sufficient to meet seedling requirements during the uptake trials. Carbon dioxide concentrations in the head-spaces of the containers at the end of the PUT were not elevated relative to air, and therefore it was assumed that O₂ limitations did not impair N uptake in any of the trials.

Roots of all three species were colonized with mycorrhizal fungi, so differences in N uptake by cedar, hemlock and salal were not the result of the absence of mycorrhizal fungi.

The results of the PUT suggested that the Magenta container system worked well and that the combination of 100 µM N nutrient solutions and moderate moisture conditions would allow for the greatest N uptake by cedar, hemlock and salal. However, high background levels of inorganic N reduced the ability to detect glycine mineralization and may have also influenced the N uptake patterns of the three species. Therefore, in subsequent N uptake trials, other substrates were used. In addition, microbial contamination may have occurred during the trial and may have increased the mineralization of the N compounds. If such mineralization occurred, the N uptake patterns of the cedar, hemlock and salal would be biased. To account for the presence and influence of foreign microbes, treatment containers without plants were included in subsequent uptake trials.
Figure 2.5. The distilled water-extractable total inorganic N (NO$_3^-$ and NH$_4^+$) in the cedar, hemlock and salal experiment containers at the end of the Preliminary Uptake Trial. The values may include $^{15}$N-labelled NO$_3^-$ and NH$_4^+$ (n=4-5, mean ± S.E.M.).
solution distilled water

Figure 2.6. Nitrate and NH$_4^+$ in the granite sand added to each container in the Preliminary Uptake Trial, determined by distilled water and nutrient solution (0 µM N) extractions (n=3, means + S.E.M.).

**Amino Acid Uptake Trial (AAUT)**

Total N assimilated by cedar, hemlock and salal seedlings in the AAUT is shown in Figure 2.7. All three species took up a portion of N from each of the five N treatments, and showed similar patterns among the treatments. Nitrate-N accounted for the largest percentage of N assimilated by all three species, and was significantly higher than the other N treatments in the cedar and hemlock plants. Relatively similar proportions of NH$_4^+$, glycine, aspartic acid and glutamic acid were taken up by all three species. These results suggest that when applied in equal amounts, NO$_3^-$-N appears to be a greater source of N to mycorrhizal cedar, hemlock and salal seedlings, while NH$_4^+$ and glycine, aspartic acid and glutamic acid are smaller sources of N. However, the measured uptake patterns may have been influenced by the mineralization of the treatment by foreign microbes, as discussed below.
Table 2.3 shows the results of deionized water extractions of the perlite from containers without plants. These estimate the amount of N in the organic N treatments that was mineralized by foreign microbes during the trial. None of the amino acid treatments had inorganic N contents significantly different from zero. Therefore, NO$_3^-$ and NH$_4^+$ in the organic N treatment containers probably was not the result of organic N mineralization, but rather resulted from measurement error, variation in the N content of the perlite, and/or the presence of NO$_3^-$ or NH$_4^+$ on the equipment. However, NH$_4^+$ contents in the NH$_4^+$ treatment containers were also not significantly different from zero and only 63% of the 0.16 mg of N applied at trial establishment was recovered in the extracts from the NO$_3^-$ treatment containers. Assuming that substantial amounts of N had not been lost through volatilization, these results indicate that the perlite substrate retained large quantities of NH$_4^+$ such that it could not be extracted with deionized water.

The capacity of perlite to chemically bond and/or retain NH$_4^+$ was tested by examining the recoverability of 100 μM and 1 mM NH$_4$NO$_3$ added to and immediately extracted from perlite (Table 2.4). Perlite appeared to retain NH$_4^+$, as only 29% and 76% of the NH$_4^+$ applied with the 100 μM and 1mM solutions, respectively, were recovered in the filtrates. The amounts of NH$_4^+$ taken out of solution during the AAUT may have been even greater because of the low solution:perlite ratio used. In the perlite extraction trial, the NH$_4$NO$_3$ solutions were added to perlite at a 10:1 (v/w) ratio. In the AAUT, this ratio was only 2.3:1, which would have increased the absorptive surface area and binding sites available to absorb or retain the applied NH$_4^+$.

The poor recovery of NH$_4^+$ from the perlite in the AAUT may invalidate the conclusions that the amino acids were not mineralized by foreign microbes prior to uptake of the treatment N by the plants. More likely, the resulting NH$_4^+$ was retained with the perlite and remained undetected. It is therefore not possible to determine the accuracy of the plant uptake results from this trial.
Figure 2.7. Total N assimilated (% of applied) by cedar, hemlock and salal seedlings in the Amino Acid Uptake Trial. Within a species, different letters indicate significantly different least square means within a species based on ANOVA (GLM procedure) ($p<0.05$, $n=2-5$, mean $\pm$ S.E.M.).
Table 2.3. Inorganic N (NO$_3^-$, NH$_4^+$ and total inorganic N) in containers without plants in the Amino Acid Uptake Trial. The values are corrected for the amounts of NO$_3^-$ and NH$_4^+$ present in the control (no N) containers without plants, and may be partially composed of $^{15}$N-labelled NO$_3^-$ and NH$_4^+$. Asterisks indicate least square means that are significantly different than zero based on t-test analysis ($p<0.05$, $n=5$, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrogen in experiment container (µg N)</th>
<th>Total inorganic N (NO$_3^-$ + NH$_4^+$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO$_3^-$</td>
<td>NH$_4^+$</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>104.0 (32.2) *</td>
<td>0</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.4 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>glycine</td>
<td>0</td>
<td>37.1 (19.3)</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>0.1 (0.1)</td>
<td>17.1 (11.4)</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>1.4 (1.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.4. Nitrate and NH$_4^+$ in the filtrates following the addition of 100 µM or 1 mM NH$_4$NO$_3$ to perlite, as a percentage of the amount applied. The results are based on the addition and extraction of 50 ml of solution added to 5 g of perlite ($n=4-5$, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Extractant</th>
<th>Percent of non-extractable N</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO$_3^-$</td>
<td>NH$_4^+$</td>
</tr>
<tr>
<td>100 µM</td>
<td>5.53 (1.40)</td>
<td>70.72 (1.31)</td>
</tr>
<tr>
<td>1 mM</td>
<td>5.32 (3.26)</td>
<td>24.23 (1.19)</td>
</tr>
</tbody>
</table>
Complex N Uptake Trial (CNUT)

Nitrogen taken up by cedar over the 106-day period of the CNUT is shown in Figure 2.8. There were no significant differences between the treatment means, and only the NO$_3^-$ and acetyl glucosamine treatments increased the total N contents of the seedlings. However, these did not differ from zero, based on t-tests (Table 2.5), indicating that there was very little assimilation of N by the cedar seedlings in any of the treatments. This is further confirmed by a comparison of the mean N concentrations in the trial seedlings compared to those measured in the pre-trial seedlings. During the trial, the seedlings increased weight (Table 2.5) and N concentrations were significantly diluted (Fig. 2.9). It is therefore probable that only small proportions of the applied N compounds were taken up by the cedar and these small amounts could not be detected with the sensitivity of the measurement technique (change in plant N content) used. Nitrogen uptake in the AAUT was easier to determine because of the $^{15}$N tracer.

Changes in seedling total N content has been used successfully by other researchers to estimate N uptake over a specified period. Bradley and Fyles (1996) estimated starting N contents from regression equations relating fresh weights to N contents and found significant increases in N contents of seven tree species. In the CNUT, however, starting N contents were calculated from fresh weights using two successive regression equations (Fig. 2.2 & 2.3). In addition, the fresh weight to dry weight regression was based on the seedling weights at the completion of the trial. Plant moisture contents are known to fluctuate with the time of year and in response to different conditions (R. Guy pers. comm.). It is therefore possible that the seedling moisture contents just after removal from the CH forest floor may have differed from those present at the end of the CNUT. This possible difference in moisture content, together with the compounded error of values produced through two regression equations probably influenced the estimated starting N values. A maximum of 0.0361 mg of treatment N was assimilated by cedar during the AAUT. Assuming similar rates of uptake, the 0.0957 mg N taken up in the CNUT would probably be lost in the error. Calculating N assimilation through estimations of changes in seedling total N was an inappropriate method to use in this trial.
Figure 2.8. Change in N content of cedar seedlings during the Complex N Uptake Trial. Values are the least square means (± S.E.M.) of the differences between N contents measured at the start and the end of the trial. There were no significant differences between the treatment least square means based on ANOVA (GLM procedure) ($p<0.05$, n=3-7).

Table 2.5. The differences between cedar seedling fresh weights and N contents at the start and end of the Complex N Uptake Trial. Asterisks indicate significant differences between trial start and end values, based on pairwise t-tests and t-test comparisons with zero ($p<0.05$, n=5-7).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seedling fresh weight (g)</th>
<th>Seedling N content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>t-test value</td>
</tr>
<tr>
<td>control</td>
<td>2.021</td>
<td>5.72 *</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>2.736</td>
<td>9.04 *</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>2.759</td>
<td>3.86 *</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>2.263</td>
<td>5.28 *</td>
</tr>
<tr>
<td>BSA</td>
<td>0.918</td>
<td>1.64</td>
</tr>
<tr>
<td>acetyl glucosamine</td>
<td>2.427</td>
<td>5.30 *</td>
</tr>
<tr>
<td>chitin</td>
<td>2.483</td>
<td>6.73 *</td>
</tr>
</tbody>
</table>
Figure 2.9. Nitrogen concentrations in cedar pre-trial seedlings (n=20) and seedlings from the Complex N Uptake Trial (n=5-7). Different letters indicate significantly different least square means based on ANOVA (GLM procedure) (p<0.05, mean + S.E.M).
In the CNUT, significant amounts of NO$_3^-$ and NH$_4^+$ were measured in the containers without plants (Table 2.6). The NO$_3^-$, NH$_4^+$, glutamic acid, acetyl glucosamine and chitin treatments all had inorganic N contents greater than zero, with at least 5% of the N applied present as NO$_3^-$ or NH$_4^+$ at the end of the trial. These estimates are probably conservative because considerable amounts of NH$_4^+$ may have been bound to exchange sites on the silica sand, and the trial examining the recovery of 1 mM NH$_4$NO$_3$ added to and immediately extracted (after 1 hour) from the sand support this conclusion. Up to 27% of the applied NH$_4^+$ was bound or not extractable from the sand (Table 2.7), and only 22% of the NH$_4^+$ added to the NH$_4^+$ containers without plants was found in the water extracts at the end of the CNUT. This indicates that a considerable portion of the NH$_4^+$ present in the containers was probably retained with the sand and not extracted with water. The high levels of NO$_3^-$ and NH$_4^+$ in the plantless containers that received organic N treatments together with the reduced extractability of NH$_4^+$ strongly suggest that mineralization occurred during the CNUT. This mineralization was probably the result of foreign microbes introduced during the trial and the N uptake results were likely confounded by compound mineralization and transformation. Thus, they do not accurately estimate the uptake of NO$_3^-$, NH$_4^+$, glutamic acid, BSA, acetyl glucosamine and chitin by cedar, hemlock and salal.
Table 2.6. Inorganic N (NO$_3^-$, NH$_4^+$ and total inorganic N) in the treatment containers without plants in the Complex N Uptake Trial corrected for the NO$_3^-$ and NH$_4^+$ present in the control (no plants and no N) containers. Asterisks indicate means that are significantly different than zero based on t-tests ($p<0.05$, $n=4-5$, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrogen in experiment container (mg N)</th>
<th>Total inorganic N (NO$_3^-$ + NH$_4^+$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO$_3^-$</td>
<td>NH$_4^+$</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>1.399 (0.029) *</td>
<td>0</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.022 (0.002) *</td>
<td>0.422 (0.028) *</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>0.013 (0.002) *</td>
<td>0.282 (0.031) *</td>
</tr>
<tr>
<td>BSA</td>
<td>0.013 (0.013)</td>
<td>0.094 (0.057)</td>
</tr>
<tr>
<td>acetyl glucosamine</td>
<td>0.002 (0.001)</td>
<td>0.249 (0.034) *</td>
</tr>
<tr>
<td>chitin</td>
<td>0.003 (0.001)</td>
<td>0.257 (0.020) *</td>
</tr>
</tbody>
</table>

Table 2.7. The percentage of applied NO$_3^-$ and NH$_4^+$ immediately extractable with deionized water following the additions of 1 mM NH$_4$NO$_3$ to the Unimum and Filter sands used in the Complex N Uptake Trial. The results are based on the addition and extraction of 50 ml of solution added to 50 g or 70 g of sand ($n=5$, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Sand</th>
<th>Percent of non-extractable N</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO$_3^-$</td>
<td>NH$_4^+$</td>
</tr>
<tr>
<td>Unimum</td>
<td>2.42 (0.66)</td>
<td>27.29 (0.33)</td>
</tr>
<tr>
<td>Filter</td>
<td>2.98 (0.76)</td>
<td>23.68 (0.20)</td>
</tr>
</tbody>
</table>
Based on the results from the PUT, it appeared that the Magenta container system worked well. Through the transplanting of mycorrhizal cedar, hemlock and salal into a sterile growth condition, it seemed possible to evaluate the abilities of the three species to utilize different organic and inorganic N compounds. Furthermore, because the test seedlings were colonized with mycorrhizal fungi found in CH forest floors it was thought that the results would provide a good understanding of the importance of organic N to cedar, hemlock and salal in CH forests.

However, the results of the subsequent AAUT and CNUT indicated that plant N uptake could not be accurately estimated with this test system. First, seedling survival steadily declined as the seedlings grew older. In the AAUT, there was severe salal mortality (Table 2.8), and in the CNUT hemlock mortality became a problem. Secondly, the mineralization of the organic N compounds by foreign microbes may have influenced the N uptake results from both trials. It could not be determined how much N was transformed by foreign microbes prior to uptake, so it was not possible to draw conclusions about organic and inorganic N uptake patterns of the three species. Thirdly, the measurement of changes in seedling total N content to evaluate N assimilated by cedar in the CNUT was not sensitive enough and uptake trends were not apparent.

Table 2.8. Mortality of cedar, hemlock and salal seedlings in the Amino Acid Uptake Trial. The values indicate the total number of seedlings that died out of a possible 10 (cedar and hemlock) or 14 (salal).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cedar</td>
</tr>
<tr>
<td>control</td>
<td>-</td>
</tr>
<tr>
<td>$\text{NO}_3^-$</td>
<td>-</td>
</tr>
<tr>
<td>$\text{NH}_4^+$</td>
<td>-</td>
</tr>
<tr>
<td>glycine</td>
<td>-</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>-</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>-</td>
</tr>
</tbody>
</table>
The poor ability of the Magenta container system to determine organic and inorganic N uptake by mycorrhizal seedlings leads to the question: What is the best system to accurately evaluate the uptake of $\text{NO}_3^-$, $\text{NH}_4^+$ and simple and complex organic N compounds by mycorrhizal plants? As outlined earlier, a completely aseptic system with plants inoculated with a single mycorrhizal fungus is one option and determines the physiological abilities of mycorrhizal plants to utilize organic N compounds. However, this approach fails to accurately assess *in situ* uptake because it ignores the diversity of fungal species typically found on root systems and the different capacities of fungi to utilize complex organic N compounds. An alternative approach to assess organic and inorganic N uptake in natural systems involves the use of N and C isotopes incorporated into treatment compounds. The isotope tracer technique allows sensitive measurement of uptake *in situ* with minimal disturbance to the system, and has been successfully used to estimate the uptake of amino acids in the field and in intact plant-soil systems (Nasholm et al. 1998; Lipson et al. 1999a; Lipson et al. 1999b; Schimel and Chapin 1996). However, it is difficult and expensive to produce isotope-labelled organic N compounds other than amino acids, so the uptake of complex forms of organic N has not yet been investigated using labelled compounds. Nonetheless, without the development of alternate techniques, the use of labelled organic N compounds appears to be the best option and the subsequent trials reported in this thesis examine organic and inorganic N uptake by cedar, hemlock and salal using the isotope tracer technique and the production of complex $^{15}$N-labelled compounds applied to intact plant – CH forest floor microcosms.
Chapter 3

The intact uptake of an amino acid by cedar, hemlock and salal growing in CH forest floor

Introduction

Observations of the growth of cedar, hemlock and salal in N-poor CH forests and on cut-overs led to the hypothesis that the three species use different forms of organic and inorganic N. By doing so, cedar, hemlock and salal would be able to access sufficient amounts of N and co-occur in these forests, a phenomenon suggested by several other studies (He et al. 1988; McKane et al. 1990; Schulze et al. 1994; Nadelhoffer et al. 1996; Schmidt and Stewart 1997). Furthermore, if the three species use different organic and inorganic N compounds, their respective productivities could also differ, especially in CH forests where the majority of soluble N in the forest floor is in an organic form (Chang 1996; K. Hannam pers. comm.). One of the ways by which cedar, hemlock and salal may use different forms of N is by absorbing organic N compounds intact. All three species may not have the capacity to take up intact organic N compounds and thus, one or two of the species may have a more limited access to organic forms of N in CH forests. Therefore, to understand the potential importance of utilizing different N sources in the growth of cedar, hemlock and salal in these forests, it is necessary to establish if all three species can absorb organic N compounds.

As outlined in Chapter 2, a good test system to evaluate N nutrition and intact organic N uptake involves the addition of isotope-labelled compounds to plant – forest floor systems. Both the mycorrhizal fungi normally associated with the plants and the saprophytic soil microbes present in the rhizosphere and bulk forest floor are included in this approach. Microbes can strongly compete with plants for N (Jackson et al. 1989; Schimel et al. 1989; Zak et al. 1990; Schimel and Chapin 1996; Norton and Firestone 1996; Nasholm et al. 1998) and should be included to accurately estimate plant access to the organic compounds. However, their presence also adds the potential confounding factor of mineralization of the organic compound prior to absorption and needs to be accounted for in assessments of intact organic N uptake. Recently, $^{15}$N and $^{13}$C isotope tracer methods incorporating both labels into the same organic compound has been used
with some success. If plant tissues contain a ratio of $^{15}\text{N}:^{13}\text{C}$ similar to that of the treatment compound, at least a portion of the organic N compound was taken up intact. Nasholm et al. (1998) estimated that a minimum of 42-91% of double-labelled glycine was absorbed intact by four boreal plant species.

This study was conducted to determine if cedar, hemlock or salal growing in CH forest floor can take up amino acids as intact molecules and used the isotope tracer technique. Double-labelled glutamic acid and $^{15}\text{N}$-labelled $\text{NH}_4^+$ were injected into forest floor surrounding potted cedar, hemlock and salal and the plants were analyzed for isotope enrichment. The questions addressed were:

- Do the three species differ in their abilities to absorb intact amino acids?
- Do contrasts in the abilities of the three species to absorb amino acids explain the different productivities of cedar, hemlock and salal in CH forests?

**Materials and Methods**

**Forest Floor Cores**

In May 1999, intact cores were harvested from three old-growth CH forests near Port McNeill. Using root saws, circular cores 15 cm wide and 13 cm deep were cut out of the forest floor. These samples consisted of the F and upper H horizons (Green et al. 1993). Microsite depressions and locations with decomposing wood were avoided, and surface litter and moss layers were brushed off before extracting the cores. The cores were carefully removed and placed in plastic pots (0.0023 m$^3$), and were then transferred to a greenhouse at the U.B.C. Botanical Gardens nursery. Within one week, each pot was planted with one two-year old cedar, hemlock or salal seedling that had been germinated and grown in CH forest floor. The polyethylene greenhouse was lined with a layer of shade cloth, and under this shade cloth, the plants received 787 $\mu$mol·m$^{-2}$·sec$^{-1}$ of photosynthetically active radiation (PAR), 40% of the PAR measured on a cloudless, sunny day. Light was measured with a Decagon Ceptometer. The seedlings were watered as necessary and were exposed to daily (24-hour) temperature ranges of 10-34 °C (June 28 – August 17).
In August 1999, the seedlings were transferred to a glass greenhouse at the U.B.C. Botanical Gardens nursery and were provided with an extended 16-hour photoperiod of 64 μmol m⁻² sec⁻¹ of photosynthetically active radiation (PAR) to prevent bud-set. In this greenhouse, the seedlings were watered as necessary and were exposed to daily (24-hour) temperature ranges of 13-39 °C (August 17-October 25).

**Preliminary Trial**

A preliminary trial was conducted on November 15, 1999 to determine a suitable harvest time and extraction procedure to use in the Main Trial. Hemlock seedlings were used and the forest floors surrounding the plants were injected with either ¹⁵N & ¹³C-labelled glutamic acid or deionized water (control). The glutamic acid was purchased from Cambridge Isotope Labs Inc. and the atom% ¹⁵N and ¹³C enrichments are presented in Table 3.1. The treatment solutions were made in bulk with autoclaved deionized water and were applied to the potted cores within one hour of preparation. A total of 60 ml of each treatment was injected into six equally spaced locations around the hemlock seedlings, 10 ml at each spot. Injections were made with 6-inch 14G Popper stainless steel blunt ended syringe needles inserted approximately 9.5 cm (3/4 of pot depth) into the forest floor. The solutions were released as the needles were withdrawn to provide homogeneous distributions of the treatments in the forest floor cores (Schimel and Chapin 1996). Any solution that leaked from the bottom of the pots after injection was collected and poured over the surface of the forest floors to ensure that the treatments were contained within the cores. A total of 0.1178 mmol N was applied with the glutamic acid treatment.

Following injection of the solutions, three hemlock seedlings were harvested at 6, 12 and 24 hours. Hemlocks receiving the control treatments were harvested at six hours. Plants were removed from the pots and all loosely adhering forest floor was shaken off the root systems. The roots were then carefully washed with tap water and the whole plants were rinsed with deionized water. After rinsing, the roots were soaked twice in 0.5 mM CaCl₂ for five minutes to remove treatment compounds adsorbed to root surfaces and present in the apoplastic free-space (Nasholm et al. 1998). The seedlings were then dried to constant weight at 70 °C prior to being weighed and ground to a fine powder in a Wiley mill followed by a Fritsch ball mill. One sub-sample from each ground sample was analyzed for whole-plant ¹⁵N and ¹³C enrichment and another was extracted with a 10 mM phosphate buffer to determine the ¹⁵N and ¹³C enrichment of the extractable fraction within the hemlock plants. The difference between the
two analyses estimated the $^{15}\text{N}$ and $^{13}\text{C}$ enrichment within the plant insoluble fraction. In the Preliminary Trial, sub-samples (50 mg) from each of the three samples (from each harvest and treatment) were composited prior to whole plant analysis or extraction. Therefore, each harvest x treatment combination was only analyzed once and the whole plant and extractable and insoluble fraction results are represented by a single value.

Table 3.1. The $^{15}\text{N}$ and $^{13}\text{C}$ atom% enrichments of the treatment compounds used in the Preliminary and Main Trials.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^{15}\text{N}$ enrichment (atom%)</th>
<th>$^{13}\text{C}$ enrichment (atom%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4$Cl</td>
<td>98.6</td>
<td>-</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>98.2-98.3*</td>
<td>98.2-98.3*</td>
</tr>
</tbody>
</table>

* In the Preliminary Trial, the glutamic acid compound was 98.3 % $^{15}\text{N}$ and $^{13}\text{C}$ enriched. The Main Trial used glutamic acid that was 98.2 or 98.3 % $^{15}\text{N}$ and $^{13}\text{C}$ enriched, depending on the block.

The extraction procedure involved the grinding of 150 mg of plant sample powder (composite of 50 mg from each of the three hemlock samples from each harvest or treatment) with 4 ml of phosphate buffer (pH 8.0) in a mortar and pestle for 45 seconds. The phosphate buffer consisted of 1639 mg and 81 mg of K$_2$HPO$_4$ and KH$_2$PO$_4$, respectively (to make 1 litre). The extraction pastes were then transferred to 15 ml Corning polystyrene centrifuge tubes and the mortars and pestles were rinsed twice successively with 5 ml and 3 ml of phosphate buffer, respectively. The two rinses were then added to the respective centrifuge tubes and the mixtures were centrifuged for 10 minutes at 4200 rpm (2134 x g). After centrifuging, the supernatants were pipetted into two sterilized 20 ml glass scintillation vials (6 ml of extract into each) and the extracts were then frozen and stored at 80°C. One set of vials was used in the analyses and one was stored as a replacement if problems occurred.
The set of vials used in the analyses were transferred into a Savant Speed Vac (reduced pressure evaporator) to reduce the extract volumes to 50-150 μl. The concentrated extracts were then pipetted into 8x5 mm tin capsules and dried at 70 °C. The scintillation vials were rinsed with 90 μl of autoclaved deionized water and the rinses were also added to the respective tin capsules and dried. Once completely dry, the tin capsules were sealed and prepared for total N, total C, $^{15}$N and $^{13}$C analysis. All analyses (whole plants and extractable fractions) were conducted at the Stable Isotope Facility at University of California (UC)-Davis.

Main Trial
The Main Trial was conducted from February 18-25, 2000 to assess the abilities of cedar, hemlock and salal to take up glutamic acid intact and to compare species-specific uptake rates of glutamic acid with those of NH$_4^+$. The trial was set up as a completely randomized block design, blocked by establishment time, and each of the eight blocks consisted of a single replicate of each treatment x species combination. The treatments were randomly assigned to potted cores within a species and at each establishment time the forest floors surrounding the cedar, hemlock or salal seedlings were injected with one of the three treatments; $^{15}$NH$_4$Cl, $^{15}$N&$^{13}$C-labelled glutamic acid and control (deionized water). The two labelled compounds were purchased from Cambridge Isotope Labs Inc. and the respective atom% $^{15}$N and $^{13}$C compound enrichments are presented in Table 3.1. A total of 0.1178 mmol of treatment N was applied to each pot.

The Main Trial used treatment application, harvesting, sample preparation and analysis methods similar to those outlined above in the Preliminary Trial. The cedar, hemlock and salal seedlings, however, were all harvested six hours after treatment injection. The harvests were conducted in numerical order to ensure a random harvesting pattern and all plants were harvested within one hour of the six-hour harvest time. Care was taken to remove as much live root as possible. However, due to the very fine roots of salal (Xiao 1994), typical of the ericaceae (Read 1991; Ehrenfeld 1992), not all of the salal root system could be recovered.

In the Main Trial, after washing and rinsing, the seedlings were not dried in an oven, but were immediately enclosed in aluminum foil, frozen in liquid N$_2$, and stored at −80 °C until they were freeze-dried. This alternative drying method was chosen to stop plant metabolism after the harvest, and reduce $^{13}$C-CO$_2$ loss through continued plant respiration during the drying process.
Following freeze-drying, the seedlings were weighed and ground to a fine powder in a Wiley mill followed by a Fritsch ball mill as for the Preliminary Trial.

Each ground sample was analyzed separately for $^{15}$N and $^{13}$C enrichment. Both whole plant samples and plant extractable fractions were analyzed. During the extractions, 35 mg of sample was extracted with 1 ml of phosphate buffer followed by two successive 1 ml rinses of the mortars and pestles with autoclaved deionized water. After centrifuging, the full volumes of the supernatants were decanted into autoclaved scintillation vials and stored at 4 °C until further use. To ensure maximum extraction of the soluble fractions within the cedar, hemlock and salal seedlings, 3 ml of the phosphate buffer was added to the centrifuged pellets, and the mixtures were stirred with a stainless steel spatula, vortexed and recentrifuged for 10 minutes at 4200 rpm (2134 x g). These supernatants were added to the respective extracts contained in the scintillation vials. The 6 ml extracts were then frozen and stored at −80 °C until being reduced in volume to 50-150 μl in the Speed Vac and dried in tin capsules. These samples were also analyzed at the Stable Isotope Facility at UC-Davis.

Roots from cedar, hemlock and salal seedlings were assessed for the presence of mycorrhizal fungi. Eight potted seedlings of each species were removed from forest floor cores and rinsed thoroughly with tap water. After rinsing, the roots were separated from the shoots and stored in FAA (formalin-acetic acid-ethanol). Cedar and hemlock roots were then cleared and stained according to modified methods outlined by Kormanik and McGraw (1984) as in Chapter 2 (pg. 31). After clearing and staining, the samples were transferred to a de-staining solution of lactic acid:glycerol:distilled water (1:1:1) and stored in this solution until the colonization assessments were completed. Salal roots did not require clearing or staining and were kept in FAA until the mycorrhizal assessments were conducted.

**Calculations**

From the analysis results estimating the atom% $^{15}$N and $\delta^{13}$C in the whole plants and extractable fractions, nmol $^{15}$N and $^{13}$C excess and %N assimilated (of applied) were calculated. The $\delta^{13}$C numbers were converted to atom% $^{13}$C using equations 1 and 2.

\[
R = (\delta^{13}C/1000 + 1) \times 0.0112372
\]

\*\* Stable Isotope Facility **at** 122 Hunt Hall, University of California, Davis, California.
Excess nmol $^{15}$N and $^{13}$C were calculated using equation 3.

\[
\text{Nmol excess} = \left( \frac{T \times A_E/100}{A_N/100 \times W_H} + \left( 1 - \frac{A_N/100}{100} \right) \times W_L \right) \times 1000000
\]

where $T$ is the total mg N or C/mg plant sample; $A_E$ is the atom% excess; $A_N$ is the sample atom%; $W_H$ is the isotope atomic weight (15 or 13); and $W_L$ is the non-isotope atomic weight (14 or 12). Atom% excess is defined as the sample atom% minus the average control atom%.

Total N assimilated was calculated using equation 1 in Chapter 2 (pg. 36), and from the results, % N assimilated of applied was calculated using equation 2 (pg. 37).

Values of the excess nmol $^{15}$N and $^{13}$C and %N assimilated in the insoluble fraction were calculated by subtracting the whole plant and extractable fraction numbers.

**Statistical Analyses**

Results of the Preliminary Trial were not statistically analyzed because the three hemlock samples in each treatment were composited prior to isotope analysis.

To confirm that at least a portion of the glutamic acid was absorbed intact in the Main Uptake Trial, $\delta^{13}$C levels in the control and glutamic acid and $\text{NH}_4^+$ treated plants were compared with ANOVA (GLM procedure). It was assumed that significantly higher $^{13}$C levels in the plants indicated that at least a portion of the applied amino acid was absorbed intact (see Discussion – pg. 68). The whole plant and extractable fraction $\delta^{13}$C means were compared by species and were analyzed as completely randomized block designs. These analyses were followed by pairwise t-test comparisons of the least square means, and the alpha level (0.05) was adjusted for the number of comparisons using Bonferroni’s adjustment (Neter et al. 1996).

To estimate the average minimum amount of glutamic acid absorbed intact by cedar, hemlock and salal, Pearson correlation analyses were initially conducted on the excess $^{15}$N and $^{13}$C concentrations to determine if the two isotopes were correlated. Correlation analyses were also conducted on the excess $^{15}$N and $^{13}$C concentrations in the plants from the $\text{NH}_4^+$ treatments to confirm the absence of a relationship between the two isotopes in the $\text{NH}_4^+$-treated plants. The extractable and insoluble fractions and whole plants of the three species were analyzed. If a
significant relationship \((p<0.05)\) was detected, \(^{13}\text{C}:^{15}\text{N}\) ratio estimates were calculated using geometric mean regressions (GMR) (Nigh 1995). GMR analysis was chosen because \(^{13}\text{C}\) and \(^{15}\text{N}\) could not be classified as dependent or independent variables. To determine the upper and lower limits of the excess \(^{13}\text{C}:^{15}\text{N}\) ratios for each plant species, 95\% confidence intervals of the ratio estimates were calculated (Nigh 1995).

To provide an indication of the partitioning of \(^{13}\text{C}\) and \(^{15}\text{N}\) within cedar, hemlock and salal tissues following glutamic acid uptake, excess concentrations of the two isotopes contained within the insoluble and extractable fractions were compared with paired t-tests. For example, the excess \(^{13}\text{C}\) present in the insoluble and extractable fractions of cedar were compared to determine if a significantly higher amount of the C isotope was partitioned to one of the fractions. SAS was used for all statistical analyses (SAS Institute 1993).

**Results**

*Preliminary Trial*

The results of the Preliminary Trial are presented in Table 3.2. Over the 24-hour trial period, the nmol \(^{15}\text{N}\) and \(^{13}\text{C}\) excess in and \(^{13}\text{C}\) assimilated values generally increased in the hemlock plants (whole) and the extractable and insoluble fractions. In contrast, the \(^{13}\text{C}:^{15}\text{N}\) excess ratios declined in the whole plants and the insoluble fractions but remained relatively unchanged in the extractable fractions during the 24-hour trial period.

*Main Trial*

Significant differences were found in the \(\delta^{13}\text{C}\) in the control and treated plants (Table 3.3). All three species had significantly larger \(\delta^{13}\text{C}\) in the extractable fractions of plants treated with glutamic acid relative to the control- and \(\text{NH}_4^+\)-treated plants indicating that cedar, hemlock and salal probably took up a portion of glutamic acid intact. Similarly, salal whole plants also had significantly higher \(\delta^{13}\text{C}\) in response to glutamic acid relative to the other treatments. In the cedar and hemlock whole plants, the \(\delta^{13}\text{C}\) levels in the glutamic acid treatments were only significantly larger than the controls.
Pearson correlation analyses of the relationships between the excess $^{15}$N and $^{13}$C assimilated in the extractable and insoluble fractions and whole plants are presented in Table 3.4. Only hemlock and salal from the glutamic acid treatments showed significant correlations between the two isotopes and these were only found in the extractable tissues and whole plants. Therefore, $^{13}$C:$^{15}$N ratios to estimate minimum intact glutamic acid uptake were only generated with GMR for these two fractions for salal and hemlock (Fig. 3.1). In the whole plants, salal had an estimated isotope ratio of 10.39 while the estimate for hemlock was 4.07. In the extractable fractions, the $^{13}$C:$^{15}$N ratio estimates were lower at 6.65 and 3.04 for salal and hemlock, respectively.

The excess $^{13}$C and $^{15}$N assimilated in the extractable and insoluble fractions of cedar, hemlock and salal in the two treatments are shown in Table 3.5 and indicate that the three species partitioned the treatment isotopes differently. In the hemlock plants receiving the glutamic acid treatment, significantly higher amounts of both isotopes were present in the extractable fraction. Similar patterns were found in the NH$_4^+$-treated hemlock. Larger amounts of the applied N were partitioned to the extractable fraction. In cedar, significantly larger amounts of $^{13}$C were present in the insoluble fraction. In the salal plants, there were no differences in the allocations of $^{13}$C and $^{15}$N in the glutamic acid treatment, but in the plants receiving the NH$_4^+$, significantly greater amounts of $^{15}$N were present in the extractable fraction.

All of the cedar, hemlock and salal plants were mycorrhizal.
Table 3.2. The $^{13}$C, $^{15}$N, C/N and % N assimilated by hemlock seedlings harvested after 6, 12 and 24 hours in the Preliminary Trial. Values are from single composite samples (3 samples in each composite) (n=1).

<table>
<thead>
<tr>
<th>Harvest Time</th>
<th>Excess $^{13}$C (nmol/mg sample)</th>
<th>Excess $^{15}$N (nmol/mg sample)</th>
<th>C/N (nmol excess/mg sample)</th>
<th>% N assimilated (% of applied)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.904</td>
<td>0.242</td>
<td>3.74</td>
<td>0.73</td>
</tr>
<tr>
<td>12</td>
<td>1.11</td>
<td>0.316</td>
<td>3.52</td>
<td>0.82</td>
</tr>
<tr>
<td>24</td>
<td>1.13</td>
<td>0.412</td>
<td>2.73</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>Extracts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.563</td>
<td>0.179</td>
<td>3.14</td>
<td>0.54</td>
</tr>
<tr>
<td>12</td>
<td>0.732</td>
<td>0.206</td>
<td>3.55</td>
<td>0.54</td>
</tr>
<tr>
<td>24</td>
<td>0.713</td>
<td>0.236</td>
<td>3.02</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Insoluble fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.342</td>
<td>0.063</td>
<td>5.45</td>
<td>0.19</td>
</tr>
<tr>
<td>12</td>
<td>0.382</td>
<td>0.110</td>
<td>3.48</td>
<td>0.28</td>
</tr>
<tr>
<td>24</td>
<td>0.415</td>
<td>0.177</td>
<td>2.35</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Table 3.3. The $\delta^{13}$C in the whole plants and extractable fractions of cedar, hemlock and salal grown in the control, NH$_4^+$ and glutamic acid treatments in the Main Trial. Different letters indicate significantly different $\delta^{13}$C treatment least square means within a species and plant fraction (whole plants or extractable fractions) based on ANOVA (GLM procedure) ($p<0.05$, n=7-8, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Whole Plants</th>
<th>Extractable Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>$\text{NH}_4^+$</td>
</tr>
<tr>
<td>cedar</td>
<td>-27.26 (0.15) b</td>
<td>-26.93 (0.16) ab</td>
</tr>
<tr>
<td>hemlock</td>
<td>-28.33 (0.24) b</td>
<td>-28.29 (0.24) ab</td>
</tr>
<tr>
<td>salal</td>
<td>-28.43 (0.18) b</td>
<td>-28.14 (0.32) b</td>
</tr>
</tbody>
</table>
Figure 3.1(a-c). Excess $^{13}$C and $^{15}$N assimilated in the extractable and insoluble fractions and whole plants of cedar, hemlock and salal grown in the glutamic acid treatments of the Main Trial. Dotted lines indicate the $^{13}$C : $^{15}$N ratio (5:1) of the injected glutamic acid and the ratio estimates for each species are based on geometric mean regression (solid lines). "NS" indicates where correlations between $^{13}$C and $^{15}$N for each species were not significant. Parentheses bracket the upper and lower 95% confidence intervals of the ratio estimates (n=7-8).
Table 3.4. Pearson correlation coefficients (r) from the correlation analyses comparing excess $^{15}$N and $^{13}$C in cedar, hemlock and salal whole plants, extractable fractions and insoluble fractions in the Main Trial. Asterisks indicate significant correlations ($p<0.05$, n=7-8).

<table>
<thead>
<tr>
<th>Species</th>
<th>GLUTAMIC ACID</th>
<th></th>
<th>NH$_4^+$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole plant</td>
<td>Extractable fraction</td>
<td>Insoluble fraction</td>
<td>Whole plant</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>cedar</td>
<td>0.201</td>
<td>0.633</td>
<td>0.308</td>
<td>0.458</td>
</tr>
<tr>
<td>hemlock</td>
<td>0.924</td>
<td>0.001*</td>
<td>0.989</td>
<td>0.0001*</td>
</tr>
<tr>
<td>salal</td>
<td>0.771</td>
<td>0.042*</td>
<td>0.791</td>
<td>0.034*</td>
</tr>
</tbody>
</table>
Table 3.5. Excess $^{15}$N and $^{13}$C in the extractable and insoluble fractions of cedar, hemlock and salal in the glutamic acid and NH$_4^+$ treatments in the Main Trial. Asterisks indicate significant differences between the excess $^{15}$N or $^{13}$C concentrations contained in the extractable and insoluble fractions within a species and treatment based on paired t-test analysis ($p<0.05$, n=7-8, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Glutamic acid</th>
<th>NH$_4^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{15}$N</td>
<td>$^{13}$C</td>
</tr>
<tr>
<td></td>
<td>(nmol·35 mg sample$^{-1}$)</td>
<td>(nmol·35 mg sample$^{-1}$)</td>
</tr>
<tr>
<td></td>
<td>Excess</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Extractable fraction</td>
<td>Insoluble fraction</td>
<td>Extractable fraction</td>
</tr>
<tr>
<td>cedar</td>
<td>0.76 (0.10)</td>
<td>0.81 (0.10)</td>
</tr>
<tr>
<td>hemlock</td>
<td>4.00 (0.73) *</td>
<td>1.73 (0.19)</td>
</tr>
<tr>
<td>salal</td>
<td>0.84 (0.24)</td>
<td>0.93 (0.23)</td>
</tr>
</tbody>
</table>

**Discussion**

*Intact glutamic acid uptake*

Cedar, hemlock and salal growing in CH forest floor were all able to take up at least a portion of the glutamic acid intact, as indicated by the higher amounts of $^{13}$C present in the extractable fractions of all three species in the amino acid treatments. A small amount of the C isotope may have entered the plants as $^{13}$C-labelled HCO$_3$ or CO$_2$, following glutamic acid metabolism or respiration by microbes. However, it is unlikely that these C sources would account for a significant proportion of the excess $^{13}$C present in the plant tissues. Any $^{13}$C-CO$_2$ would be heavily diluted in the background of CO$_2$ present in the forest floor (R. Guy *pers. comm.*), and the acidic nature of the forest floor (pH 3.65-4.21) (Prescott and Weetman 1994) would reduce the amount of $^{13}$CO$_2$ dissolved and present in soil solution as H$^{13}$CO$_3$. Consequently, only trace amounts of uncoupled $^{13}$C probably entered cedar, hemlock and salal. Therefore, the majority of $^{13}$C was most likely taken up as glutamic acid.

To estimate the minimum amounts of applied amino acid taken up intact, previous studies have used the excess $^{13}$C and $^{15}$N present in the extractable fraction of test plants (Nasholm et al. 1998). The $^{13}$C:$^{15}$N ratio from this fraction is thought to more accurately estimate intact uptake.
because a large proportion of the recently assimilated N and C is probably still in an extractable form (A. Nordin pers. comm.). In addition, only a comparatively small amount of total plant C is water soluble and therefore treatment $^{13}$C would be less hidden in the large C content and the variability in $^{13}$C natural abundance of the plants. A comparison of the excess $^{13}$C and $^{15}$N in the whole plants and extractable fractions in this trial support this rationale. As indicated by Figure 3.1 (a-c), the variability in the whole plant excess $^{13}$C and $^{15}$N measurements was much higher than that of the extractable fraction. Therefore, the excess $^{13}$C and $^{15}$N present in the extractable fraction was used to estimate the minimum intact uptake of glutamic acid.

A comparison of the excess $^{13}$C:$^{15}$N ratio in plant extractable fractions and the 5:1 C:N ratio of glutamic acid indicates that an average of 61% and 100% of the amino acid was taken up intact by hemlock and salal, respectively. Based on the 95% confidence interval lower limit, the two species took up a minimum of 52% and 69% of the N as amino acid. The amount of intact glutamic acid uptake by cedar could not be estimated. The correlations between $^{13}$C and $^{15}$N in both the extractable fractions and whole plants were not significant. However, as discussed earlier, this lack of correlation does not indicate that the amino acid was not absorbed in an intact form, but rather suggests that a comparatively small amount of the glutamic acid was taken up by cedar and that the $^{13}$C and $^{15}$N isotopes were quickly separated following uptake. Because of low glutamic acid uptake by cedar, the excess $^{13}$C and $^{15}$N measurements were probably influenced to a greater degree by the background variability in the natural abundances of $^{13}$C and $^{15}$N, and following absorption of the amino acid, cedar partitioned significantly higher amounts of $^{13}$C to the insoluble fraction, while the $^{15}$N was allocated equally to the two fractions. Thus, a correlation between the two isotopes in the extractable fraction, could not be detected. This isotope separation did not occur in the hemlock and salal plants. Therefore, it appears that although intact uptake occurred, the double-labelled amino acid tracer technique was not able to estimate the intact glutamic acid uptake by cedar during a 6-hour period. Measurements conducted at 12 or 24 hours may have been more suitable.

Differences in the estimates of glutamic acid uptake by hemlock and salal suggest that a larger proportion of the amino acid was absorbed intact by salal, and Nasholm et al. (1998) found similar results in their comparison of glycine uptake by the ericaceous shrub, Vaccinium myrtillus and two conifers, Picea abies and Pinus sylvestris. Ninety-one and 42% of the glycine was taken up intact by the shrub and trees, respectively. Therefore mineralization of the amino
acid prior to uptake may have occurred to a greater extent for the trees. However, as discussed by Nasholm et al. (1998), excess $^{13}$C:$^{15}$N ratios may not provide exact measurements of intact uptake because they can be influenced by the metabolism of the amino acids following uptake.

Following absorption, amino acids can go through different metabolic pathways in plants. Many of these pathways involve CO$_2$ production and release and therefore, the $^{13}$C content and $^{13}$C:$^{15}$N ratio can decrease. Schimel and Chapin (1996) examined the uptake of single-labelled $^{15}$N, $^{13}$C-1 glycine and aspartate by two arctic tundra plant species in situ, and found no detectable $^{13}$C after one day. Despite the absence of elevated $^{13}$C levels in the plant tissues, they argued that a large proportion of the amino acid was taken up intact and that the $^{13}$C was probably lost through metabolism and $^{13}$C-CO$_2$ release. In their examination of ubiquitously-labelled glycine uptake by four different boreal plant species, Nasholm et al. (1998) found that the estimates of intact uptake differed for each species and that *Deschampsia flexuosa* and *V. myrtillus* had lower $^{13}$C:$^{15}$N slope ratios after 24 hours than after 6 hours. They suggested that the differences in the estimates of intact glycine uptake both between species and with time resulted from the metabolism of the amino acid through serine synthesis. The glycine may have been metabolized differently by each plant species resulting in varying degrees of $^{13}$C-CO$_2$ release. As the trial progressed, the assimilated amino acid could have been metabolized further and more $^{13}$C-CO$_2$ would have been lost. Glutamic acid may have undergone similar catabolism in this current trial.

A main pathway for glutamic acid metabolism is the formation of $\alpha$-ketoglutarate, an intermediate compound in the tri-carboxylic acid (TCA) cycle which occurs in the mitochondria of plant cells (Taiz and Zeiger 1991). Glutamic acid is deaminated to form $\alpha$-ketoglutarate and during one turn of the TCA cycle, two carboxyl groups are cleaved as CO$_2$. Barring re-assimilation, this CO$_2$ is lost from the plant tissues. Therefore, if even a small proportion of the double-labelled glutamic acid taken up by the plants during the trial were to enter the TCA cycle, the $^{13}$C enrichment and the $^{13}$C:$^{15}$N ratio would decrease. In the Preliminary Trial, a decline in the ratios of excess $^{13}$C:$^{15}$N assimilated in the whole plants and insoluble fractions of hemlock was seen during the 24-hour trial period. It is unlikely that mineralization of the glutamic acid prior to uptake of the uncoupled $^{15}$N accounted for the lower excess $^{13}$C:$^{15}$N ratios because the isotope ratios in the extractable fractions remained relatively constant at 3-3.5 during the 24-hour trial period. Instead, the pattern of ratio decline suggests that some of the glutamic acid-$^{13}$C was
metabolized through a metabolic pathway such as the TCA cycle and was lost from the plants. A similar loss of $^{13}$C-CO$_2$ may have also occurred in the hemlock during the Main Trial, and may account for the lower estimates of intact glutamic acid uptake by hemlock. In contrast, salal may have metabolized less glutamic acid through catabolic pathways like the TCA cycle, so the average $^{13}$C:$^{15}$N ratio estimate would not be as affected.

Regardless of this inability to accurately estimate the exact amount of intact uptake accurately, it is apparent that cedar, hemlock and salal growing in CH forest floor are all able to take up intact amino acids. Thus the intact uptake of simple organic N compounds such as amino acids does not appear to be a mechanism that allows cedar, hemlock or salal to access N unavailable to one or both of the other two species.

The finding that cedar, hemlock and salal all absorbed intact glutamic acid in the presence of microbial competition, suggest that amino acids may be a significant sources of available N to all three species in CH forests. Up to 30 different amino acids have been identified in the soil (Kowalenko 1978; Stevenson 1994), and in water-extractions from the “F” horizons of three old-growth CH forest floors, total protein amino acids averaged 511 ng N·g soil$^{-1}$ compared to an average NH$_4^+$ concentration of 516 ng N·g soil$^{-1}$ (K. Hannam pers. comm.). There are at least 3-4 different membrane-bound transport proteins responsible for the uptake of acidic (1), basic (1) and neutral (2) amino acids in plants (Sauer and Tanner 1985; Wyse and Komor 1984; Bush 1993) and 5-16 carriers responsible for amino acid uptake in fungi (Chalot and Brun 1998), which would facilitate the simultaneous absorption of many amino acids.

**Summary**

This trial demonstrated that cedar, hemlock and salal growing in CH forest floors are able to absorb amino acids intact, so amino acids should be considered available sources of N to the three species in CH forests. Because cedar, hemlock and salal did not differ in their capacities to absorb amino acids intact, amino acid uptake does not appear to be a mechanism by which the three species in CH forest floors access different N sources. A larger variety of amino acids and organic N compounds should be examined to better understand if cedar, hemlock and salal differ in their abilities to access and take up organic and inorganic N compounds and if N-form
partitioning allows cedar, hemlock or salal to access different N sources and different amounts of N in CH forests.
Chapter 4

The importance of organic and inorganic N compounds in the nutrition of cedar, hemlock and salal growing in CH forest floor

Introduction

Cedar, hemlock and salal growing in CH forests may access and utilize different forms of organic and inorganic N. This may occur by directly taking the compounds themselves up or by supporting microbial communities that transform and supply the compound N in a form that is more easily absorbed by the plants. The findings presented in Chapter 3 established that cedar, hemlock and salal growing in CH forest floor can absorb glutamic acid intact, and therefore intact uptake of simple organic N compounds does not appear to be a mechanism by which the three species access different N forms. However, amino acids only represent 6-16% of the soluble organic N (SON) extracted with water from CH forest floors (K. Hannam pers. comm.). Consequently, it is necessary to evaluate the abilities of cedar, hemlock and salal to utilize more complex organic N compounds.

Laboratory experiments indicate that mycorrhizae are necessary to take up larger organic N compounds (Abuzinadah et al. 1986; Abuzinadah and Read 1989a; Finlay et al. 1992), and mycorrhizal type and fungal species can influence this ability. Some ericoid and ectomycorrhizal fungi can use proteins as N sources (Abuzinadah and Read 1986a; Abuzinadah and Read 1989b; Xiao 1994; Bending and Read 1996a; Bending and Read 1996b), and ericoid fungi are able to mobilize proteins bound to tannins (Bending and Read 1996a). However, only three of the eighteen ectomycorrhizal fungi tested by Bending and Read (1996a) could access the tannin-bound N, suggesting a lower ability of ectomycorrhizal plants to access the more complex compounds. The capacities of VA mycorrhizal plants or fungi to take up complex organic N compounds have not been studied, but it is hypothesized that these mycorrhizal types have limited abilities to absorb organic N compounds (Michelsen et al. 1998; Read 1991). Cedar, hemlock and salal have VA, ecto and ericoid mycorrhizal associations, respectively, and so availability of different organic N forms to the three species in CH forests may vary.
Like mycorrhizae, the saprophytic microbial communities in rhizospheres may influence the ability of associated plants to access complex organic N compounds. Several studies have shown that microbes can increase N availability to plants, and that the plant-microbe relationship is not obligately competitive. Abuzinadah and Read (1989b) found that *O. griseum* supplied N from bovine serum albumin (BSA) to non-mycorrhizal silver birch (*Betula pendula*) growing in sterile perlite. Non-mycorrhizal birch grown aseptically showed very poor growth and N concentrations. Similarly, Andersson et al. (1997) found that non-mycorrhizal *P. sylvestris* growing in non-sterile peat could take up as much organic $^{15}N$ as seedlings inoculated with the mycorrhizal fungus *P. involutus* and growing in the same media. However, Abuzinadah and Read (1989b) also tested N uptake by non-mycorrhizal birch grown with the fungi *H. ericae* and *Ulocladium botrytis* and found that the plants did not absorb more N when the two saprophytic fungi were present. This suggests that microorganisms differ in their abilities to take up and transfer N and so, depending on the species composition, some rhizosphere microbial communities may share a mutualistic relationship with associated plants (Abuzinadah and Read 1989b; Bradley et al. 1997a). Microbial community composition and functional diversities often differ in the rhizospheres and soil surrounding different plant species (Jones and Richards 1977; Amora-Lazcano et al. 1998; Grayston et al. 1996; Grayston and Campbell 1996; Priha and Smolander 1999), and so, the abilities of the microbial communities to supply N to the associated plant may also vary. In CH forests, the compositions of the rhizosphere microbial communities affiliated with cedar, hemlock and salal may differ as may their potential to supply N to the plants, leading to differences in the capabilities of the three species to access organic N.

Both intact uptake of more complex organic N compounds through mycorrhizal roots and the absorption of N made available to plants through microbial metabolism of the compounds may be important in determining the abilities of cedar, hemlock and salal to access different N forms in CH forests.

A pot study was conducted to examine the capacities of cedar, hemlock and salal growing in CH forest floor to utilize a variety of inorganic and organic N compounds and to determine if differences in access to these compounds could explain the relative productivities of the three species on CH sites. The study tested the hypothesis that the three species differ in their abilities to access different N forms, specifically that salal would take up more organic N, cedar would take up more NO$_3^-$-N, and hemlock would take up more NH$_4^+$-N.
Nitrogen compounds of varying complexity were injected into the forest floor surrounding the seedlings and the amount of applied N taken up was measured for 20 days to determine how access to the treatment N changed with time. In addition, forest floor microbial community sizes and metabolic abilities were assessed to determine the potential importance of the communities in supplying N to cedar, hemlock and salal.

**Materials and Methods**

*Test seedling preparation*

In May, June and August 1998, intact forest floor cores were extracted from five old-growth CH forests near Port McNeill. Using root saws, cores 15 cm wide and 13 cm deep were cut out of the forest floor as described in Chapter 3 (pg. 56). The potted cores were transferred to the horticulture greenhouse at U.B.C. and within one week, each was planted with two one-year-old cedar, hemlock or salal seedlings that had been germinated and grown on CH forest floor. The potted seedlings were watered as necessary and were exposed to daily (24-hour) temperature ranges of 10.4-31.9 °C (May – August). At the end of August, the seedlings were transferred to the South Campus Forest Nursery Facility where they were kept outside over the winter. In June 1999, the seedlings were transferred to polyethylene greenhouses at the U.B.C. Botanical Gardens nursery, and were exposed to the light, temperature and moisture regimes outlined for the potted seedlings in Chapter 3 (pg. 56/57).

In August 1999, the cedar, hemlock and salal potted seedlings used in the forest floor microbial community assessments were transferred to a glass greenhouse at the U.B.C. Botanical Gardens nursery. They were provided with an extended photoperiod of 64 μmol·m⁻²·sec⁻¹ of PAR to prevent bud-set. The seedlings were watered as necessary and exposed to a daily (24-hour) temperature range of 13-39 °C (August 17-October 25).

*N Uptake Trial*

The N Uptake Trial was a completely randomized block design, blocked by establishment time. Seven blocks composed of single replicates of each treatment were established from July 12-27, 1999; each block consisting of cedar, hemlock and salal seedlings transplanted into forest floor cores collected at the same time. Blocks 1-2 used cores collected in May, blocks 3-5 used cores...
collected in June, and blocks 6-7 used cores collected in August. Restricting the seedlings in each block by core collection time eliminated the influences of differing amounts of growth time in each core.

At each establishment time, one of six $^{15}$N-enriched treatment solutions, Ca(NO$_3$)$_2$, (NH$_4$)$_2$SO$_4$, glutamic acid, plant protein, plant protein-tannin complex and control (deionized water), were injected into the forest floor substrates surrounding the cedar, hemlock or salal seedlings. The Ca(NO$_3$)$_2$, (NH$_4$)$_2$SO$_4$ and glutamic acid were purchased from Cambridge Isotope Laboratories Inc., the plant protein was produced from proteins extracted and purified from $^{15}$N-enriched barley shoots, and the protein-tannin complex was made from condensed tannins extracted and purified from salal foliage and flower heads. To determine $^{15}$N isotopic enrichment, samples of the protein and tannin-protein complex were analyzed for total N and $^{15}$N at the Stable Isotope Facility at UC–Davis. The $^{15}$N-enrichments of all the treatment compounds are shown in Table 4.1.

Table 4.1. The $^{15}$N atom% enrichments of the treatment compounds used in the N Uptake Trial.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^{15}$N enrichment (atom%)</th>
<th>N content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>98.3</td>
<td>95.0</td>
</tr>
<tr>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>98.5</td>
<td>98+</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>98.5</td>
<td>98+</td>
</tr>
<tr>
<td>protein</td>
<td>30.21</td>
<td>12.4</td>
</tr>
<tr>
<td>protein-tannin complex</td>
<td>40.43</td>
<td>6.6</td>
</tr>
</tbody>
</table>
(a) Compound Preparation

*Plant protein preparation*

Barley was grown for two weeks in silica sand from Target Products Ltd. and was watered with modified Johnson’s solution (Epstein 1972) enriched with $^{15}$N-labelled (98+ atom%) Ca(NO$_3$)$_2$ and (NH$_4$)$_2$SO$_4$. After harvesting, the shoots were separated from the roots and rinsed with deionized water. After being patted dry, the shoots were frozen in liquid N$_2$ and the soluble protein fraction was extracted using methods outlined by Gegenheimer (1990).

Barley tissue was ground with a mortar and pestle and extracted with a 50 mM Tris-HCl homogenizing buffer (pH 7.5) enriched with 1.5% polyvinylpyrrolidone (PVPP), 5 mM dithiothreitol (DTT) and 0.1 mM EDTA, antiphenolic, reducing, and metal chelating agents, respectively. The homogenate was then filtered through two layers of Miracloth and the collected filtrate was centrifuged at 8850 rpm (9474 x g) for 15 minutes to remove debris. Polyethyleneimine (PEI) was added (0.3% w/v) to the decanted supernatant to remove nucleic acids and phospholipids, and the solution was re-centrifuged at 8850 rpm (9474 x g) for 15 minutes. The supernatants were then added together and the protein fraction was precipitated with (NH$_4$)$_2$SO$_4$ in two steps. To remove impurities, the solution was initially brought to 25% saturation with (NH$_4$)$_2$SO$_4$ and centrifuged at 9900 rpm (11855 x g) for 10 minutes. The supernatant was then increased to 85% (NH$_4$)$_2$SO$_4$ saturation and allowed to stand for 30 minutes to allow for equilibration between the salt and the proteins, prior to centrifuging at 9900 rpm (11855 x g) for 15 minutes. During the (NH$_4$)$_2$SO$_4$ additions, the pH was maintained at 7.5 by the addition of 1M NaOH as necessary. The temperature of the solutions was maintained between 0-4 °C during all stages of the protein extraction and purification. The supernatant was then decanted and the protein precipitate was resolubilized in Tris-HCl buffer (without DTT) and dialyzed in 3,500 MW dialysis tubing against sterile deionized water overnight in the refrigerator (below 4 °C). The sterile water was changed five times during the dialysis to maximize the removal of small impurities and salts. After dialysis, the protein solution was freeze-dried, and the dried protein powder was stored below 0 °C until further use. The chemical purity of the protein powder was assessed using the Bradford protein assay (Bradford 1976) and gel electrophoresis (Sambrook et al. 1989). Both tests indicated a high protein purity. The Bradford assay, conducted with a Biorad kit and an immunoglobulin (IGG) standard, indicated 100% protein content. The gel electrophoresis used the agarose quantification of nucleic acids method involving the staining (ethidium
bromide at 0.5 µg·ml\(^{-1}\)) of a 0.8% gel run for 30 minutes at 100 volts, and did not show detectable levels of nucleic acids.

Salal tannin preparation

Purified condensed tannins from salal were provided by Dr. Caroline Preston at the Pacific Forestry Centre in Victoria, B.C. The tannins were prepared from salal foliage and flower heads using standard methods described in Preston (1999). Briefly, samples were pre-extracted with hexane, followed by an extraction with 70% (v/v) acetone. The extracts were then washed with CHCl\(_3\) and ethyl acetate and condensed by rotary evaporation. The condensed extract was freeze-dried then purified by chromatography. Sugars and low-MW phenolics were removed using methanol, followed by acetone (70% v/v) to elute the tannins. The extracts were then rotary evaporated to remove the acetone and the remaining solution was freeze-dried to produce purified condensed tannin powder.

Plant protein-tannin complex preparation

The protein-tannin complex was made using a modification of methods outlined by Lewis and Starkey (1968). Briefly, 4 g of protein and 1.3 g of tannin were dissolved in 400 ml and 130 ml of 0.05 M acetate buffer (pH 5.0), respectively. The two solutions were combined and refrigerated at 4 °C for 30 minutes before being centrifuged at 6,000 rpm (4355 x g) for 10 minutes. The precipitated protein-tannin pellets were freeze-dried and stored below 0 °C until further use.

At the time of establishment of each block, each treatment (Ca(NO\(_3\))\(_2\), (NH\(_4\))\(_2\)SO\(_4\), glutamic acid, protein, protein-tannin complex and deionized water) was applied to four pots of each plant species to produce a total of 504 experimental units (3 species x 6 treatments x 4 harvest times x 7 blocks). The treatment solutions were prepared with autoclaved deionized water and were applied within one hour of preparation. The Ca(NO\(_3\))\(_2\), (NH\(_4\))\(_2\)SO\(_4\) and glutamic acid treatments were prepared as bulk solutions and the protein and protein-tannin treatments were prepared individually due to their low solubilities. The protein and protein-tannin treatments were well mixed into suspension prior to application. In each pot, a total of 60 ml of solution was applied to six evenly-spaced locations around the seedling, 10 ml of solution at each spot, as described in Chapter 3 (pg. 57). Flasks holding the protein and protein-tannin treatments were rinsed twice with deionized water and the rinses were applied to the forest floor surfaces of the respective
pots. Solution that leaked from the bottom of the pots was collected in individual trays and were re-applied to the forest floor surface. These measures were taken to ensure that the treatments were fully contained within the cores. Each pot received 0.1178 mmol of N with the (NH₄)₂SO₄, glutamic acid, protein, and protein-tannin treatments, but Ca(NO₃)₂ was applied at an equivalent of 0.1604 mmol N because of an error in product data sheet.

Cedar, hemlock and salal seedlings were harvested from the cores 1, 3, 7 and 20 days after treatment injection and in the order in which they were injected. All harvests occurred within three hours of the specified harvest time. Whole plants were harvested and care was taken to remove as many of the live roots as possible. It was not possible to recover all the salal roots due to the hair-like structure of the roots (Xiao 1994), so a portion of the salal roots was not included in the samples. Following harvest, the plants were rinsed with tap water to remove all particles of forest floor adhering to the roots. The roots were then soaked twice successively in 0.5 mM CaCl₂ for five minutes (Nasholm et al. 1998) to remove labelled N adsorbed to root surfaces and present in the free apoplastic space. The two seedlings from each pot were combined and the whole plants were dried at 70 °C to constant weight.

The cedar, hemlock and salal seedlings from day-3 harvests were separated into roots and shoots before being dried. These seedlings were used to assess shoot and root weights and three (hemlock) or four (cedar and salal) sets of each species receiving the NO₃⁻ treatment were analyzed to provide indications of N allocation patterns to plant shoots and roots. The salal measurements were used to calculate a correction factor in N assimilated due to root loss. The NO₃⁻ treatment was chosen because NO₃⁻ reduction usually occurs in the roots of most conifers and ericaceous plants (Smirnoff et al. 1984). The NO₃⁻ treatment would therefore provide an upper level of N loss by the salal seedlings. Based on the average proportion of treatment N contained in the roots in the N Uptake Trial and the salal shoot:root (including rhizomes) ratio measured by Messier (1992), the total N uptake values for salal would only be increased, on average, 1.19x by including the unharvested root fraction. This increase would not substantially alter the results nor was it known how the different N compounds were partitioned within the plants, so the salal N uptake measurements were not adjusted.

The dried tissues were cooled in a dessicator and then allowed to equilibrate with the moisture in the air before weighing. This prevented bias in the isotope analysis due to moisture absorption.
during grinding and sample preparation. After weighing, the plants were ground to a fine 
powder by a Wiley mill followed by a Fritsch ball mill. Ground samples were then analyzed for 
total N and $^{15}$N contents at the Stable Isotope Facility at UC-Davis. 
From the atom% $^{15}$N values, total amounts of N assimilated by the cedar, hemlock and salal 
seedlings in each treatment were calculated using equation 1 in Chapter 2 (pg.36). 

Atom% $^{15}$N values for whole plants were generated from root and shoot atom% $^{15}$N using 
equation 2. 

$$\text{(2) } \text{atom}\%_{wp} = \frac{(\text{atom}\%_s \times X_s) + (\text{atom}\%_r \times X_r)}{X_s + X_r}$$ 
where atom%$_{wp}$ is the whole plant atom % $^{15}$N; atom%$_s$ is the shoot atom % $^{15}$N; and atom%$_r$ is 
the root atom % $^{15}$N. 

Total nmol N assimilated was calculated and results are expressed as percentages of N applied 
(nmol assimilated/nmol N applied *100). 

To confirm the presence of mycorrhizal fungi, the root systems of seven cedar, hemlock and 
salal seedlings were assessed. Seedlings from the forest floor cores used in the microbial and 
forest floor chemical analyses (outlined below) were rinsed thoroughly with tap water and the 
roots were separated from the shoots and preserved in FAA. The cedar and hemlock root 
systems were cleared and stained according to modified methods outlined by Kormanik and 
McGraw (1984) as in Chapter 2 (pg. 31). Salal roots did not require clearing or staining and 
were kept in FAA until root colonization was assessed as described in Chapter 2. 

*Forest floor microbial assessments* 
In October 1999, six pots (forest floor cores harvested in May) of cedar, hemlock and salal, were 
used to assess for differences in the forest floor microbial communities associated with the three 
plant species. These planted cores were chosen because the plants had grown for the longest 
time period (17 months). Each core was removed from the pot and all forest floor material 
within 1 cm a root or loosely adhered to root surfaces was shaken into a plastic bag. The cores 
were fully occupied by cedar, hemlock and salal roots, so forest floor within 1 cm of the roots 
was considered to be within a zone of root influence (RIZ) and similar to the rhizosphere in 
biological characteristics (C. Chanway *pers. comm.*). Similar conclusions were reached by Priha
et al. (1999a) who showed that the amounts of microbial C were equivalent in the rhizospheres and bulk soils from *B. pendula* and Scots pine *P. sylvestris* seedlings grown for four months in soil. The bagged forest floors were sieved through 5 mm followed by 2 mm sieves to remove the fine roots. Sparling et al. (1985) and West and Sparling (1986) found that the inclusion of fine roots significantly affected chloroform fumigation estimates of microbial N and glucose-induced respiration, respectively. Therefore, care was taken to remove as much fine root biomass as possible with the 2 mm sieving (Sparling et al. 1985). The samples from the sieved forest floors were used in the assessments of microbial biomass size and the metabolic abilities of the microbial communities.

(a) Microbial Biomass

To determine if the microbial communities in the CH forest floors planted with cedar hemlock and salal differed in size, microbial biomass N contents were estimated using the chloroform fumigation-extraction technique (Brookes et al. 1985) modified by the use of 1M KCl as the extractant (Amato and Ladd 1988; Cabrera and Beare 1993; Horwath and Paul 1994). Two sets of 20 g (fresh weight) samples were prepared from each of the eighteen forest floors. The first set was extracted with 100 ml of 1M KCl, shaken for one hour and filtered through pre-soaked (in 1M KCl for one hour) Whatman #42 filter paper. The second set of samples was placed in 100 ml beakers and transferred into dessicators lined with wet paper towels. Each dessicator contained a beaker with 50 ml of ethanol-free chloroform (CHCl₃) and anti-bumping chips. The dessicators were sealed and evacuated until the CHCl₃ boiled. After two minutes of boiling, the valves were closed and the samples were incubated at 25 °C for five days (Davidson et al. 1989). Following the incubation, the paper towel and CHCl₃ were removed and the dessicators were evacuated eight times to remove residual CHCl₃ from the forest floor samples. The samples were then extracted with 100 ml of 1 M KCl as described for the first sample set. Both sets of samples were stored in the refrigerator below 4 °C until analyzed.

The KCl extracts from the fumigated and non-fumigated forest floor samples were then digested in sulphuric acid and TKN was measured as outlined in Kalra and Maynard (1991). The difference between the total N contents in the fumigated and non-fumigated forest floor extracts is an estimate of the N contained within the microbial biomass (Brookes et al. 1985). Conversion factors as outlined by Brookes et al. (1985) to determine actual biomass sizes were
not applied because the microbial composition of CH forest floors is not known (Horwath and Paul 1994). The TKN analyses were conducted at Soilcon Laboratories.

(b) Microbial Community Metabolic Abilities (MCMA)
To determine if the microbial communities in the forest floors planted with cedar, hemlock or salal differed in their metabolic capabilities, functional diversities of the microbes in the cedar, hemlock and salal forest floors were assessed by measuring the increase in respiration in response to the addition of organic N compounds similar to those applied in the N Uptake Trial. The increase in CO$_2$ production was used as an index of the ability of the microbial biomass to mineralize the compounds and potentially provide mineral N for plant uptake. The eighteen sieved forest floors (outlined above) were used in this analysis and sat at room temperature (20 °C) for 48 hours to allow the microbial populations to stabilize before starting the trial (M. Holmes pers. comm.). The forest floors were kept in open plastic bags that were slightly closed to prevent moisture loss. Moisture contents were determined by drying 10 g fresh weight samples at 105 °C for 24 hours. After 48 hours, deionized water was added to adjust the moisture content of each forest floor to 81% (w/w), which was the largest water content among the samples. Typically, soil or forest floor samples are brought to 60% moisture content prior to conducting microbial respiration assessments to optimize microbial activity (Kuzyakov 1996). However, in this trial samples were not allowed to air-dry for more than 48 hours to prevent excessive alteration of the microbial communities in the forest floors. The field moisture contents of these forest floors, measured in June and July, ranged from 77-84% (K. Hannam pers. comm.), so 81% is within the range normally experienced by microbes in CH forest floors during the growing season. Five samples (equivalent to 1.9 g dry weight) of each of the 18 forest floors were transferred to 500 ml wide-mouthed canning jars.

One of four treatment solutions or deionized water (control) was added to each of the five samples. Glutamic acid, BSA, tannin-BSA, and globulins (from curcurbit seed) were dissolved in autoclaved deionized water and 1 ml of each was dispensed into the appropriate forest floor sample to provide equivalents of 4 mg C·g forest floor$^{-1}$ (dry weight). Vesterdal (1998) showed that forest floor microbes fully utilized organic compounds when added at these concentrations. Following treatment additions, the forest floors were mixed with stainless steel spatulas to ensure homogeneous distribution of the treatments and the jars were sealed with airtight lids. The BSA
and globulins were purchased from Sigma-Aldrich, the glutamic acid was from Fisher Scientific and the tannin-BSA was prepared using the condensed salal tannins and methods previously described (pg. 78).

To measure CO₂ production, 1 ml aliquots of gas were removed from the jar headspaces (477 ml) with a syringe and were directly injected into a Li-Cor LI-800 infrared gas analyzer (IRGA) linked to a Waters 746 Data Module integrator. Gas was sampled 1, 5, 15, 25, 35, 45, 55, 65 and 75 hours after sealing the jars. After 75 hours, the lids were removed, the jars were left open for one hour and air was circulated in each jar for 15 seconds before resealing. A preliminary trial indicated that this cleared the headspace of accumulated CO₂. Carbon dioxide was also measured 5, 10, 15 and 20 days after treatment application, and after each sampling, the jar lids were removed for one hour and flushed for 15 seconds. The jars were kept in the dark, at 20 °C throughout the trial.

Carbon dioxide contents within the jars were determined from the area-based integrator values. At each sampling time, standard curves were produced from known volumes of 1510 ppm CO₂ injected into the IRGA. From these curves, the CO₂ contents in the 1 ml samples were generated and total CO₂ contents for the jars were calculated based on the 477 ml headspace volume. This headspace volume was calculated assuming a constant 81% moisture content and an organic matter bulk density of 130 kg·m⁻³. Carbon dioxide values were converted to micrograms C-CO₂ assuming the CO₂ was acting as an ideal gas (24.05 L CO₂·mol⁻¹).

Cumulative and hourly CO₂ production was calculated from the CO₂ measurements and both were adjusted for ambient CO₂ concentrations and CO₂ withdrawn during previous CO₂ sampling periods. Hourly CO₂ production was calculated using equation 3.

\[ T = (\text{hour}Y - \text{air}Y + (\text{CO}_2X / 477.285)) - (\text{hour}X - \text{air}X) \]

Where \(\text{hour}Y\) is the CO₂ content of the jar head-space; \(\text{air}Y\) is the CO₂ content of the control (air only) jar head-space; \(\text{CO}_2X\) is the CO₂ produced during the previous measurement period; \(\text{hour}X\) is the CO₂ content of the jar head-space at the last measurement time; and \(\text{air}X\) is the CO₂ content of the control (air only) jar head-space at the last measurement time.
Forest Floor Characteristics

Nutrient concentrations and pH were measured in the eighteen forest floors samples described earlier (pg. 80). Nitrate, NH$_4^+$ and SON concentrations were measured in the 1 M KCl extracts from the first set (unfumigated) of samples used in the microbial biomass assessments. Total N concentrations were determined by TKN analysis (Kalra and Maynard 1991). Total C was measured by combustion, as outlined by Lavkulich (1981). The nutrient analyses were conducted at Soilcon Laboratories.

The pH of the forest floors was measured on 10 g (fresh weight) samples shaken in 25ml deionized water for one hour. The samples were allowed to stand for ten minutes prior to measurement with a Hanna Instruments pH meter (HI 9025).

Statistical Analyses

To determine differences in the amounts of Ca(NO$_3$)$_2$, (NH$_4$)$_2$SO$_4$, glutamic acid, protein, protein-tannin treatment N taken up by cedar, hemlock and salal at each harvest time, N uptake measurements were analyzed as a completely randomized block design and were compared by species and harvest day. Because the total amount of N added differed between NO$_3^-$ and the other treatments, N uptake was calculated as percent assimilated (% of applied). These values were then compared using ANOVA (GLM procedure) followed by pairwise t-test comparisons of the least square means. The alpha level (0.05) was adjusted for the number of comparisons using the Bonferroni’s adjustment (Neter et al. 1996). All data was square root(arcsin) transformed prior to analysis to meet the assumptions of normality and equality of variances, except the cedar - day 3, cedar - day 20, hemlock - day 3, salal - day 7 and salal - day 20 values. Hemlock - day 3 and salal - day 20 were arcsin transformed. Original values are reported in the tables and figures presented in the Results.

To estimate the relative importance of organic N in the total N nutrition of cedar, hemlock and salal, the N assimilated (% of applied) values were summed or all the treatments and for only the organic N treatments. These two summed values were then expressed as a ratio (organic N/total N assimilated), and the ratios for each species were compared by harvest day using ANOVA (GLM procedure) followed by pairwise t-test comparisons and adjusted alpha level, as outlined above.
To estimate differences in the amounts of N assimilated in the shoots and roots of cedar, hemlock and salal, the N values from the NO$_3^-$-treatment plants harvested on day 3 were analyzed with paired t-tests.

Pairwise t-tests were also used to compare cedar, hemlock and salal shoot and root dry weights in all treatments harvested at day 3. Shoot:root ratios of each species were first tested and no significant treatment influences were detected. So the day-3 harvest shoot and root weights were pooled for each species. Differences between the shoot:root ratios of the three species were then tested using ANOVA (GLM procedure) followed by pairwise t-test comparisons and adjusted alpha level, as previously outlined.

The effect of the cedar, hemlock and salal seedlings on microbial and forest floor properties was tested using ANOVA (GLM procedure) followed by pairwise t-test comparisons and adjusted alpha levels, as outlined above. Ammonium, NO$_3^-$, TKN and SON concentrations, C/N ratio and microbial biomass N were all analyzed in this way. Soluble organic N was log-transformed prior to analysis but the original values are reported in the Tables. All statistical analyses of the microbial and forest floor data used an initial (prior to Bonferroni’s adjustment) alpha level of 0.10. SAS was used for all plant and forest floor analyses (SAS Institute 1993).

**Results**

*N Uptake Trial*

The five N treatments differed in their importance as N sources for cedar, hemlock and salal during the first 24 hours. However, the uptake trends among the species were similar (Fig. 4.1). More of the inorganic N compounds (NO$_3^-$ and NH$_4^+$) were taken up by all three species, with significantly greater amounts of the treatment NO$_3^-$ and NH$_4^+$ being assimilated relative to the N from the organic N treatments. In the organic N treatments, there were no significant differences between the percentages of glutamic acid-, protein- and protein-tannin-N assimilated by cedar. Hemlock and salal took up larger amounts of glutamic acid- relative to protein-tannin-N.

Similar trends were seen for the remainder of the trial. Cedar, hemlock and salal absorbed significantly higher amounts of the applied N in the inorganic N treatments. After day 1, more
Nitrate-N was taken up by cedar, followed by NH$_4^+$-N and N from the organic N compounds (Fig. 4.2 a-b). There were no statistical differences between the proportions of N taken up by cedar in the organic N treatments, but there was a trend of increasing importance of glutamic acid- and protein-N relative to protein-tannin-N over time. Nitrate-N was also taken up in the largest amounts by salal, but differences between the NO$_3^-$ and NH$_4^+$ treatments were significant only on the third day (Fig. 4.3 a-b). Uptake of N by salal did not differ among the organic N, except on day 3, when the absorption of glutamic acid-N was higher than that of protein- and protein-tannin N. In general, availability of glutamic acid- and protein-N to salal increased relative to protein-tannin-N during the 20 days. In hemlock, there were no differences in the amounts of applied N absorbed in the NO$_3^-$ and NH$_4^+$ treatments, but NH$_4^+$ was a larger source of N until day 7, after which NO$_3^-$ was a greater source of N (Fig. 4.4 a-b). On days 3 and 7, a significantly larger amount of glutamic acid-N was absorbed by hemlock relative to protein-tannin N. The protein-tannin treatment was not a large source of N to any of the species. Less than 2% of the N applied in this form was assimilated during the 20 days. Blocks were significant in the analyses of hemlock – day 3 harvest and salal – day 20 harvest (Appendix I).

Figure 4.5 shows the N absorbed by the plants from the organic N treatments as a proportion of the N absorbed from all the treatments during the 20-day trial. Although no significant differences in the ratios were detected, organic N appeared to account for more of the treatment N assimilated by hemlock (days 1-3) and salal (all 20 days) relative to cedar. Blocks were significant for the day 1 harvests (Appendix I).

Shoot dry weights were significantly greater than root dry weights in all three species and salal shoot:root ratios were greater than those of cedar and hemlock (Table 4.2). A larger amount of the treatment N was contained within the roots of all three species in the NO$_3^-$ treatments (Table 4.3).

All cedar, hemlock and salal root samples were mycorrhizal and therefore it was assumed that the plants used in the N Uptake Trial were also mycorrhizal and would accurately reflect the abilities of cedar, hemlock and salal to access organic N in CH forest floors.
Figure 4.1. Treatment N taken up by cedar, hemlock and salal during the first 24 hours of the N Uptake Trial. Different letters indicate significantly different least square means within a species based on ANOVA (GLM procedure) ($p<0.05, n=5-7, \text{mean} \pm \text{S.E.M.}$)
Figure 4.2 (a-b). Treatment N assimilated by cedar during the N Uptake Trial. Different letters indicate significantly different least square means within a harvest day based on ANOVA (GLM procedure) ($p<0.05$, $n=5-7$, mean + S.E.M.). Unless otherwise stated, there were no significant differences between the organic N treatments.
Figure 4.3 (a-b). Treatment N assimilated by salal during the N Uptake Trial. Different letters indicate significantly different least square means within a harvest day based on ANOVA (GLM procedure) ($p<0.05$, n=5-7, mean ± S.E.M.).
Figure 4.4 (a-b). Treatment N assimilated by hemlock during the N Uptake Trial. Different letters indicate significantly different least square means within a harvest day based on ANOVA (GLM procedure) ($p<0.05$, $n=5-7$, mean + S.E.M.).
Figure 4.5. The proportion of treatment N assimilated by cedar, hemlock and salal from the organicN compounds. Each mean is calculated as: sum of organic N treatments taken up (% of applied) / sum of all N treatments taken up (% of applied) at specific harvest day. There were no significant differences between least square means within a harvest day based on ANOVA (GLM procedure) ($p<0.05$, $n=5-7$, mean ± S.E.M.).
Table 4.2. Shoot and root dry weights and shoot:root ratios of cedar, hemlock and salal seedlings. Values are calculated from all the plants from the day 3 harvests. Different letters indicate significantly different means within species (shoot and root weights) and between species (shoot:root) based on paired t-test and ANOVA (GLM procedure), respectively ($p<0.05$, $n=42$, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Shoot wt. (g)</th>
<th>Root wt. (g)</th>
<th>Shoot:Root ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>cedar</td>
<td>5.15 (0.43) a</td>
<td>3.69 (0.32) b</td>
<td>1.44 (0.04) b</td>
</tr>
<tr>
<td>hemlock</td>
<td>2.85 (0.23) a</td>
<td>1.86 (0.13) b</td>
<td>1.51 (0.05) b</td>
</tr>
<tr>
<td>salal</td>
<td>7.64 (0.62) a</td>
<td>1.60 (0.14) b</td>
<td>4.93 (0.17) a</td>
</tr>
</tbody>
</table>

Table 4.3. Nitrogen assimilated in the roots and shoots of cedar, hemlock and salal treated with $\text{NO}_3^-$ and harvested on day 3 in the N Uptake Trial. Different letters indicate significantly different means within species based on paired t-test analysis ($p<0.05$, $n=4$, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>N assimilated (nmol N·mg tissue$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cedar</td>
<td>shoot</td>
<td>4.32 (1.45) a</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>10.37 (2.31) b</td>
</tr>
<tr>
<td>hemlock</td>
<td>shoot</td>
<td>1.38 (0.23) a</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>3.76 (1.23) b</td>
</tr>
<tr>
<td>salal</td>
<td>shoot</td>
<td>2.77 (1.02) a</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>5.30 (1.12) b</td>
</tr>
</tbody>
</table>
Forest Floor Characteristics
The chemical characteristics of the CH forest floors in which cedar, hemlock and salal grew are given in Table 4.4. The pH, SON, TKN and C/N were not significantly different. Nitrate and NH$_4^+$ concentrations were significantly higher in cedar than in salal forest floors.

Forest floor microbial assessments
(a) Microbial biomass
The amount of N in the microbial biomasses in the CH forest floors in which cedar, hemlock and salal grew were not significantly different (Table 4.5). However, N contents were greatest in salal followed by hemlock and cedar samples, in descending order. Results from the MCMA also support this trend. Glutamic acid can be used as an alternate substrate to glucose in substrate-induced respiration (SIR) analyses to assess microbial biomass sizes (Hopkins et al. 1994; Degens and Harris 1997; Lipson and Monson 1998). The amounts of CO$_2$ produced during the first hour after the additions of glutamic acid were not significantly different, and mean CO$_2$ production was largest in the salal forest floors.

(b) Microbial Community Metabolic Abilities (MCMA)
Carbon dioxide production by the control forest floors (deionized water added) estimates the basal respiration rates of the aerobic microbes (Fig. 4.6). During the first day, the respiration rates of the hemlock and cedar samples were approximately equivalent and the CO$_2$ production rates in the salal forest floors were lowest. For the remainder of the measurement period, cedar forest floors maintained a higher rate of CO$_2$ production than did the hemlock and salal forest floors.
Table 4.4. Chemical characteristics of CH forest floor cores planted with cedar, hemlock and salal (plants grew in pots for 17 months). Different letters indicate significantly different least square means based on ANOVA (GLM procedure) ($p<0.10$, $n=5-6$, mean (S.E.M.)).

<table>
<thead>
<tr>
<th>Species</th>
<th>pH</th>
<th>$\text{NH}_4^+$ ($\mu g$ N $g^{-1}$ forest floor$^{-1}$)</th>
<th>$\text{NH}_3^+$ ($\mu M$)</th>
<th>$\text{NO}_3^-$ ($\mu g$ N $g^{-1}$ forest floor$^{-1}$)</th>
<th>$\text{NO}_2^-$ ($\mu M$)</th>
<th>Soluble organic N ($\mu g$ N $g^{-1}$ forest floor$^{-1}$)</th>
<th>Total N (TKN) (g N $g^{-1}$ forest floor$^{-1}$)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>cedar</td>
<td>4.56 (0.11) a</td>
<td>5.03 (1.01) a</td>
<td>108.90 (21.25)</td>
<td>0.29 (0.06) a</td>
<td>6.34 (1.39)</td>
<td>117.30 (24.91) a</td>
<td>0.0111 (0.0005) a</td>
<td>50.00 (2.46) a</td>
</tr>
<tr>
<td>hemlock</td>
<td>4.41 (0.09) a</td>
<td>3.14 (0.21) ab</td>
<td>63.58 (3.39)</td>
<td>0.26 (0.07) ab</td>
<td>5.09 (1.39)</td>
<td>158.22 (29.57) a</td>
<td>0.0098 (0.0006) a</td>
<td>56.41 (4.18) a</td>
</tr>
<tr>
<td>salal</td>
<td>4.30 (0.04) a</td>
<td>2.76 (0.38) b</td>
<td>89.24 (14.03)</td>
<td>0.08 (0.05) b</td>
<td>2.38 (1.51)</td>
<td>137.17 (33.61) a</td>
<td>0.0123 (0.0004) a</td>
<td>49.56 (1.49) a</td>
</tr>
</tbody>
</table>
Table 4.5. Nitrogen contained within the microbial biomass in the CH forest floor cores in which cedar, hemlock and salal grew, and microbial CO₂ production during the first hour following the addition of glutamic acid to samples from the forest floor. No significant differences between treatment least square means were detected based on ANOVA (GLM procedure) \((p<0.05, n=5-6 \text{ for N, } n=6 \text{ for CO}_2, \text{ mean (S.E.M.)})\).

<table>
<thead>
<tr>
<th>Species</th>
<th>Microbial biomass N ((\mu g \text{ N g forest floor}^{-1}))</th>
<th>CO₂ produced in one hour in response to glutamic acid ((\mu g \text{ C-} \text{CO}_2 \text{ g forest floor}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>cedar</td>
<td>291.68 (25.16) a</td>
<td>50.62 (4.90) a</td>
</tr>
<tr>
<td>hemlock</td>
<td>312.98 (53.60) a</td>
<td>44.97 (11.63) a</td>
</tr>
<tr>
<td>salal</td>
<td>332.66 (34.16) a</td>
<td>60.20 (7.57) a</td>
</tr>
</tbody>
</table>

Figure 4.6. Basal respiration rates during a 20-day incubation in the CH forest floors in which cedar, hemlock and salal grew \((n=6, \text{ mean } \pm \text{ S.E.M.})\).
The stimulation of CO₂ production (above basal respiration rates) by the organic N treatments in the cedar, hemlock and salal forest floors during the initial 75 hours following treatment application is shown in Figure 4.7(a-c). The forest floors from all three species showed large respiratory responses to glutamic acid that lasted for about 55 hours. Tannin-BSA, globulins and BSA treatments caused different respiration responses in the cedar, hemlock and salal forest floors. During the first 15 hours, the tannin-BSA treatment increased respiration in the forest floors of all three species. However, respiration in the cedar forest floor declined after 15 hours and was lower than basal respiration for the remainder of the 75-hour period. The respiration in salal and hemlock forest floors was stimulated for at least 25 hours by the tannin-BSA treatments but thereafter, CO₂ production declined to basal respiration levels. Globulins and BSA initially caused a decrease in cedar forest floor respiration relative to basal respiration rates for 75 hours. Similarly, CO₂ production in hemlock forest floors in response to the two protein treatments was depressed during the first 35 hours, but then increased to approximate the basal rates. The respiration of salal forest floors following the addition of globulins and BSA was relatively constant and slightly above basal levels during the 75-hour period.

Total cumulative CO₂ production (minus basal respiration) in the cedar, hemlock and salal forest floors over the 20-day trial period is shown in Figure 4.8 (a-c). Positive slopes indicate CO₂ production rates above basal respiration, negative slopes indicate CO₂ production below basal rates and horizontal lines indicate CO₂ production equivalent to the amount produced by basal respiration. The respiration responses of the cedar, hemlock and salal forest floors to glutamic acid began to differ after 75 hours. Carbon dioxide production in the cedar samples paralleled basal respiration rates. The respiration rates of salal forest floors were similar to, if not slightly higher than basal respiration levels and the hemlock forest floor showed elevated CO₂ production in response to glutamic acid for the full 20 days. Hemlock and salal forest floors also showed sustained higher respiration rates in response to the two proteins and the tannin-BSA treatments. In contrast, only the globulins increased the CO₂ production in cedar forest floors after 75 hours. Respiration in response to BSA was similar to basal respiration rates but appeared to decline after the tenth day. The addition of tannin-BSA, however, caused a sustained depression in CO₂ production in cedar forest floors for the duration of the trial.
Figure 4.7 (a-c). Carbon dioxide production in CH forest floors (in which cedar, hemlock and salal grew) in response to the addition of glutamic acid, globulins, BSA and tannin-BSA. The zero line represents basal respiration rate (with addition of deionized water only) (n=6, mean ± S.E.M.).
Figure 4.8 (a-c). Total CO₂ produced in CH forest floors (in which cedar, hemlock and salal grew) over 20 days in response to the addition of glutamic acid, globulins, BSA and tannin-BSA. The zero line represents total CO₂ produced by basal respiration (with addition of deionized water only) (n=4-6, mean ± S.E.M.).
Discussion

Methodological considerations
The results of studies using \(^{15}\)N stable isotope tracers to determine N uptake and partitioning within plant soil or forest floor microcosms must be interpreted with caution (Jackson et al. 1989; Schimel et al. 1989; Buchmann et al. 1995; Atkin 1996; Schimel and Chapin 1996). As outlined by Buchmann et al. (1995), when analyzing plant uptake results in trials employing tracers, it is necessary to consider: the amount of N added relative to the N present in the soil/forest floor; the \(^{15}\)N tracer dilution by these background N pools; and the microbial transformation of treatment compounds. These considerations allow for the adjustment of tracer results to more accurately estimate plant uptake and to put the plant uptake patterns in the context of microbial activity and the transformation of applied N compounds prior to uptake.

In the current trial, the N treatments were applied at rates equivalent to 14.18 and 10.42 \(\mu\)g N·g⁻¹ of forest floor and \(\text{NO}_3^-\) and \(\text{NH}_4^+\), respectively, and increased the forest floor KCl-extractable \(\text{NH}_4^+\) pools by 2-4 times and the \(\text{NO}_3^-\) pools by 49-177 times. However, it has been suggested that deionized water extractions may provide better estimates of the free \(\text{NH}_4^+\) pool available to plants (Buchmann et al. 1995). Water typically extracts less \(\text{NH}_4^+\) because it does not remove as many of the cations adsorbed to exchange sites, whereas KCl extractions include these attached ions. Amounts of \(\text{NH}_4^+\) extracted with deionized water and 1 M KCl were compared in a sub-trial conducted on CH forest floor samples from 12 cores planted with cedar, hemlock and salal (four of each species). Water or KCl was added to sub-samples from each of the forest floors, the mixtures were shaken for one hour and were gravity-filtered through pre-rinsed (1 M KCl) Whatman #42 filter paper. On average, water extracted only 40.5% of the \(\text{NH}_4^+\) present in the KCl extractions (Appendix I). With this conversion, the \(\text{NH}_4^+\) pool sizes in the forest floors outlined in Table 4.4 were increased 5-9 times with the treatment additions. The background concentrations of glutamic acid, proteins and protein-tannin complexes in the forest floors were not measured, so increases in pool size with the treatment additions could not be determined for these compounds.

It has been suggested that large differences in the sizes of N pools, as seen in the N Uptake Trial, can bias uptake results by unequally diluting the \(^{15}\)N tracer in the background pools (Buchmann et al. 1995). In this current study, \(\text{NO}_3^-:\text{NH}_4^+\) ratios, using the \(\text{NH}_4^+\) values corrected for the KCl
extractant, ranged from 1:7–1:14 and therefore $^{15}$N tracers were diluted in background N by the inverse of the pool ratios ($^{15}$NO$_3$/NO$_3$: $^{15}$NH$_4^+$/NH$_4^+$ = 7:1–14:1). According to Buchmann et al. (1995), plant uptake rates should be adjusted to compensate for these changes because, assuming that the relative uptake rates remain unchanged following $^{15}$N compound additions, the amount of $^{15}$N detected in the plants from the different treatments would probably be heavily influenced by isotope dilution in background N pools. The N pool ratios (for example NO$_3^-$: NH$_4^+$) should be multiplied by the corresponding plant N uptake ratios to provide accurate uptake estimates. Figure 4.9 shows a comparison of measured and adjusted (for tracer dilution) plant NH$_4^+$ and NO$_3^-$ uptake after one day. According to these calculations, a much larger proportion of NH$_4^+$ was absorbed by cedar, hemlock and salal, suggesting that the importance of NH$_4^+$ as an N source was underestimated. However, this “correction” for tracer dilution does not take into account the importance of N compound cycling or turnover rates, and it has recently been suggested that evaluations of flux rates or turnover rates are much more important than background concentrations when evaluating N available for plant uptake (Jackson et al. 1989; Davidson et al. 1990; Hart et al. 1994a; Leadley et al. 1997). Stark and Hart (1997) showed that despite low or undetectable soil NO$_3^-$ concentrations, production rates of NO$_3^-$ in soils/forest floors can be 27-304 mg N m$^{-1}$·day$^{-1}$. Therefore, in plant uptake trials conducted over a 24-hour time period, the sizes of the N pools that the plant is exposed to can be considerably larger than the pools measured at one point in time.

Field rates of gross N cycling (mineralization, nitrification, NH$_4^+$ consumption and NO$_3^-$ consumption) in old-growth CH forest floors (F horizon) were determined by R. Bradley (pers. comm.) in July 1997 (Table 4.6). Although gross cycling rates vary with space and time, the field rates indicate that the production and turnover of mineral N forms in CH forests is fast and that more NO$_3^-$ can be produced than NH$_4^+$ over 24 hours. Therefore, if total N pools are adjusted to include N production over a 24-hour period, estimates of pool size can be dramatically changed. For example, assuming that the N cycling rate estimated in CH forests were similar to those in the forest floor cores used in this trial, the NH$_4^+$ and NO$_3^-$ pool sizes in the cores during a 24-hour period were almost equivalent (Fig. 4.10). Therefore, adjusting the uptake measurements for static N pool size probably introduces unnecessary bias into the results. For this reason, the plant uptake measurements of NH$_4^+$ and NO$_3^-$ in the N Uptake Trial were not adjusted to account for tracer dilution and may accurately represent the relative uptake rates of the two compounds.
Figure 4.9. Measured and adjusted $\text{NO}_3^-$ and $\text{NH}_4^+$ taken up by cedar, hemlock and salal during the first 24 hours of the N Uptake Trial. The adjusted $\text{NH}_4^+$ uptake values are calculated by multiplying plant $\text{NO}_3^-$:$\text{NH}_4^+$ uptake ratios with the corresponding forest floor $\text{NO}_3^-$:$\text{NH}_4^+$ concentration ratios (corrected for KCl extraction) and applying the product to the measured plant $\text{NO}_3^-$ and $\text{NH}_4^+$ uptake.
Figure 4.10. Estimates of the 24-hour pool sizes of $\text{NO}_3^-$ and $\text{NH}_4^+$ in the CH forest floor cores in the N Uptake Trial. Values were calculated from the measured $\text{NO}_3^-$ and $\text{NH}_4^+$ concentrations (adjusted for KCl extractant) and field gross $\text{NO}_3^-$ and $\text{NH}_4^+$ production rates (R. Bradley *pers. comm.* - Table 6).
Table 4.6. Gross N cycling rates and N concentrations in old-growth CH forest floors and N concentrations in CH forest floor cores.

<table>
<thead>
<tr>
<th>N form</th>
<th>Production (nitrification or mineralization) (μg N·g forest floor⁻¹·day⁻¹)</th>
<th>Consumption (μg N·g forest floor⁻¹·day⁻¹)</th>
<th>Field concentration (μg N·g forest floor⁻¹)</th>
<th>Pot concentration (μg N·g forest floor⁻¹·day⁻¹)</th>
<th>Mean Residence Time (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻</td>
<td>1.19</td>
<td>2.21</td>
<td>0.240</td>
<td>0.08-0.28</td>
<td>0.065-0.24</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.36</td>
<td>2.71</td>
<td>2.45</td>
<td>2.76-5.03</td>
<td>7.6-14.0</td>
</tr>
<tr>
<td>SON</td>
<td>-</td>
<td>-</td>
<td>169.09</td>
<td>117.30-158.22</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Gross rates from R. Bradley (*pers. comm.*) (n=3). Rates were measured in forest floor samples using the isotope dilution technique (Hart et al. 1994b). To estimate gross mineralization and NH₄⁺ consumption estimates, 0.0914 mg N·g forest floor⁻¹ was added and for gross nitrification and NO₃⁻ consumption, 1.259 mg N·g forest floor⁻¹ was added. Consumption may be overestimated due to increased immobilization with increased substrate supply (Davidson et al. 1990; Hart et al. 1994b).

2 Field forest floor N concentrations (average of N concentrations in “F” and “Hr” horizons) (n=3) in 2 M KCl extracts from Chapter 5.

3 Potted CH forest floor core N concentrations in 1 M KCl (range encompasses the mean values for the cedar, hemlock and salal pots).

4 NO₃⁻ and NH₄⁺ concentrations (Table 4.4) divided by gross nitrification and mineralization rates, respectively (Hart et al. 1994a). The MRT for NH₄⁺ may be overestimated because the N concentrations were calculated from 1 M KCl extracts and therefore include NH₄⁺ bound to exchange sites. Concentrations in CH forest floor solutions are probably lower.
Amino acids, although still largely unquantified in natural systems, exhibit similar cycling characteristics to mineral N. Forest floor/soil pool sizes are small (Stevenson, 1994; Lipson and Monson 1998) and turnover rates high (Stevenson, 1994; Jones 1999). Kuzyakov (1996) found that the half-life of amino acids in solution at 5 °C was 2.9 hours, and Kielland (1995) estimated that glutamic acids were turned over after six hours in an arctic heath soil incubated at 20 °C. Therefore, although glutamic acid concentrations in CH forest floors in August have been measured at 9.29 and 2.38 ng N·g forest floor$^{-1}$ in the F and upper H horizons (K. Hannam pers. comm.), respectively, glutamic acid probably cycles very quickly. Therefore, the estimates of N uptake in the glutamic acid treatments should also not be adjusted for tracer dilution. Such adjustments would likely add unnecessary error to the uptake estimates. There is no published research to date that has comprehensively examined the pool sizes or cycling rates of proteins and protein-tannin complexes. Therefore, it was not possible to make any adjustments to the measurement of plant uptake of these compounds.

A third factor that is necessary to consider in the interpretation of tracer N uptake studies is the role of microbial immobilization and transformations of the N compounds prior to plant uptake (Buchmann et al. 1995). In the N Uptake Trial, a main objective was to determine if cedar, hemlock and salal differ in their abilities to utilize organic and inorganic N compounds, and how access to the N changed during a 20-day period. As previously outlined this utilization could have involved intact uptake of the compounds by mycorrhizal roots and/or the activity of rhizosphere microbial communities and the uptake of the treatment N incorporated into different chemical forms. Both mechanisms of N acquisition probably occurred during the 20-day trial period. During a 48-hour period, Norton and Firestone (1996) estimated that 27% of the N applied as NH$_4^+$ was nitrified prior to uptake by ponderosa pine (Pinus ponderosa) seedlings. Similar conclusions were drawn by Davidson et al. (1990) and Schimel et al. (1989) who estimated that 12-46% and 30% of added NH$_4^+$ was consumed by microbes and nitrified during 24-hour and 8-hour measurement periods, respectively. In the N Uptake Trial, although a large pulse of N was added, relative to background pools, the amount applied with the treatments represented only 2.9-4.5% of the N contained within the microbial communities associated with cedar, hemlock and salal. In a fertilization experiment, Jonasson et al. (1996) found that the microbial biomass was able to double its N content without increasing biomass C. Similarly, Chang (1996) showed that 29 kg N·ha$^{-1}$ applied as (NH$_4$)$_2$SO$_4$ to a seven-year-old regenerating forest on a CH site was immobilized in the microbial biomass within 24 hours. In the N Uptake
Trial, approximately 0.9 kg N-ha\(^{-1}\) was applied to each pot. Therefore, it is likely that the treatment N compounds were quickly immobilized by the microbes and that some of this released N was in a different chemical form prior to plant uptake. The importance of the microbial community in determining the form and amount of N available for plant uptake probably increased as the trial progressed, and after the first day, a large proportion of the N taken up by the plants had probably passed through microbes.

Although large amounts of the applied N compounds were probably transformed prior to plant uptake, the cedar, hemlock and salal plants may have also absorbed a portion of the N compounds intact, especially during the first day of the trial. Intact uptake of the N compounds during the first 24 hours is supported by the results of this and other studies. The trial described in Chapter 3 demonstrated that all three species have the ability to take up amino acids intact. Also, the relative amounts of treatment N absorbed during the first day of the N Uptake Trial are much larger than those that were taken up during the remainder of the trial (days 3-20). This is especially evident in the organic N treatments, which showed comparatively low N availabilities after day 1, and probably indicates microbial immobilization of these organic compounds. Schimel and Chapin (1996) also considered it unlikely that a significant portion of the N immobilized during the first 24 hours is released and made available to plants for uptake. During the first 25 hours of the MCMA in this trial, only 17-20% of the C applied with glutamic acid and 0.6-5% of tannin-BSA was respired, and the microbial CO\(_2\) production in response to the protein treatment (globulins and BSA) additions was initially either depressed (cedar and hemlock) or relatively unchanged (salal). As discussed below, these CO\(_2\) evolution pattern probably indicate microbial biomass synthesis or energy production, and it is unlikely that much, if any, N was released and made available for plant uptake. Therefore, at least some of each N compounds was probably taken up intact, mostly during the first 24 hours. Proteins and protein-tannins are thought to require degradation by exo-enzymes produced by mycorrhizal fungi prior to uptake (Bajwa and Read 1985; Leake and Read 1989; Leake and Read 1990; Finlay et al. 1992; Bending and Read 1995; Bending and Read 1996a; Bending and Read 1996b; Chalot and Brun 1998). However, in the N Uptake Trial, the plant-mycorrhizal system was considered a single unit and therefore uptake of the cleaved amino acids or small N forms was considered intact uptake.
Pot trials have often been criticized for the removal of the plant-forest floor or soil microcosm from the field and the influences of field conditions on processes. However, it is often necessary to create a controlled environment within which to assess relationships and processes and pot trials can provide good test units (Priha et al. 1999a). In this trial, separation of the cedar, hemlock and salal plants allowed for a comparison of species-specific N uptake patterns and plant-microbe interactions. This assessment would be very difficult to conduct in the field. However, when interpreting the results and extrapolating the trends to the field, it is necessary to know the pot trial conditions and substrate characteristics compared with the field situation.

The potted forest floor cores provided a restricted volume for root growth, and the cedar, hemlock and salal roots fully occupied the substrate. Most of the forest floor was no more than 1 cm from a root. The presence of roots from a single species and the full occupation of the forest floor core probably created conditions similar to those found in the rhizospheres of the three species (C. Chanway pers. comm.). Therefore, the plant uptake patterns and microbial biomass characteristics probably more accurately reflect rhizosphere conditions than those in the bulk soil.

The addition of N compounds into the restricted pot volume and the recycling of leached solution back into the pots may have produced an unrealistic situation and influenced plant uptake. Nutrients were not allowed to move laterally or vertically out of the system. The restricted N compound movement together with the full root occupation and the elevated temperatures may have resulted in overestimates of plant N uptake. Measurements of N uptake were as high as 83.9% which may be a result of the conditions. However, the relative rankings of the N forms absorbed by cedar, hemlock and salal were probably not affected by the restricted movement of the nutrients.

**N Uptake Trial**

Cedar, hemlock and salal growing in CH forest floor did not show different patterns of N utilization. During the 20-day measurement period, the organic and inorganic N treatment compounds were taken up in similar proportions by all three species. Nitrate and NH$_4^+$ accounted for the largest amounts of applied N absorbed by the end of the first 24 hours, and for the duration of the trial, these mineral N treatments remained the greatest sources of N.
Although significantly more NO$_3^-$-N was taken up by cedar after day 1, NO$_3^-$-N was also a larger source for salal and eventually for hemlock some time after day 3.

Small amounts of the applied organic N compounds were used by cedar, hemlock and salal relative to the mineral N forms, and the glutamic acid-, protein- and protein-tannin-N sources were ranked in the same order of uptake for all three species. Glutamic acid-N was taken up in the largest proportions followed by protein-N and protein-tannin-N. Although significantly larger amounts of applied glutamic acid-N relative to protein-tannin-N were absorbed by hemlock until day 7 and by salal until day 3, these differences are probably not biologically significant. The proportions of applied glutamic acid-N and protein-tannin-N taken up by cedar, hemlock and salal during the first day only differed by 0.8-1.5%, and by day 20, the range of differences between the two compounds was 3.8-6.7%. An understanding of the relative concentrations and cycling rates of the glutamic acid, protein and protein-tannin compounds in CH forest floors may reveal that the apparently small differences in the uptake of organic N compounds by cedar, hemlock and salal actually translate into significant distinctions in N form acquisition. However, without a better examination of the chemical compositions of the DON pool in CH forests, this cannot be substantiated.

The three species also took up similar amounts of organic N relative to the total amount of N absorbed in all the treatments, the. Although the organic N treatments constituted a larger proportion of the total N absorbed by hemlock on days 1-3 and for salal during the full trial period, the differences between the species were not significant. Therefore organic N compounds may not be more important sources of N to salal than to the other two species.

These results strongly suggest that cedar, hemlock and salal do not differ in their abilities to utilize N compounds. Therefore, accessing different N forms does not appear to be a mechanism by which the three species acquire more N or N from different sources in CH forests.

Despite the apparent lack of distinction in the patterns of N uptake by cedar, hemlock and salal, some interesting trends relating to N cycling in CH forests were revealed in the data, and warrant further discussion. Firstly, after day 1, NO$_3^-$-N and NH$_4^+$-N maintained much higher availabilities to the plants than did than organic N compounds. A maximum of 8% of the applied organic N compounds relative to a maximum of 84% of mineral N was utilized by the plants.
during the full trial period. This lower availability of the organic N forms suggests that a large proportion of the organic treatment compounds were immobilized in the forest floors.

Microbes in the forest floor may have been responsible for much of the immobilization of the organic N compounds. As discussed earlier, most of the applied NO$_3^-$ and NH$_4^+$ were probably quickly absorbed by the microbes and a large proportion of the organic N compounds, especially the proteins and glutamic acid, may have also been taken up by the forest floor microbiota. However, unlike the mineral N forms that were rapidly released and made available for plant uptake, the organic N compounds were retained within the microbial biomass, suggesting that the organic N compounds were used in biomass synthesis. With the immobilization of the organic N in the microbial biomass, the N in these compounds would not be mineralized over the short term, and turnover, predation, mineralization and decline of the microbial biomass and/or microbial products would release N potentially available for plant uptake (Jonasson et al. 1996). The comparatively low amounts of organic N uptake by the plants over 20 days support this interpretation.

Respiration in the cedar, hemlock and salal forest floors also support this interpretation. Proteins initially depressed CO$_2$ production in the cedar and hemlock samples and this lower respiration rate may have been a result of the direct usage of amino acids and peptides from the proteins in the synthesis of new microbial cells and biomass (Dawes 1989; Jingguo and Bakken 1997). Proteins are typically the largest fraction of N-containing compounds in microbial cells (Paul and Clark 1996), and in fungal cells, 60-70% of the total N is accounted for by proteinaceous compounds (Cochrane 1958). Thus, using these monomeric compounds would reduce the energy demand for the construction of new peptide and amino acid molecules, and CO$_2$ production would decrease. Eventually, respiration would be expected to increase as a result of the increased microbial biomass, and this was seen in the cedar and hemlock forest floors after 25 and 35 hours, respectively.

Unlike the proteins, glutamic acid immediately stimulated respiration in the forest floors of all three species, and respiration remained elevated for 55 hours. This suggests that the glutamic acid may have been degraded and/or mineralized, but it is probable that a large proportion was used in microbial biomass synthesis. As outlined in Chapter 3, glutamic acid can be deaminated and enter the TCA cycle as α-ketoglutarate. In this energy cycle, which is present in the cells of
most aerobic microbes (Brock 1994), CO₂ is generated. Therefore, respiration would increase with assimilation of glutamic acid by forest floor microbes.

In contrast to the protein and glutamic acid treatments, the N in the protein-tannin complex did not increase in availability to cedar, hemlock and salal plants during the trial period. This suggests that the microbes quickly immobilized and retained the N contained within these compounds, or that both plants and microbes had limited access to these compounds after the first day. Tannins form strong complexes with proteins that are resistant to microbial degradation (Benoit et al. 1968; Lewis and Starkey 1968; Northup et al. 1995) and are involved in the formation of recalcitrant humus (Field and Lettinga 1992; Northup et al. 1995). Condensed tannins are particularly resistant to microbial decomposition and effectively bind proteins when exposed to acidic conditions (Benoit et al. 1968; Lewis and Starkey 1968). Despite the poor ability of microbes to degrade tannins, Benoit et al. (1968) showed that during a 21-day period, 3% of the C applied as a complexed wattle tannin-gelatin compound (1:4 tannin:protein ratio) was respired by a mixed soil microbial community. The majority of this respiration occurred during the first 24 hours, as also found by Lewis and Starkey (1968). Similar CO₂ production patterns were seen in the MCMA in the cedar, hemlock and salal forest floors treated with the protein-tannins in our trial, suggesting that the microbes metabolized a pulse of the protein-tannin compounds shortly following compound addition. Condensed tannins range in size from 800-6000 MW (Foo and Porter 1980) and the smaller MW tannins are more easily degraded by microbes (Field and Lettinga 1992). The greater stimulation of microbial respiration during the first 15-45 hours of the MCMA and the aforementioned trials may have been a result of the utilization of the smaller MW tannin compounds. Some of the bound N contained within these compounds may have been accessed by the microbes and may have been slowly released and made available for plant uptake through mineralization and/or microbial turnover during the trial. However, based on the plant uptake results, the amounts released were small.

After the first 15-45 hours, microbial respiration in response to protein-tannins either paralleled that found in the protein treatments (hemlock and salal forest floors), or was depressed (cedar forest floors). It appears that the rhizosphere microbes associated with hemlock and salal may have had continued access to some of the C in the protein-tannins and that a portion of the N contained within the compounds that were absorbed may have been released. During the 20-day
trial, hemlock and salal absorbed three times as much as was taken up by first day, and a portion of this assimilated N may have been of microbial origin. However, during the trial, only totals of 1.0 and 1.3% of the N applied with the protein-tannin treatments were taken up by the hemlock and salal, respectively, further suggesting a limited ability of both the microbes and plants to access the protein-tannin compounds.

In contrast to the hemlock and salal forest floors, the respiration in the cedar forest floors was depressed 15 hours after the addition of protein-tannin, and this reduced CO$_2$ production was sustained for the 20-day measurement period. Tannins are inhibitory to some microbes (Benoit et al. 1968; Jalal and Read 1983; Field and Lettinga 1992; Kolodziej et al. 1999), so the reduced respiration in the cedar forest floors may have been due to the negative effect of tannins on microbial activity. Either the metabolic by-products of protein-tannin degradation generated during the first 15 hours (Jalal and Read 1983) and/or certain protein-tannin compounds could have caused the inhibition of microbial respiration. Regardless, after the first 15 hours, the protein-tannin compounds were probably poorly metabolized by the microbes in the cedar forest floors indicating a limited ability of cedar rhizosphere microbes to access the bound N after the first day. Also, 61% of the protein-tannin-N taken up by cedar had been absorbed by the first 24 hours further supporting the poor ability of the microbes in the cedar forest floors to access the tannin-bound proteins and release N for plant uptake.

A second interesting trend apparent in the N Uptake Trial data was the different availabilities of NO$_3^-$-N and NH$_4^+$-N during the 20-day period. Although both mineral N compounds were large sources of N to cedar, hemlock and salal, they behaved differently. The applied NO$_3^-$-N was consistently the largest source of N for cedar and salal after day 1 and for hemlock after day 7. The availability of NH$_4^+$-N for cedar and hemlock plateaued after day 7. Salal, however, appeared to have continued access to the NH$_4^+$-N throughout the trial. The differences in the availabilities of the N in these two compounds to cedar, hemlock and salal strongly suggest that NO$_3^-$ and NH$_4^+$ were not cycled the same way. These cycling differences of the inorganic N forms could be a function of the chemical mobility, binding and fixation and microbial immobilization of the two compounds.

Ammonium and NO$_3^-$ differ in their mobility and binding in forest floors. Nitrate is relatively mobile and its diffusivity is an order of magnitude greater than that of NH$_4^+$ in soil solution (Paul...
and Clark 1996). Substantial amounts of NH\textsubscript{4}\textsuperscript{+} can be adsorbed onto cation exchange sites in forest floors (Nye and Tucker 1977; Leadley et al. 1997). A comparison of the NH\textsubscript{4}\textsuperscript{+} concentrations in the 1 M KCl and distilled water extracts indicated that approximately 60% of NH\textsubscript{4}\textsuperscript{+} in CH forest floors was adsorbed to exchange sites (Appendix I). Ammonium can also be chemically fixed in forest floors. Schimel and Firestone (1989) found that roughly 20% of added NH\textsubscript{4}\textsuperscript{+} was fixed by abiotic mechanisms and could not be extracted with 1 M KCl. Therefore, although some of the adsorbed treatment NH\textsubscript{4}\textsuperscript{+} was probably released during the 20-day trial to maintain an equilibrium with the solution, a combination of chemical adsorption to exchange sites and fixation probably resulted in a reduction in the pool size of available treatment NH\textsubscript{4}\textsuperscript{+}-N relative to the pool of available N in the NO\textsubscript{3}\textsuperscript{-} treatments.

Microbial activity may have also contributed to the differences in the mineral N availability over time. Several studies have examined the abilities of soil microbes to assimilate NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+}. Rice and Tiedje (1989) found suppression of microbial NO\textsubscript{3}\textsuperscript{-} assimilation in agricultural soil slurries at low concentrations of NH\textsubscript{4}\textsuperscript{+}, but when NH\textsubscript{4}\textsuperscript{+} was absent, NO\textsubscript{3}\textsuperscript{-} was assimilated immediately and at a constant rate. Based on these results, many have attributed high levels of NO\textsubscript{3}\textsuperscript{-} assimilation to microsites with low NH\textsubscript{4}\textsuperscript{+} and high C availabilities (Rice and Tiedje 1989; Schimel et al. 1989; Davidson et al. 1992; Hart et al. 1994a). However, Rice and Tiedje (1989) also found that the uptake of NO\textsubscript{3}\textsuperscript{-} by three different soil bacteria was influenced differently by the presence of NH\textsubscript{4}\textsuperscript{+}. Azotobacter vinelandii did not reduce NO\textsubscript{3}\textsuperscript{-} absorption until NH\textsubscript{4}\textsuperscript{+} concentrations reached a very low level and above this concentration, NO\textsubscript{3}\textsuperscript{-} uptake was not affected until after five minutes and was only reduced by 24%. Thus, NO\textsubscript{3}\textsuperscript{-} assimilation in forest floors or soils with inhibitory levels of NH\textsubscript{4}\textsuperscript{+} may occur at microsites with certain conditions and may also be a function of microbial composition. Some microorganisms may be better able to assimilate NO\textsubscript{3}\textsuperscript{-} than others. Therefore, in the potted CH forest floor cores in the N Uptake Trial, different microbial populations may have been responsible for assimilating larger proportions of the NO\textsubscript{3}\textsuperscript{-} or NH\textsubscript{4}\textsuperscript{+} compounds. These different populations may also have had different cycling rates or may have released different N products. A shorter immobilization period or faster turnover time could provide more N to the plants. Thus NO\textsubscript{3}\textsuperscript{-}-N may have been immobilized for a shorter time than NH\textsubscript{4}\textsuperscript{+}-N. In addition, the N compounds released from the NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} assimilating microbes may have differed with the NO\textsubscript{3}\textsuperscript{-}N products being more easily accessed and/or more available for plant uptake than the NH\textsubscript{4}\textsuperscript{+}-N products. Therefore, differences in
microbial activities may have influenced NO$_3^-$-N and NH$_4^+$-N uptake by cedar, hemlock and salal.

Regardless of the slight differences between the NO$_3^-$-N and NH$_4^+$-N availabilities throughout the trial, the two inorganic N compounds were large sources of available N for cedar, hemlock and salal. Nitrogen fluxes in the field, without the addition of fertilizers, probably rarely equal the amount of N applied in our trial, but even if the fast cycling rates and relatively constant availabilities of NO$_3^-$-N and NH$_4^+$-N occur to a small extent (ie. are not just artifacts of the large N pulses), these findings could have significant implications for N cycling and plant-microbe interactions in CH forests. More work into the cycling and long-term availability of NO$_3^-$ and NH$_4^+$ for plant uptake is necessary to determine to what degree the observed trends occur in the field.

*Forest floor microbial community assessments*

The objectives in assessing the microbial communities in the cedar, hemlock and salal forest floors were to determine the importance of the microbes in making N available for plant uptake and to ascertain whether the communities associated with the three species differed in their capacities to affect N availability. By evaluating microbial biomass sizes and the abilities of the microbial communities to metabolize and/or mineralize the organic N compounds, differences in the communities associated with cedar, hemlock and salal could be detected. In addition, the methods of N acquisition by cedar, hemlock and salal, in part, could be explained. For example, if the addition of proteins immediately stimulated microbial respiration in the forest floors of one of the plant species, and if the same species showed relatively large uptake of protein, a substantial proportion of the applied protein-N may have been mineralized and taken up in a non-protein form. Although the influences of anaerobic microbiota could not be evaluated with the MCMA, this assessment was thought to provide an indication of the potential differences in the whole microbial communities in cedar, hemlock and salal forest floors.

Although no differences were detected in the sizes of the microbial biomass, the CO$_2$ production patterns in response to the additions of different N forms indicated that the composition of the microbes in the cedar, hemlock and salal forest floors were probably different. The four organic N treatments induced similar respiration responses in the hemlock and salal forest floors, but CO$_2$ production in the cedar samples was very different. Other field
and pot studies have shown that microbial community compositions and/or functions can differ in soils under different plant species (Jones and Richards 1977; Turner and Franz 1985; Killham 1990; Bradley and Fyles 1995; Bradley et al. 1997b; Priha et al. 1999a; Priha et al. 1999b; Priha and Smolander 1999), and it appears that the cedar, hemlock and salal planted in CH forest floor core may have also influenced the forest floor microbial communities.

Differences in the microbial communities associated with the three species are further supported by the basal respiration results. The cedar control samples showed higher CO₂ production rates for the full 20-day trial period, whereas the hemlock and salal forest floor basal respiration rates were lower and declined earlier. Plants allocate up to 40-70% of fixed C to the roots and 2-10% of this fixed C is allocated to exudates (Grayston et al. 1996), and plant species differ in both the quantity and composition of root exudates released into the surrounding floor (Smith 1976; Smith 1969; Grayston et al. 1996). These differences in exudates create conditions which favour different microbial populations (Bansal and Mukerji 1994), and it has been suggested that plants release exudates to stimulate microbial activity and mineralization of N (Bradley and Fyles 1995; Bradley et al. 1997a; Bradley et al. 1997b), therefore potentially supporting a mutualistic relationship with the associated microbial community. The forest floor surrounding cedar in the N Uptake Trial had higher basal respiration rates and larger extractable inorganic N concentrations, and cedar absorbed a larger proportion of mineral N than did the other two species. Thus, through the release of exudates, cedar may support an active microbial community that differs in species composition to the microbes associated with hemlock and salal, and this community may in turn mineralize more N. Other plants, like the ericaceous shrub *Kalmia angustifolia*, are thought to directly access N through the fungal partner (Bradley et al. 1997b), which may also be the case for salal. However, the results of our trial are only suggestive of a difference in the microbial communities in the cedar, hemlock and salal forest floors. Further work identifying the microbial populations, their abilities, and their possible interactions with the plant roots is necessary to substantiate these suggestions.

**Summary**

Cedar, hemlock and salal seedlings growing in CH forest floor did differ in their abilities to access organic and inorganic N compounds. Whether through the intact uptake of the different
forms of N by mycorrhizal roots or the support of rhizosphere microbial communities that supplied N to the plants, all three species preferentially utilized NO₃⁻-N and NH₄⁺-N sources throughout the 20-day trial. The applied glutamic acid, protein and protein-tannin compounds were taken up by cedar, hemlock and salal, but only small amounts of these N forms were used by the plants over the 20-day period. It therefore appears that cedar, hemlock and salal do not partition access to N in CH forests by utilizing different forms of N preferentially. The hypotheses that the three species differ in their abilities to utilize different N compounds and that salal accesses a greater proportion of organic N forms while hemlock and cedar take up more NH₄-N and NO₃⁻-N, respectively, were rejected. However, it was apparent that the rhizosphere microbial communities associated with cedar, hemlock and salal are different and these communities may be important in the nutrition of the three species in CH forests. A more thorough examination of the microbial community composition and functions is needed to determine the relative importance of mycorrhizae versus saprophytic microbes in providing nutrients to cedar, hemlock and salal in CH forests.
Chapter 5

The vertical fine root distributions of cedar, hemlock and salal in old-growth CH forests

Introduction

Fine roots are responsible for most nutrient and water uptake by plants (Bowen 1984; Landsberg and Gower 1997), and therefore are very important in determining the productivity and growth of plants in terrestrial ecosystems. In forests, fine roots of different species often overlap, occupying the same organic or mineral soil horizon (Jonsson et al. 1988). However, various degrees of vertical and horizontal separation in distribution patterns have also been found (Grier et al. 1981; Persson 1983; Strong and La Roi 1983a; Gholz et al. 1986; Jonsson et al. 1988; Manning and Barbour 1988), and have led to the postulation that such differences in the arrangement of fine root systems can influence competition for shared resources and can facilitate access to different spatially separated pools of resources (Yeaton et al. 1977; Manning and Barbour 1988; Franco and Nobel 1990). In such systems, the sums of the plant productivities can exceed those found in monocultures or mixtures of plants rooting in the same soil horizons (Berendse 1979; Berendse 1981; Wilson 1988). Differences in the spatial structure of rooting systems has therefore been suggested as one of the mechanisms by which plant species limited by the same resource can grow together (Berendse 1979; Schulze et al. 1994; Nadelhoff er et al. 1996; Schmidt and Stewart 1997). Plants that differ in their fine root distributions may also have higher aboveground productivities than species with overlapping root systems (Yeaton et al. 1977).

Differences in the vertical distribution patterns of cedar, hemlock and salal fine roots may be important to the growth of the three species in CH forests. In these N-limited systems, cedar, hemlock and salal fine roots may be able to spatially separate N acquisition by accessing N from different horizons, thereby reducing competition for this resource. The results presented in the preceding chapters indicate that species-specific abilities to take up different N forms do not appear to be an important mechanism influencing plant access to N. However, differentially tapping into the N in the forest floor and soil horizons may allow cedar, hemlock and salal to
acquire more N and may be partially responsible for differences in the productivities of the three species in CH forests.

This hypothesis was tested by examining the distributions of cedar, hemlock and salal fine root biomass in the forest floor and upper mineral soil horizons in three old-growth CH forests, and relating these patterns to the sizes and concentrations of N in the respective horizons. More specifically, the questions addressed were:

- Do the three species differ in their fine root density and total biomass distribution patterns? Do the N forms differ in concentrations and content by horizon?
- Are the cedar, hemlock and salal fine root distributions correlated with N forms contained in the forest floor and upper mineral soil horizons?
- Could the species-specific vertical fine root distribution patterns, in part, explain the differences in the aboveground productivities of cedar, hemlock and salal in CH forests?

**Materials and Methods**

*Root sample collection*

On September 3-5, 1999 forest floor and mineral soil samples were collected from three different old-growth CH forests. At each site, 7-8 potential sampling locations were chosen according to specific criteria. There had to be at least one stem of hemlock, cedar and salal within 5 m of the sampling spot, and no other tree species or dominant shrub larger than 1 m in height could be present within 5 m or 2 m of the spot, respectively. From the 7-8 potential locations, five were randomly selected, and at each, a pit at least 0.75 m wide and 1 m long was dug to the depth of the hardpan layer. In all pits, this impervious layer was more than 10 cm into the mineral soil. On the exposed profile surfaces, the thicknesses of the F, Hr, Hh forest floor horizons (Green et al. 1993) were measured and then samples aligned vertically (15 x 15 cm) were collected from each of the four horizons. The entire depths of the F and Hr horizons were included in the samples. In the Hh horizons more than 15 cm deep, only the top 15 cm was collected, and in the mineral soils, only the top 10 cm was sampled. When decaying wood was encountered in a horizon, an alternate sampling location in the pit was randomly chosen. No distinctions were
made between the Ae and B mineral horizons. This sampling method was chosen over the soil coring method because of the large sampling depths (up to 93 cm).

**Root separation and cleaning**

The forest floor and mineral soil samples were put into plastic bags, stored in a cooler, transported back to U.B.C, and maintained around 4 °C until processed. In the lab, each sample was weighed and hand-sorted to remove all visible roots, and then sieved through 5 mm and 2 mm pores successively to remove the remaining root fragments. The mineral soils samples only received the 5 mm sieving and the coarse fragments (> 5 mm) remaining in the sieve were measured and subtracted from the sample volumes. Following separation from the forest floor or mineral soil, the roots were carefully rinsed with tap water to remove all visible particles of soil or forest floor. Care was taken to recover as many root fragments as possible during the sorting process. The samples could not be wet-sorted because the sieved forest floors and mineral soils were also used for the N analyses. The biomass of this unrecovered root fraction was probably minimal.

During the rinsing process the roots were sorted according to species and status (live or dead). The three species were easy to distinguish due to distinct root morphologies (Persson 1978; Vogt et al. 1981; Persson 1983). Hemlock has a distinct ectomycorrhizal branching pattern and many of the roots were covered in fungal mantles. Cedar, which is vesicular-arbuscular mycorrhizal, has thicker, paler roots with long laterals, and salal roots are white and very thin, a root morphology characteristic of the ericaceae (Read 1991; Ehrenfeld et al. 1992; Xiao 1994).

Live or dead status of the roots was more difficult to determine, as has been discussed by others (Majdi and Persson 1995). The criteria used in this study were root condition and colour (Persson 1983; Jonsson et al. 1988; Tufekcioglu et al. 1999). If the roots were pliable and the cortex was still white, they were considered to be alive or recently dead, and were composited. These roots were then dried to constant weight at 70 °C and divided into two size classes based on fresh diameters; fine roots (≤ 2 mm) and coarse (> 2 mm) roots. Only fine roots were weighed and included in the analyses. To determine cedar, hemlock and salal dry-to-fresh diameter conversions, the diameters of roots sampled from three four-year old seedlings of each species were measured before and after drying at 70 °C. Five root samples with diameters around 2 mm and similar degrees of suberization were taken from each seedling to produce
average estimates of diameter loss upon drying (Appendix II). Variability in diameter loss with
drying was very low and the standard errors were never larger than 2% of the means. Therefore,
the root samples sorted after drying probably provided accurate estimates of cedar, hemlock and
salal fine root biomasses. All the roots were processed and drying within eleven days of

Forest floor/Mineral soil nitrogen assessments
Following the removal of roots and sieving, the forest floor and mineral soil samples were stored
at 4 °C until assessed for concentrations of total N and extractable NO₃⁻-N, NH₄⁺-N and organic
N (SON). Five grams of each sample were dried to constant weight at 70 °C and ground in a
Wiley mill. Equivalent weights of each sample were then composited by site and horizon and
analyzed for total Kjeldahl N (TKN). Briefly, the twelve samples were digested with sulphuric
acid in a block digester at 360 °C for one hour to release all bound NH₄⁺ ions. The digests were
then analyzed for NH₄⁺ on a Lachat autoanalyzer to determine total N contents. The TKN
analyses were conducted at the Soil Science Laboratory at U.B.C.

To estimate extractable NO₃⁻ and NH₄⁺ and SON contents, 50 ml of 2 M KCl was added to 5 g of
each forest floor or mineral soil sample, and the mixtures were shaken for one hour. The extracts
were then combined by site and horizon and were filtered through pre-soaked (2 M KCl)
Whatman #42 filter paper. The twelve composite samples were then filter-sterilized through
Gelman 0.45 µm micropore syringe attachments connected to 60 ml plastic syringes and frozen
until analyzed. To estimate moisture content, approximately 10 g of each forest floor or mineral
soil sample was weighed before and after drying for 24 hours at 105 °C. All drying and
extractions were completed within five days of initial collection. Extract solutions were frozen
until analyzed.

The frozen samples were thawed on February 29th, 2000 and NO₃⁻-N and NH₄⁺-N concentrations
were measured on sub-samples with the autoanalyzer, and total soluble N contents were
determined using a modified version of the persulphate oxidation technique outlined by Cabrera
and Beare (1993). Briefly, 10 ml of persulphate solution (15 g NaOH, 40 g low-N K₂O₃S₂ and
15 g boric acid dissolved in 1 L deionized water) was added to 5 ml of extract contained in 40 ml
glass vials. The vials were then tightly capped, weighed and autoclaved at 121 °C for 45
minutes. After autoclaving, the vials were re-weighed to adjust for any evaporative water loss
and were diluted with 5 ml of deionized water prior to being analyzed for \( \text{NO}_3^-\) concentrations with the autoanalyzer. Soluble organic N was calculated as the difference between the N measured following the persulphate oxidations and the inorganic N (\( \text{NO}_3^-\)-N and \( \text{NH}_4^+\)-N) measured in the non-autoclaved samples. All the extract analyses were conducted at the Environmental Engineering Laboratory at U.B.C.

**Bulk densities**

To determine forest floor and mineral soil horizon bulk densities, 3-5 samples of each horizon were collected from two pits in each CH forest site on June 5-6, 2000. Before removing the samples, the forest floor volumes were determined by a 15 cm wide x 15 cm long metal plate and a ruler to determine sample thickness. For the mineral soils, the size of samples were measured volumetrically by pouring a recorded amount of water into a plastic bag lining the hole left after the sample was removed. All forest floor and mineral soil samples were weighed in the lab and large roots or pieces of wood (>1 cm diameter) were removed. The mineral soils were sieved through 5 mm pores to remove all coarse fragments and roots. The volumes of the removed wood/root and mineral coarse fragments were measured and subtracted from the sample volumes. The samples were then dried to constant weight at 70 °C. Roots less than 1 cm in diameter were considered integral components of the forest floor and were therefore included in the sample fresh and dry weights and the estimates of forest floor bulk densities.

**Statistical analyses**

The N contents and cedar, hemlock, salal and total fine root abundances in the different forest floor and mineral soil horizons were analyzed by N form and species as completely randomized block designs. Values were expressed as densities/concentrations (\( \mu \text{g} \cdot \text{g}^{-1} \) and \( \text{kg} \cdot \text{m}^{-3} \)) and total weights (\( \text{g} \cdot \text{m}^{-2} \)) and differences among these mean values were determined using ANOVA (GLM procedure) followed by pairwise t-test comparisons of the least square means. The alpha level (0.05) was adjusted for the number of comparisons using Bonferroni's adjustment (Neter et al. 1996). Site x horizon interactions were also tested using the sampling errors for the cedar, hemlock and salal root analyses. The forest floor and soil total N and extract samples were composited at each site, so Tukey test for non-additivity was used to determine significant site x horizon interactions (Steel and Torrie 1960). All the root measurements required transformation prior to analysis. The cedar and hemlock root densities (\( \text{kg} \cdot \text{m}^{-3} \)) and the cedar and salal total root biomasses (\( \text{g} \cdot \text{m}^{-2} \)) required log \((x+1)\) transformation. Square root transformations were applied...
to the salal root densities and hemlock total biomass measurements. Original values are reported in the tables and figures in the Results.

To determine relationships between N forms (total N, NO$_3^-$, NH$_4^+$ and SON) and cedar, hemlock, and salal fine roots, Spearman rank correlations were applied to the N concentrations and root densities (g·m$^{-3}$ - for N or kg·m$^{-3}$ - for roots) and total N contents and root biomasses (g·m$^2$) for each horizon using average root biomasses for each site. Spearman rank correlations were also used to determine correlations between the vertical fine root distributions of cedar, hemlock and salal, using individual root values from each pit. This type of correlation analysis was chosen because the root biomass data were not normally distributed; Spearman rank correlations are similar in strength to Pearson correlation analyses for the analysis of such data (Conover 1980). An alpha level of 0.05 was used, and all analyses were conducted with SAS (SAS Institute 1993).

Results

The physical characteristics of the forest floor and mineral soil horizons in the three old-growth CH forests are presented in Table 5.1. The Hh was the thickest forest floor horizon with average depths of 0.21-0.39 m at the three sites. This horizon also had the largest bulk densities and weights ranging from 110-136 kg·m$^{-3}$ and 288-439 Mg·ha$^{-1}$, respectively. The F horizon was consistently the thinnest, and usually had the lowest densities and total weights. On average, this horizon was 3 cm thick and had bulk densities and total weights of 73 kg·m$^{-3}$ and 23 Mg·ha$^{-1}$, respectively. The entire forest floors varied in total depth from 0.19–0.70 m and had an average dry weight of 485 Mg·ha$^{-1}$. The bulk densities of the upper mineral soils were much greater than the forest floors densities and were 690±125 kg·m$^{-3}$ at site 1, 373±84 kg·m$^{-3}$ in site 2 and 682±52 kg·m$^{-3}$ at site 3.
Table 5.1. Characteristics of the forest floor and mineral soil horizons in the three old-growth CH forests. Each value is the mean (S.E.M.) of 3-5 pits.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Mean thickness (m)</th>
<th>Thickness range (m)</th>
<th>Bulk density (kg·m⁻³)</th>
<th>Weight (Mg·ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SITE 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.03 (0.00)</td>
<td>0.02-0.04</td>
<td>69.64 (6.26)</td>
<td>20.89 (2.20)</td>
</tr>
<tr>
<td>Hr</td>
<td>0.13 (0.03)</td>
<td>0.10-0.18</td>
<td>75.39 (5.73)</td>
<td>95.49 (20.10)</td>
</tr>
<tr>
<td>Hh</td>
<td>0.39 (0.16)</td>
<td>0.07-0.60</td>
<td>112.52 (29.25)</td>
<td>438.84 (182.94)</td>
</tr>
<tr>
<td>Total forest</td>
<td>0.47 (0.09)</td>
<td>0.28-0.73</td>
<td></td>
<td>552.91 (162.48)</td>
</tr>
<tr>
<td><strong>SITE 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.04 (0.00)</td>
<td>0.03-0.04</td>
<td>73.10 (6.00)</td>
<td>26.32 (1.79)</td>
</tr>
<tr>
<td>Hr</td>
<td>0.11 (0.01)</td>
<td>0.08-0.15</td>
<td>85.84 (15.60)</td>
<td>92.70 (9.94)</td>
</tr>
<tr>
<td>Hh</td>
<td>0.34 (0.04)</td>
<td>0.26-0.44</td>
<td>110.49 (10.05)</td>
<td>377.88 (39.28)</td>
</tr>
<tr>
<td>Total forest</td>
<td>0.49 (0.04)</td>
<td>0.40-0.59</td>
<td></td>
<td>496.90 (42.46)</td>
</tr>
<tr>
<td><strong>SITE 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.03 (0.00)</td>
<td>0.02-0.04</td>
<td>75.83 (3.98)</td>
<td>22.75 (2.40)</td>
</tr>
<tr>
<td>Hr</td>
<td>0.12 (0.03)</td>
<td>0.05-0.20</td>
<td>78.93 (4.94)</td>
<td>93.13 (21.38)</td>
</tr>
<tr>
<td>Hh</td>
<td>0.21 (0.05)</td>
<td>0.09-0.38</td>
<td>135.99 (9.96)</td>
<td>288.30 (73.91)</td>
</tr>
<tr>
<td>Total forest</td>
<td>0.41 (0.08)</td>
<td>0.19-0.70</td>
<td></td>
<td>404.19 (64.68)</td>
</tr>
</tbody>
</table>

Nitrogen concentrations and contents differed by horizon (Table 5.2). When expressed per unit forest floor or mineral soil weight, SON concentrations were significantly higher in the F horizons and total N concentrations were higher in the F, Hr and Hh horizons relative to the mineral soil. On a volume basis (g·m⁻³), there were no significant differences in SON and total N concentrations, although average concentrations of both N forms were consistently highest in mineral soils. There were significantly higher amounts of total N and SON in the Hh horizons relative to the mineral soil, Hr and F horizons, and SON contents were lowest in the F horizons (Table 5.2). Nitrate and NH₄⁺ contents and concentrations did not differ by horizon and did not show any clear trends. SON concentrations (g·m⁻³) were significantly different by site, and significant site x horizon interactions for SON and NH₄⁺ concentrations and NO₃⁻ and NH₄⁺ contents (Appendix II).
Table 5.2. Total N, soluble organic N and extractable NO$_3^-$ and NH$_4^+$ in the forest floor and upper 10 cm of mineral soil in old-growth CH forests. Values are expressed by weight ($\mu$g·g$^{-1}$), volume (g·m$^{-3}$) and area (g·m$^{-2}$). Significant differences between the means within N form and unit of measurement are indicated by different letters ($p<0.05$, n=3, mean (S.E.M.)) based on ANOVA (GLM procedure). ND indicates that NO$_3^-$ was below the detection limit (0.010 ppm NO$_3^-$-N).

<table>
<thead>
<tr>
<th>Soluble Organic N</th>
<th>F</th>
<th>Hr</th>
<th>Hh</th>
<th>Ae/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(uM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.06 (4.05)</td>
<td>-</td>
<td>11.76 (0.59)</td>
<td>16.17 (3.41)</td>
<td>18.59 (5.56)</td>
</tr>
<tr>
<td>0.44 (0.11) c</td>
<td>1.38 (0.01) bc</td>
<td>4.73 (0.14) a</td>
<td>1.86 (0.56) b</td>
<td></td>
</tr>
<tr>
<td>(g·m$^{-3}$)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.11 1 (3.526)</td>
<td>-</td>
<td>5.971 (4.758)</td>
<td>ND</td>
<td>1.279 (1.279)</td>
</tr>
<tr>
<td>0.015 (0.010)</td>
<td>0.021 (0.017)</td>
<td>ND</td>
<td>0.010 (0.010)</td>
<td></td>
</tr>
<tr>
<td>0.001 (0.000)</td>
<td>0.003 (0.002)</td>
<td>ND</td>
<td>0.001 (0.001)</td>
<td></td>
</tr>
<tr>
<td>(g·m$^{-2}$)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55.133 (6.027)</td>
<td>-</td>
<td>57.669 (30.646)</td>
<td>15.040 (4.858)</td>
<td>46.905 (21.581)</td>
</tr>
<tr>
<td>0.162 (0.015)</td>
<td>0.207 (0.111)</td>
<td>0.100 (0.039)</td>
<td>0.407 (0.191)</td>
<td></td>
</tr>
<tr>
<td>0.005 (0.004)</td>
<td>0.026 (0.014)</td>
<td>0.028 (0.006)</td>
<td>0.041 (0.019)</td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(uM)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>707.92 (37.87)</td>
<td>-</td>
<td>755.61 (42.07)</td>
<td>1125.59 (54.65)</td>
<td>1519.57 (350.75)</td>
</tr>
<tr>
<td>(g·m$^{-3}$)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.61 (1.52) b</td>
<td>88.42 (2.00) b</td>
<td>348.48 (44.94) a</td>
<td>151.96 (35.08) b</td>
<td></td>
</tr>
<tr>
<td>(g·m$^{-2}$)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant differences between sites (blocks) based on ANOVA (GLM procedure)
** significant site x horizon interactions based on Tukey test for non-additivity.

Cedar, hemlock and salal fine root biomass distributions in the forest floor and mineral soil horizons are presented in Table 5.3 and Figure 5.1. Fine root data are presented as densities (kg·m$^{-3}$) to evaluate species-specific differences in root concentrations, and as total biomasses (g·m$^{-2}$) in each horizon to determine differences in cedar, hemlock and salal fine root biomass allocation patterns. Hemlock had significantly higher root densities in the F and Hr than in the Hh and upper Ae/B horizons, and salal had higher densities in the F and Hr relative to the upper mineral soil horizons. Cedar fine root densities were not significantly different among horizons, but the highest average concentrations were in the Hh horizon. The distributions of total fine root biomass also differed among the species. Cedar had significantly larger amounts of fine roots in the Hh than in the upper mineral and F horizons. Salal had higher amounts in the Hr and Hh than in the upper Ae/B horizons, and hemlock had significantly larger fine root biomass in...
the Hr horizons than in the other horizons. In the analyses of salal fine root densities and cedar and salal total biomasses, sites (blocks) were significant, but there were no significant site x horizon interactions (Appendix II).

Spearman rank correlations indicated significant relationships between fine root distributions and N concentrations and contents in CH forest floor and mineral soil horizons. Salal fine root densities were negatively correlated with total N and SON concentrations (Fig. 5.2 & 5.3). Hemlock fine root densities and total root biomass were also negatively correlated with total N. In cedar, root biomass was positively correlated with total N and SON contents. There were no significant relationships between inorganic N (NO$_3^-$ and NH$_4^+$) concentrations or total N contents and cedar, hemlock and salal fine root distributions (Fig. 5.4).

Spearman correlation analyses of cedar, hemlock and salal fine root densities and total biomasses determined relationships between cedar, hemlock and salal fine root distributions. Hemlock and salal root densities were positively correlated, and cedar total root biomass in each horizon was positively correlated that of salal (Table 5.4).
Table 5.3. Cedar, hemlock and salal fine roots (densities and total biomasses) in the forest floors and upper 10 cm of mineral soil in old-growth CH forests. Values are presented on volume (kg·m⁻³) and area (g·m⁻²) basis. Different letters represent significant differences between the least square means within a species and unit of measurement. Asterisks indicate where significant blocks were determined by ANOVA (GLM procedure) (p<0.05, n=12-15, mean (S.E.M.)). No significant site x horizon interactions were detected.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Cedar (kg·m⁻³)</th>
<th>Cedar (g·m⁻²) *</th>
<th>Hemlock (kg·m⁻³)</th>
<th>Hemlock (g·m⁻²)</th>
<th>Salal (kg·m⁻³) *</th>
<th>Salal (g·m⁻²) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.979 (0.187)</td>
<td>31.806 (6.193) c</td>
<td>2.889 (0.117) a</td>
<td>90.769 (1.748) b</td>
<td>0.580 (0.079) a</td>
<td>19.887 (2.839) ab</td>
</tr>
<tr>
<td>Hr</td>
<td>1.348 (0.234)</td>
<td>160.692 (30.299) ab</td>
<td>3.328 (0.211) a</td>
<td>446.743 (20.813) a</td>
<td>0.375 (0.056) a</td>
<td>53.106 (9.703) a</td>
</tr>
<tr>
<td>Hh</td>
<td>1.677 (0.102)</td>
<td>508.732 (37.075) a</td>
<td>0.318 (0.083) b</td>
<td>37.151 (10.449) b</td>
<td>0.288 (0.052) ab</td>
<td>106.415 (22.509) a</td>
</tr>
<tr>
<td>Ae/B</td>
<td>1.161 (0.057)</td>
<td>116.080 (5.701) b</td>
<td>0.457 (0.097) b</td>
<td>45.730 (9.654) b</td>
<td>0.081 (0.014) b</td>
<td>8.060 (1.439) b</td>
</tr>
</tbody>
</table>
Figure 5.1. Proportions of cedar, hemlock and salal fine roots in the forest floor and upper 10 cm of mineral soil in old-growth CH forests. Values are based on the least square means in Table 3. For each species, root densities (a) and total root biomass (b) in each horizon are expressed as percentages of the summed values for all four horizons.
Figure 5.2. Relationships between soluble organic N (SON) concentration and fine root density (a-c) and SON content and fine root biomass (d-f). Asterisks indicate significant correlations \((p<0.05)\), and the "\(r\)" is the Spearman rank correlation coefficient with the corresponding \(p\)-value.
Figure 5.3. Relationships between total N concentration and fine root density (a-c) and total N content and fine root biomass (d-f). Asterisks indicate significant correlations ($p<0.05$), and "$r$" is the Spearman rank correlation coefficient with corresponding $p$-value.
Figure 5.4. Relationships between inorganic N (NH$_4^+$-N and NO$_3^-$-N) concentration and fine root density (a-c) and total inorganic N content and fine root biomass (d-f).
Table 5.4. Spearman rank correlations of cedar, hemlock and salal fine root abundances. Asterisks indicate significant correlations at \( p<0.05 \) (\( n=54-57 \)).

<table>
<thead>
<tr>
<th>Unit of measurement</th>
<th>Comparison</th>
<th>Correlation coefficient</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg·m(^{-3})</td>
<td>cedar - hemlock</td>
<td>-0.007</td>
<td>0.960</td>
</tr>
<tr>
<td></td>
<td>cedar - salal</td>
<td>0.242</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>hemlock - salal</td>
<td>0.344</td>
<td>0.009*</td>
</tr>
<tr>
<td>g·m(^{-2})</td>
<td>cedar - hemlock</td>
<td>-0.013</td>
<td>0.925</td>
</tr>
<tr>
<td></td>
<td>cedar - salal</td>
<td>0.494</td>
<td>0.0001*</td>
</tr>
<tr>
<td></td>
<td>hemlock - salal</td>
<td>0.201</td>
<td>0.144</td>
</tr>
</tbody>
</table>

**Discussion**

**Methodological considerations**

Samples were collected at the beginning of September and a main objective of the study was to characterize cedar, hemlock and salal vertical fine root distributions in CH forests, and determine if the three species differed in their distribution patterns. Although fine root biomass in conifers is commonly believed to be bimodal with peaks in the spring and the fall (Vogt et al. 1981; Mitchell 1995), many studies of fine root biomass and productivity do not support this assumption. Seasonal standing root biomass can be unimodal with peaks in the spring (Herman 1977; Keyes and Grier 1981; Burton et al. 2000), summer (Gholz et al. 1986; Burton et al. 2000) and fall (Vogt et al. 1981). Others have also shown no significant changes in fine root biomass over the year (Keyes and Grier 1981; Nadelhoffer et al. 1985; Gholz et al. 1986) or high intra-annual variability (Persson 1978). Many authors have therefore suggested that the occurrence of maximum fine root biomass is not predictable and is heavily influenced by environmental factors (Persson 1983; Fogel 1991; Fredericksen and Zedaker 1995; Landsberg and Gower 1997; Cote et al. 1998). However, very little is known about how fine root distributions change during the year. Vogt et al. (1981) found seasonal fluctuations in fine root distributions in a 180-year-old *Abies amabilis* stand, with larger amounts of fine roots in the forest floor than in the mineral A horizon during the autumn. At other times of the year, larger amounts of fine roots were in the mineral soil. Cedar, hemlock and salal fine root distributions in CH forests may also change...
during the year, but this could not be evaluated because of the large time commitment required
for such a study. September was chosen as the sampling month because it is at the end of the
aboveground growth period, and so would capture fine root production and distribution over the
growing season. Also, if cedar, hemlock and salal fine root production in CH forests increases in
the fall, these new roots would have also been included.

Although the full depth of the F and Hr horizons were collected, only the top 15 cm of Hh was
sampled which may not have accurately estimated the average densities and total amounts of fine
roots in the this horizon. Roots have been observed to concentrate in the interfaces between
horizons (Strong and La Roi 1983b) and decrease in abundance with depth within a horizon.
Therefore, the calculations of fine root biomass in the Hh horizon may have been over-estimated.
To account for this potential bias, root biomasses were re-calculated using the fine root densities
of the mineral soils, if they were less than the measured Hh densities. It was assumed that the
densities in the Hh horizon below 15 cm would not be lower than those in the Ae/B. These re­
calculated estimates provide a conservative estimate of total fine root biomass in the Hh horizons
(Appendix II). Even with these adjustments, the results were nearly identical. Thus, the data
presented in this chapter accurately represent the trends of cedar, hemlock and salal fine root
biomass distributions in CH forests.

The roots of cedar, hemlock and salal in CH forests are colonized by mycorrhizal fungi. Xiao
and Berch (1996) and T. Allen (pers. comm.) showed that salal fine roots from CH forests are
infected with ericoid mycorrhizae, and most of the hemlock fine roots collected in this study had
ectomycorrhizae fungal mantles and stunted lateral roots. Almost all the roots of cedar seedlings
harvested from CH forests were VA mycorrhizal (B. Gilbert pers. comm.). Mycorrhizal hyphae
typically extend beyond the root surfaces and facilitate the exploitation of a larger volume of
soil/forest floor (Paul and Clark 1996). It is therefore important to consider the potential
influence of hyphal extension into different floor or soil horizons not included in the samples
analyzed. Ericoid mycorrhizal hyphae extend only a few mm from the root surface (Read 1991;
Paul and Clark 1996), whereas ecto and VA mycorrhizal fungi have much more extensive hyphal
systems. Ectomycorrhizal fungi fan out from infected root tips, while VA fungi extend from
infection points along the root surface and can have 20-200 entry points per cm of root (Read
1991). Conservative estimates of up to 300 m of hyphae per metre of colonized root for both
fungal types have been put forward (Tinker et al. 1992), although estimates as high as
3992 m·m⁻¹ (Abbott et al. 1992) and 10⁵ m·m⁻¹ (Read 1991) have been reported for VA and ectomycorrhizae, respectively. Mycorrhizal hyphae were not measured in our study, so the potential extension of hyphae into horizons above or below the location of the host root is not known. This may have confounded the correlations between root distributions and N concentrations and contents, but further work is necessary to determine if fungal hyphae from mycorrhizae influenced the results.

*Horizon characteristics, nitrogen and fine roots*

The average thickness of the forest floors in the three CH forests was 0.46 m, corresponding to an average weight of 485 Mg·ha⁻¹. These values are beyond the upper ranges reported for similar ecosystems. Forest floor weights ranging from 3.55–149.5 Mg·ha⁻¹ have been reported for boreal and temperate deciduous and coniferous forests (see Vogt et al. 1986), and slightly higher forest floor depths were measured in eleven Douglas-fir/western hemlock stands in Washington and Oregon (0.03–0.17 m). The largest corresponding forest floor weight was 221 Mg·ha⁻¹. In old-growth CH forests, Keenan et al. (1993) and deMontigny (1992) found average forest floor depths of 0.28 cm and 0.23 cm, respectively and the measured depths in this study fall within their reported ranges.

A total of 6.1 Mg·ha⁻¹ of total N was measured in the forest floor and upper 10 cm of mineral soil horizons of these CH forests. Only 1.4% of the total N was soluble, and only 1.2% of this soluble fraction was in inorganic forms (NO₃⁻ and NH₄⁺). The NO₃⁻, NH₄⁺, SON and total N concentrations in the F and Hr horizons were similar to the values measured in previous studies of old-growth CH forests (Prescott et al. 1993b; K. Hannam *pers. comm.*), as were the total N concentrations in the upper 10 cm of the mineral soils (Keenan et al. 1993). Concentrations of SON, extractable NO₃⁻ and NH₄⁺ and total N did not change significantly with depth, though the largest average levels of SON, NH₄⁺ and total N were present in the upper 10 cm of the Ae/B horizon. Therefore, there does not appear to be a N concentration gradient with depth. The presence of large amounts of soluble N in the mineral soil in CH forests suggests there may be losses of N from these systems and most of this N loss occurs as SON. The total fine root densities were lowest in the mineral soil, and thus roots probably do not capture a lot of the N moving down through the mineral soil. In an examination of N losses from thirteen old-growth temperate forests in Chile, Hedin et al. (1995) found that an average of 176.2 μg·L⁻¹ of dissolved N was present in streams draining the forests and that 95% of this N was SON. Similar losses
may be occurring from CH sites, but further work using lysimeters or stream analysis is necessary to test this hypothesis.

The average total fine root biomass of cedar, hemlock and salal in the forest floor and upper 10 cm of mineral soil was 817, 620 and 187 g·m$^{-2}$, respectively. These values are similar to previous reports of fine root biomasses (≤2-3 mm) of conifers (Vogt et al. 1981; Nadelhoffer et al. 1985), ericaceous shrubs (Persson 1978; Persson 1983), and coniferous forests (Vogt et al. 1986). The salal root biomass is comparatively low because of the species’ very thin, hair-like fine root structure (Xiao 1994). Salal fine roots have a much large surface-area to weight ratio than that of conifers, so weight is a poor indicator of the relative absorptive surface areas of the three species, and therefore should only be compared within a species. Fine roots of all three species were concentrated in the forest floors horizons, with 86%, 93% and 96% of the fine root biomass of cedar, hemlock and salal in the forest floors, respectively. Grier et al. (1981) and Vogt et al. (1987) showed a shift in fine root distribution from mineral to forest floor horizons with increased stand age and forest floor development. Ehrenfeld et al. (1992) reported that 30-60% of fine root biomass was in the organic horizons. Therefore, it is not surprising that the majority of the cedar, hemlock and salal fine roots were located within the thick forest floors in the CH forests.

Cedar, hemlock and salal fine root correlations

Cedar, hemlock and salal showed distinct vertical fine root distribution patterns. Hemlock and salal roots were concentrated in the upper forest floors while cedar roots were distributed evenly among all horizons. Similar distribution trends have been reported in other studies. In old-growth CH forests, Chang and Handley (2000) observed that salal and cedar rooted mainly in the F and H, respectively. Ecto and ericoid mycorrhizal fine roots are often concentrated in surface soil layers (see Read 1991). Cedar, hemlock and salal total fine root biomass distribution patterns were also different. Cedar and salal both had larger amounts of fine roots in the Hh while hemlock had more roots in the Hr horizons. The high root weights in the Hh horizons were largely a function of the depth, but differences in the biomass distributions of cedar, hemlock and salal fine root were still apparent.

Distinct vertical distributions of cedar, hemlock and salal fine roots in CH forest floors and mineral soils suggest that there may be differences in the amounts of inter-specific root
interaction and competition for resources. Salal and hemlock fine roots were concentrated in the upper forest floor horizons and the Spearman correlations indicated that the fine root density distributions of the two species were correlated. This indicates that salal and hemlock fine roots intermingle and have overlapping root depletion zones. This being the case, the two species would be forced share common pools of resources, including N (Fitter 1976; Berendse 1979; Fredericksen and Zedaker 1995). As presented in Chapter 4, hemlock and salal have similar abilities to utilize different forms of organic and inorganic N, and therefore, both species are probably competing for the same N resource. Based on the differences in the growth of the two species in CH forests, salal appears to be capturing more of this shared pool of available N.

Several studies, conducted with restricted rooting environments, have shown that plant species differ in their abilities to capture resources when grown in mixtures. For example, when Agrostis tenius and Lolium perenne were planted together, P uptake and relative growth rate of A. tenius was significantly reduced, but L. perenne was unaffected (Fitter 1976). Similarly, when paired combinations of Festuca ovina, Nordus stricta and Deschampsia flexuosa were planted together, the N and K contents and total biomass of N. stricta were reduced relative to the other two grass species (Wilson 1989). Therefore, salal and hemlock may differ in their abilities to capture nutrients such as N, and salal may access a larger proportion of the shared resources. This hypothesis is supported by the findings of Chang (1996) that on a four-year-old regenerating CH forest sites, the salal-dominated understory took up thirteen times as much fertilizer $^{15}$N as did hemlock. When the aboveground component of the understory was removed, $^{15}$N uptake by hemlock increased by nine times.

Salal may access a greater amount of N through one or more mechanisms. It may have a higher growth rate and represent a larger N sink (A. Glass pers. comm.), and so may take up more of the available resource. Alternatively, with an efficient N uptake system, salal may be able to reduce nutrient concentrations to levels that are inaccessible to hemlock (Tilman 1982; Tilman 1987; Hodge et al. 1999). Thirdly, salal may have greater access to the shared N pool through its extensive, fibrous fine root system, a characteristic common to the ericaceae (Read 1991; Ehrenheld et al. 1992; Xiao 1994). Lastly, salal may interfere with hemlock fine root activity and growth through the production of allelochemicals. Kalmia angustifolia root extracts (Mallik 1987) and phenolic acids contained within root extracts (Vaughan and Ord 1991) inhibit the root growth of black spruce (Picea mariana) and peas (Pisum sativum), respectively, and the results
from several field experiments have suggested that allelopathy or root interference were responsible for the reduced growth of individual plant species in mixtures (Fitter 1976; Callaway et al. 1991). Similarly, based on chemical analyses, deMontigny (1992) suggested that salal may have allelopathic effects on conifer growth in CH forests. Mycorrhizal fungi can also be sensitive to allelochemicals, and therefore indirect inhibition of the root-associated fungus may affect the productivity of the host plant. Xiao (1994) found that the growth of three ectomycorrhizal fungi found in association with hemlock roots was inhibited by one or more of four different ericoid mycorrhizal fungi isolated from salal roots in CH forests. Therefore, interactions between the ericoid and ectomycorrhizae associated with the fine roots of hemlock and salal may influence the productivities of the two plant species in CH forests.

In contrast to hemlock and salal, cedar fine roots were evenly distributed in the CH forest floor and mineral soil horizons and the cedar fine root density distributions were not significantly correlated with those of the other two species. However, the salal-cedar root density correlations were almost significant ($p=0.07$), suggesting a stronger relationship between the root distributions of salal and cedar than between the two conifers. In addition, cedar and salal total root biomass distributions were strongly correlated because both species had larger amounts of fine roots present in the Hh horizons. The greater similarities between cedar and salal fine root distributions suggest there is some root overlap, however, this overlap is probably small compared to that between salal and hemlock fine roots. Salal probably shares more competitive interactions with hemlock than it does with cedar. This interpretation is supported by Fraser et al. (1995) who showed that salal vigour was negatively correlated with hemlock growth but did not appear to adversely affect the productivity of cedar in regenerating CH forests. Therefore, because cedar may not experience the same degree of competitive interaction, and by having larger amounts of fine roots in the lower organic and mineral soil horizons, cedar probably accesses N that is less exploited by salal and hemlock. Other studies have also found spatial separation of fine root systems in mixed stands (Mikola et al. 1966; McQueen 1968; Persson 1983; Strong and La Roi 1983a; Gholz et al. 1986; Jonsson et al. 1988; Fredericksen and Zedaker 1995; Mou et al. 1995; Coners et al. 1998), grasslands (Caldwell et al. 1996), and deserts (Yeaton et al. 1977; Manning and Barbour 1988), and several of these showed that plants with spatially separated fine roots had higher productivity levels (Yeaton et al. 1977; Manning and Barbour 1988; Caldwell et al. 1996). The same phenomena may occur in cedar in CH
forests, and the spatial arrangement of fine roots may contribute to the better growth of cedar than hemlock in these forests.

**Roots and N correlations**

Although the mechanism(s) responsible for the distribution patterns of cedar, hemlock and salal fine roots could not be tested in this study, nutrient availabilities are known to influence fine root growth. Under field and greenhouse conditions, fine roots tend to proliferate in areas with high nutrient concentrations (Coutts and Philipson 1977; Eissenstat and Caldwell 1988; see Robinson 1994; Eckhard et al. 1997; Hodge et al. 1999) and similar influences would have stimulated the concentration of salal and hemlock roots in the upper forest floor horizons. However, the N in CH forest floors and mineral soils did not appear to strongly affect fine root distribution patterns. Hemlock and salal roots were negatively correlated with total N concentrations and salal fine roots also showed a negative relationship with SON concentrations in the horizons. In contrast, cedar fine root biomass distribution was positively correlated with total N and SON contents, however this was probably a function of the Hh horizon thickness. Negative relationships between fine root distributions and indices of N availability such as N concentrations and net nitrification and mineralization rates have been found in other studies (Aber et al. 1985; Nadelhoffer et al. 1985; Bhatti et al. 1998). However, the negative correlations may be a result of fast and thorough uptake of N present in the upper forest floor horizons with higher fine root densities (Caldwell et al. 1996; Bhatti et al. 1998). The measurements may therefore estimate the amounts of N remaining following active absorption and poorly evaluate N availability.

Recently, research has started to examine gross N cycling and has suggested that N turnover rates are better indicators of N availability to plants than are static N concentration measurements (Hart et al. 1994a). Using the isotope dilution technique (Hart et al. 1994b), Bradley (pers. comm.) determined that 1.19 and 0.36 µg·g⁻¹·day⁻¹ NO₃⁻ and NH₄⁺, respectively, were produced in the upper CH forest floors (see Chapter 4). Therefore, approximately 16–578% more NO₃⁻ and NH₄⁺ may have been produced over a 24-hour period then were measured in the 2 M KCl extracts from this study. Furthermore, these production rates show that the NO₃⁻ pool may be larger than that of NH₄⁺, the inverse of which was suggested by the extraction results in this trial. Although Bradley (pers. comm.) did not characterize organic N cycling or changes in N turnover with forest floor and mineral soil depth, it is apparent the N pool sizes experienced by plant roots are probably different then the concentrations measured in the KCl extractions. By examining
gross rates of N production, much better estimates of nutrient availability can be gained and such measurements would not be biased by plant uptake. The N concentration – fine root density and N content – fine root biomass correlations presented in our study probably do not accurately evaluate the relationship between fine root allocation patterns and N availability. Further work examining gross N cycling rates in the forest floor and mineral soil horizons in CH forests is needed to gain a better understanding of the availability of inorganic and organic N to cedar, hemlock and salal on these sites.

Factors influencing root distributions

Also, nutrient availability may not be the main factor influencing the vertical distributions of cedar, hemlock and salal fine roots in CH forests. As outlined in the literature review, variables such as genetics, stage of stand development, soil conditions, and competition can affect fine root growth patterns (Herman 1977; Vogt et al. 1981; Fogel 1983; Persson 1983; Gale and Grigal 1987; Hayes and Seastedt 1987; Mou et al. 1995; Parker and Van Lear 1996; Bhatti et al. 1998), and some of these may be important determinants in CH forests. Plant root distributions can be largely determined by genetics (Herman 1977; Vogt et al. 1981; Persson 1983; Gale and Grigal 1987). Some species are phenotypically plastic and will alter rooting distributions in response to changes in environmental or soil conditions, while others are more rigid in root allocation patterns (Pulling 1918; Presston 1942). Cedar, hemlock and salal may therefore differ in the degree to which their fine root distribution patterns are genetically determined. The vertical fine root concentrations and allocation patterns of the three species may be products of low phenotypic plasticity or response to conditions found in the rooting environment in CH forests. Other published studies have not examined the vertical rooting patterns of cedar, hemlock or salal in different forests, therefore the inherent variability of the rooting patterns of each species is unknown.

(i) Stand Age

Stand age or stage of development can also influence fine root distribution (McQueen 1968; Grier et al. 1981; Vogt et al. 1981). The old-growth CH forests on northern Vancouver Island are more than 1000 years old (Keenan et al. 1993) and have accumulated thick forest floors. Thus, the vertical fine root patterns of cedar, hemlock and salal in these forests may be the result of the developed forest floors. In younger forests with less developed organic substrates, the relative vertical rooting patterns of the three species may be different. Chang and Handley
(2000) examined three- and ten-year-old regenerating CH forests and noted that salal and cedar were both rooted in the H horizon. Only in the old-growth forests were the species reported to separate vertical rooting distributions. However, Chang and Handley (2000) only noted rooting patterns in the forest floors, and the three species, especially in the regenerating forests, may have had different amounts of fine roots in the mineral soils. Nevertheless, the differences in cedar, hemlock and salal fine root biomass and density distribution patterns in CH forests may largely be a function of stand development and forest floor depth.

(ii) Soil Conditions
Factors such as soil moisture content can also affect the distribution of fine roots (Rytter and Hansson 1996; Xu et al. 1997; Bhatti et al. 1998). Dry conditions can stop roots from growing and cause dieback (Landsberg and Gower 1997), and an overabundance of water can also alter fine root distributions. CH forests on northern Vancouver Island, receive an average of 1700 mm of precipitation annually (Prescott and Weetman 1994), and the gravimetric moisture contents of the forest floors measured in September, were up to 84% (w H2O/w total) and increased with depth. It is therefore possible that water, and most probably a water overabundance, influences cedar, hemlock and salal root allocation patterns in these forests. Cedar is flood-tolerant and frequently grows in wet soil moisture conditions. In contrast, western hemlock is most frequently found in fresh to moist soils (Klinka and Worrall 1989). Therefore, the relatively even distribution of cedar fine roots throughout the forest floor and upper mineral soil horizons and the concentration of hemlock roots in the surface horizons in CH forests may be a product of the species-specific abilities to grow in different soil moisture conditions.

(iii) Competition
Inter-specific root competition is thought to influence fine root distributions (Mikola et al. 1966; Herman 1977; Caldwell et al. 1996). Roots in close proximity can be forced to utilize the same resource from a common pool (Grubb 1994) and may interact and negatively influence the growth of the other plant (Schoener 1983). Both can potentially influence fine root distribution patterns. It is possible that competition between cedar, hemlock and salal in CH forests induces the fine root distribution patterns found in these forests. Cedar may have a greater proportion of fine roots in the lower organic and mineral soil horizons in response to the competitive pressures.
Summary

Regardless of the mechanism responsible, it is apparent that cedar, hemlock and salal have different vertical patterns of fine root distributions in CH forest floor and mineral soil horizons. Salal and hemlock fine root depletion zones overlap, with roots of both species concentrated in the upper forest floor horizons. Thus these two species are probably competing for the same resources, and based on the productivities of the two species, salal appears to be the more effective competitor. In contrast, cedar fine roots are evenly distributed, and are probably not subject to the same amount of competition. As a result, cedar may have greater access to a larger proportion of N available in the lower organic and mineral soil horizons. Therefore, species-specific fine root distribution patterns and the resulting spatial separation of N acquisition may be important in CH forests. Furthermore, the distributions of fine roots may contribute to the differences in productivities of cedar, hemlock and salal in these forests.
Chapter 6

Conclusions and Recommendations for Future Research

Cedar, hemlock and salal growth in CH forests on northern Vancouver Island is N-limited. Despite this nutrient limitation, the three species grow together in the old-growth forests and differ in their respective productivities. These growth trends may be a function of the abilities of the three species to utilize different N sources. Cedar, hemlock and salal may differ in their access to the form of N (organic and inorganic) or may access N in different forest floor and mineral soil horizons. In this thesis, four trials were conducted to examine the importance of these two mechanisms in providing different sources and amounts of N to the three species. Species-specific abilities to utilize a variety of organic and inorganic N forms were measured, and the vertical distribution patterns of cedar, hemlock and salal fine roots in old-growth CH forests were characterized.

N form

Cedar, hemlock and salal all had the capacity to absorb glutamic acid intact and appeared to be able to access more complex organic N compounds including proteins and proteins bound to tannins. Therefore, in CH forests where the majority of N present in the forest floors and mineral soils is in an organic form, organic N compounds may serve as sources of N to the three species. Cedar, hemlock and salal did not differ in their preferences for N forms or their abilities to access different N compounds. Nitrate and NH$_4^+$ accounted for the largest proportions of N absorbed by the three species in the 24 hours following treatment application, and these compounds were also taken up in the largest quantities during a 20-day period. These results suggest that organic N compounds are less important sources of N to cedar, hemlock and salal, and 24 hours after application, are largely unavailable to the plants and are probably utilized by the microbial biomass in CH forest floor. Thus, cedar, hemlock and salal do not appear to differ in their access to and capacity to utilize different N compounds, so N form is not a mechanism by which cedar, hemlock and salal in CH forests utilize different N sources and capture larger amounts of N.
**N in different forest floor and soil horizons**

An examination of the vertical distributions of cedar, hemlock and salal fine roots in three old-growth CH forests showed that the three species have distinct fine rooting patterns, and therefore, may spatially separate N acquisition and utilize N from different forest floor and mineral soil horizons. The density distributions of salal and hemlock roots in forest floor and mineral soil horizons were correlated, with the roots of both species concentrated in the F and Hr horizons. In contrast, cedar fine roots were evenly distributed in all four horizons and the root densities of cedar were not correlated with those of the other two species. Therefore, hemlock and salal probably draw upon common N pools and have more competitive interactions than cedar, which accesses more N in lower horizons. Of the two conifers, the growth of cedar would be affected less by competition and N limitation than that of hemlock, and the relative productivities of these two species in CH forests support this interpretation.

The main conclusions from this work are that spatially separating N acquisition may be a more important mechanism than N form for accessing N in CH forests. Differences in both the fine root distributions and competitive abilities of cedar, hemlock and salal may influence the relative productivities of the three species in these forests.

**Future research**

The results from this thesis are more suggestive than conclusive and require confirmation through similar trials and additional studies to test the validity of the interpretations. An area unexplored in this thesis, yet integral to the N nutrition in CH forests, is an understanding of the microbial component of N cycling. Studies have shown that \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) production and consumption are rapid (Jackson et al. 1989; Schimel and Firestone 1989; Davidson et al. 1990; Davidson et al. 1992; Hart et al. 1994a), however, little is known about the cycling rates of organic N compounds. In CH forests, organic N compounds are the main forms of N experienced by cedar, hemlock and salal roots, and organic N dominates the soluble N fraction. Furthermore, the three species appear to be able to access different organic N compounds. Therefore, it is necessary to learn more about organic N cycling in CH forests.

The pot trial described in Chapter 4 included an assessment of metabolic abilities of the saprophytic microbes found in the forest floors surrounding cedar, hemlock and salal roots. The three communities had different basal respiration rates and also responded differently to the
additions of a variety of organic N compounds. The cedar forest floors showed more distinct CO₂ production patterns. These differences were interpreted as the product of the microbial community composition suggesting that the community associated with cedar is different from those associated with hemlock and salal. As discussed in Chapter 4, these differences in rhizosphere microbial communities may have implications for the mechanism of nutrient acquisition. For example, cedar may support a more active microbial community that provides more mineral N for uptake, making it less affected by low N availability and competition for the same N resource. A more comprehensive examination of the compositions of the microbial communities associated with cedar, hemlock and salal together with an assessment of the metabolic abilities of the identified microbes would test this hypothesis and perhaps redefine our characterization of the interactions shared between free-living microbes and plant roots.

The vertical distribution patterns of cedar, hemlock and salal fine roots were only suggestive of differential access to N. Nitrogen concentrations (NO₃⁻, NH₄⁺, SON and total N), in general, were poorly correlated to fine root distribution patterns, and spatial separation of N uptake by the three species was not measured. Also, hemlock and salal fine roots were concentrated in the same horizons and the relative productivities of the two species were used to infer which species probably captured more of the N in these horizons. To validate these interpretations, it would be necessary to track N uptake through the roots. In addition, to understand why salal is able to capture more N, further investigations into the ecophysiological mechanisms that facilitate this greater acquisition are necessary. As discussed in Chapter 4, the potential mechanisms employed by salal may include allelopathy (Fitter 1976; Mallik 1987; Callaway et al. 1991; Vaughan and Ord 1991; deMontigny 1992; Xiao 1994), a greater sink strength (A. Glass pers. comm.), better exploitation of total forest floor/mineral soil volume due to an extensive fine root system, and the physiological capacity to take up N at low soil N concentrations (Tilman 1982; Tilman 1987; Hodge et al. 1999). Salal may have one or more of these characteristics and these mechanisms could be investigated further.
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### APPENDIX I

ANOVA tables showing significant blocking effects detected in the analyses conducted in Chapter 4 (GLM procedure).

#### Hemlock Day 3 Harvest

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#### Organic N/Total N taken up Day 1 Harvest

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<td>0.0348</td>
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<tr>
<td>Treatment</td>
<td>2</td>
<td>0.024365</td>
<td>0.012183</td>
<td>2.68</td>
<td>0.1127</td>
</tr>
</tbody>
</table>
Ammonium concentrations in CH forest floor cores planted with cedar, hemlock and salal determined by 1M KCl and deionized water extractions. Approximately 10 g forest floor (fresh weight) was extracted with 50 ml of water or KCl, shaken for one hour, and the gravitation filtrated extracts were analyzed for NH$_4^+$ on the autoanalyzer at the Soil Science Laboratory at U.B.C.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Species</th>
<th>Water extraction (µg N·g forest floor$^{-1}$)</th>
<th>1 M KCl extraction NH$_4^+$·N (µg N·g forest floor$^{-1}$)</th>
<th>Water extraction/KCl extraction</th>
<th>KCl extraction/Water extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>cedar</td>
<td>0.38</td>
<td>2.92</td>
<td>0.13</td>
<td>7.72</td>
</tr>
<tr>
<td>2</td>
<td>cedar</td>
<td>0.38</td>
<td>4.20</td>
<td>0.09</td>
<td>11.12</td>
</tr>
<tr>
<td>3</td>
<td>cedar</td>
<td>15.21</td>
<td>57.20</td>
<td>0.27</td>
<td>3.76</td>
</tr>
<tr>
<td>4</td>
<td>cedar</td>
<td>1.39</td>
<td>10.70</td>
<td>0.13</td>
<td>7.68</td>
</tr>
<tr>
<td>5</td>
<td>hemlock</td>
<td>2.47</td>
<td>3.61</td>
<td>0.68</td>
<td>1.46</td>
</tr>
<tr>
<td>6</td>
<td>hemlock</td>
<td>1.03</td>
<td>4.60</td>
<td>0.22</td>
<td>4.47</td>
</tr>
<tr>
<td>7</td>
<td>hemlock</td>
<td>1.34</td>
<td>2.26</td>
<td>0.59</td>
<td>1.69</td>
</tr>
<tr>
<td>8</td>
<td>hemlock</td>
<td>0.88</td>
<td>4.12</td>
<td>0.21</td>
<td>4.71</td>
</tr>
<tr>
<td>9</td>
<td>salal</td>
<td>1.90</td>
<td>3.91</td>
<td>0.48</td>
<td>2.06</td>
</tr>
<tr>
<td>10</td>
<td>salal</td>
<td>1.13</td>
<td>1.62</td>
<td>0.70</td>
<td>1.43</td>
</tr>
<tr>
<td>11</td>
<td>salal</td>
<td>2.36</td>
<td>2.40</td>
<td>0.98</td>
<td>1.02</td>
</tr>
<tr>
<td>12</td>
<td>salal</td>
<td>0.75</td>
<td>2.03</td>
<td>0.37</td>
<td>2.71</td>
</tr>
</tbody>
</table>

Averages: 0.41  4.15

Concentrations of NH$_4^+$·N extracted in 1 M KCl and adjusted for the deionized water/KCl extraction correction factor

<table>
<thead>
<tr>
<th>Species</th>
<th>Extracted NH$_4^+$·N (µg N·g forest floor$^{-1}$)</th>
<th>Adjusted NH$_4^+$·N (µg N·g forest floor$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cedar</td>
<td>5.03</td>
<td>2.04</td>
</tr>
<tr>
<td>hemlock</td>
<td>3.14</td>
<td>1.27</td>
</tr>
<tr>
<td>salal</td>
<td>2.76</td>
<td>1.12</td>
</tr>
</tbody>
</table>
APPENDIX II

ANOVA table showing a significant blocking effect for soluble organic nitrogen (kg·m⁻³) (GLM procedure).

<table>
<thead>
<tr>
<th>Soluble Organic Nitrogen (kg·m⁻³)</th>
<th>Df</th>
<th>Type III SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon</td>
<td>3</td>
<td>76.722</td>
<td>25.574</td>
<td>1.43</td>
<td>0.3241</td>
</tr>
<tr>
<td>Site</td>
<td>2</td>
<td>248.427</td>
<td>124.214</td>
<td>6.94</td>
<td>0.0275</td>
</tr>
<tr>
<td>Experimental Error</td>
<td>6</td>
<td>107.401</td>
<td>17.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>432.55</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results from the Tukey 1 degree of freedom test for non-additivity conducted on the nitrogen values from the root distribution trial.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calculated F value</th>
<th>Critical F value</th>
<th>Significant site x horizon interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>SON (µg·g⁻¹)</td>
<td>3.1600</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>SON (kg·m⁻²)</td>
<td>0.016</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>SON (kg·m⁻³)</td>
<td>14.72</td>
<td>6.61</td>
<td>**</td>
</tr>
<tr>
<td>NH₄⁺ (µg·g⁻¹)</td>
<td>2.41</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>NH₄⁺ (kg·m⁻²)</td>
<td>6.64</td>
<td>6.61</td>
<td>**</td>
</tr>
<tr>
<td>NH₄⁺ (kg·m⁻³)</td>
<td>11.02</td>
<td>6.61</td>
<td>**</td>
</tr>
<tr>
<td>NO₃⁻ (µg·g⁻¹)</td>
<td>2.98</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻ (kg·m⁻²)</td>
<td>41.46</td>
<td>6.61</td>
<td>**</td>
</tr>
<tr>
<td>NO₃⁻ (kg·m⁻³)</td>
<td>2.33</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>Total N (µg·g⁻¹)</td>
<td>0.000004</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>Total N (kg·m⁻²)</td>
<td>0.47</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>Total N (kg·m⁻³)</td>
<td>1.59</td>
<td>6.61</td>
<td></td>
</tr>
</tbody>
</table>
Significant site x horizon interactions found in the analyses of N form concentrations and contents in CH forest floor and mineral soil horizons based on Tukey test for non-additivity.
ANOVA tables showing significant blocking effects for fine root densities (kg·m⁻³) and biomass (kg·m⁻²) (GLM procedure).

### Cedar fine roots (g·m⁻²)

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Type III SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon</td>
<td>3</td>
<td>59.392</td>
<td>19.797</td>
<td>12.08</td>
<td>0.0059</td>
</tr>
<tr>
<td>Site</td>
<td>2</td>
<td>18.566</td>
<td>9.283</td>
<td>5.67</td>
<td>0.041</td>
</tr>
<tr>
<td>Horizon x Site (experimental error)</td>
<td>6</td>
<td>9.832</td>
<td>1.639</td>
<td>1.19</td>
<td>0.3308</td>
</tr>
<tr>
<td>Sampling Error</td>
<td>42</td>
<td>57.881</td>
<td>1.378</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>146.325</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Salal fine roots (g·m⁻²)

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Type III SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon</td>
<td>3</td>
<td>32.779</td>
<td>10.926</td>
<td>37.64</td>
<td>0.0003</td>
</tr>
<tr>
<td>Site</td>
<td>2</td>
<td>28.121</td>
<td>14.061</td>
<td>48.43</td>
<td>0.0002</td>
</tr>
<tr>
<td>Horizon x Site (experimental error)</td>
<td>6</td>
<td>1.742</td>
<td>0.290</td>
<td>0.23</td>
<td>0.9660</td>
</tr>
<tr>
<td>Sampling Error</td>
<td>42</td>
<td>53.912</td>
<td>1.284</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>112.344</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Salal fine roots (kg·m⁻³)

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Type III SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon</td>
<td>3</td>
<td>1.484</td>
<td>0.495</td>
<td>13.98</td>
<td>0.0041</td>
</tr>
<tr>
<td>Site</td>
<td>2</td>
<td>1.053</td>
<td>0.526</td>
<td>14.88</td>
<td>0.0047</td>
</tr>
<tr>
<td>Horizon x Site (experimental error)</td>
<td>6</td>
<td>0.212</td>
<td>0.035</td>
<td>0.48</td>
<td>0.8216</td>
</tr>
<tr>
<td>Sampling Error</td>
<td>45</td>
<td>3.335</td>
<td>0.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>6.134</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Cedar, hemlock and salal root diameters used to determine the fine root fractions. These dry sample values are equivalent to 2 mm diameters in fresh samples (mean ± S.E.M). Five to seven roots (around 2 mm in diameter) similar in degree of suberization to samples collected from the CH sites, were selected from each of three cedar, hemlock and salal seedlings. The seedlings were four years old and had been germinated and grown in CH forest floor. Two sets of samples (suberized and brown roots) were selected from the cedar plants to account for the two forms of large cedar roots found in the field. Each root sample was measured eight times (four times at each end) before and after drying at 70 °C to a constant weight.

<table>
<thead>
<tr>
<th>Root condition</th>
<th>Number of samples</th>
<th>Dry sample diameter equivalent to 2mm fresh sample diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>suberized</td>
<td>18</td>
<td>1.52 ± 0.033</td>
</tr>
<tr>
<td>brown (not fully suberized)</td>
<td>17</td>
<td>1.75 ± 0.015</td>
</tr>
<tr>
<td>suberized</td>
<td>17</td>
<td>1.73 ± 0.010</td>
</tr>
<tr>
<td>suberized</td>
<td>18</td>
<td>1.69 ± 0.021</td>
</tr>
</tbody>
</table>

The results of ANOVA (GLM procedure) of the adjusted cedar, hemlock and salal fine root biomass present in the forest floor and mineral soil horizons. The amounts of roots in the Hh horizon were adjusted for a potential reduction in rooting density at depths greater than 15 cm. Below 15 cm, Ae/B rooting densities were used to calculate root biomass. When the Ae/B root densities were greater than or equal to the Hh root densities, the measured Hh densities were applied. Different letters indicate significantly different means within a species based on ANOVA (GLM procedure) (p<0.05. n=12-15, means)

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Cedar (g·m⁻²)</th>
<th>Hemlock (g·m⁻²)</th>
<th>Salal (g·m⁻²) **</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>31.81 c</td>
<td>90.77 b</td>
<td>20.54 ab</td>
</tr>
<tr>
<td>Hr</td>
<td>148.39 ab</td>
<td>446.74 a</td>
<td>55.30 a</td>
</tr>
<tr>
<td>Hh</td>
<td>286.60 a</td>
<td>32.78 c</td>
<td>36.30 a</td>
</tr>
<tr>
<td>Ae/B</td>
<td>114.47 b</td>
<td>45.73 bc</td>
<td>8.06 b</td>
</tr>
</tbody>
</table>