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Organic and inorganic nitrogen nutrition of western red cedar, western hemlock and salal in mineral N-limited cedar–hemlock forests

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Abstract Western red cedar (*Thuja plicata* Donn.), western hemlock (*Tsuga heterophylla* Raf. Sarge) and salal (*Gaultheria shallon* Pursh) are the main species growing in cedar–hemlock forests on Vancouver Island, Canada. Based on the dominance of organic N in these systems, we tested the hypotheses that: (1) organic N can be utilized by the three plant species; and (2) salal, which is ericoid mycorrhizal and has high tannin concentration in its tissues, would absorb more N from the complex organic N compounds than the other two species. The abilities of cedar, hemlock and salal to take up ^{15}N,^{13}C-labelled glutamic acid were measured and the capacities of the three species to use nitrate (NO_3^-), ammonium (NH_4^+), glutamic acid, protein and protein–tannin N were compared over a 20-day period. Based on ^{13}C enrichment, all three species absorbed at least a portion of glutamic acid intact. Cedar, hemlock and salal also showed similar patterns of N uptake from the NO_3^-, NH_4^+, glutamic acid, protein and protein–tannin treatments. The largest proportions of applied N were taken up from the NO_3^- and NH_4^+ treatments while smaller amounts of N were absorbed from the organic N compounds. Thus organic N was accessed to a modest degree by all three species, and salal did not have a greater capacity to utilize protein and protein–tannin–N.

Keywords Protein · Tannin · Mycorrhizae · Isotope

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Introduction

Mycorrhizal and non-mycorrhizal plants from a variety of ecosystems have shown the capacity to take up organic forms of N, without the necessity of mineralization prior to uptake. Nasholm et al. (1998) examined the uptake of ^{15}N,^{13}C-labelled glycine by two trees, a shrub and a grass in a boreal forest, and all four were found to contain enriched levels of the two isotopes, strongly suggesting that at least a portion of the amino acid was absorbed intact (Nasholm et al. 1998). Similar investigations have been conducted with plants in arctic tundra (Schimel and Chapin 1996), boreal (Nordin et al. 2001; Persson et al. 2003), alpine (Lipson and Monson 1998; Lipson et al. 1999; Miller and Bowman 2003), and sub-tropical Eucalyptus forest (Turnbull et al. 1995) and agricultural (Nasholm et al. 2000) systems, and all the examined species demonstrated the ability to take up amino acids. Based on uptake kinetics and soil amino acid concentrations, simple organic N compounds are thought to meet 50–100% of the N required by plants in alpine ecosystems (Lipson et al. 2001) and account for 10–82% of N potentially absorbed by plants in arctic ecosystems (Chapin et al. 1993; Kielland 1994). In some studies, applied amino acids were taken up in similar quantities as NH_4^+ or NO_3^-, further highlighting the potential importance of simple organic N in some systems (Schimel and Chapin 1996; Nasholm et al. 2000; Nordin et al. 2001; Miller and Bowman 2003).

More complex organic compounds may also be important sources of N. In controlled laboratory studies using plants infected with single strains of fungi, ecto- and ericoid mycorrhizal plants were able to grow on a variety of organic N compounds including amino sugars (Kerley and Read 1995), chitin (Kerley and Read 1995), peptides (Bajwa and Read 1985) and larger proteins (Abuzinadah and Read 1986; Finlay et al. 1992). Griffiths and Caldwell (1991) showed that ectomycorrhizal fungi found in coniferous forests were able to utilize N from insoluble protein–tannin complexes. However, four ectomycorrhizal fungi studied by Bending and Read (1996) did not show

this capacity. Only the two ericoid mycorrhizal fungi, *Oidiodendron griseum* and *Hymenoscyphus ericaceae*, were able to mobilize N from tannin–BSA compound.

The ability to access more complex organic N compounds may be an adaptation to nutrient poor sites with low amounts of mineral N but large accumulations of N in organic forms. In an edaphic gradient of coastal terraces in southern California, Northup et al. (1995a,b) found significant positive relationships between tannin concentrations and soil dissolved organic N. They hypothesized that the production of foliar tannins and formation of insoluble tannin–protein complexes in the soil enabled N retention on site and limited access of these compounds to soil biota with the enzymatic capacity to break down the complexed proteins. Ericoid and to a lesser extent ectomycorrhizal fungi have the ability to produce such enzymes. Thus plants, by virtue of their associations with these fungi, would have the capacity to mobilize N from these compounds and be able to dominate nutrient-poor sites. It has also been suggested that the ability of plants to utilize a variety of organic N sources allows them to co-exist in N-poor environments by partitioning the forms of N absorbed (Stewart et al. 1993; Schulze et al. 1994; Nadelhoffer et al. 1996; Schmidt and Stewart 1997; Nordin et al. 2001). Plants with the capacity to mobilize N from organic sources could utilize the organic N compounds on site, while other plants would rely more on mineral forms of N. Although these hypotheses are exciting and could account for N uptake in systems with large organic matter accumulations and low amounts of mineral N, the abilities of plants growing in such systems to take up organic N compounds larger than amino acids have not been widely tested.

In this study we examined the ability of western red cedar, western hemlock and salal to access organic N from a variety of sources ranging in complexity. Cedar, hemlock and salal which are vesicular arbuscular (VA), ecto- and ericoid mycorrhizal, respectively, are the three main species found growing in old-growth cedar–hemlock forests on northern Vancouver Island, British Columbia. Cedar and hemlock occupy the dominant and co-dominant canopy layers in the overstorey, and the ericaceous shrub salal forms a dense understorey layer. The vegetation in these forests are underlain by thick forest floors (0.19–0.73 m) with low extractable mineral N concentrations (0.63 kg N ha^{-1} of NO_3^- and NH_4^+) but high concentrations of soluble organic N (4,310 kg N ha^{-1}) (Bennett et al. 2002). Based on the dominance of organic N in this ecosystem, we hypothesized that: (1) simple organic N compounds can be absorbed intact by cedar, hemlock and salal growing in cedar–hemlock forest floor; (2) organic N compounds are sources of N to all three species growing in these forest floors; and (3) organic N accounts for a larger proportion of N absorbed by salal than for the other two species. Salal has high tannin concentrations in its tissues (Preston 1999) and shares ericoid mycorrhizal associations. Two potted seedling experiments were conducted to test these hypotheses and involved the injection of ^{15}N or double-labelled ^{15}N,^{13}C organic and inorganic N compounds into intact cores of cedar–hemlock forest floor planted with cedar, hemlock and salal. The plants were harvested over the course of 20 days to determine uptake of N and C from the applied compounds.

Materials and methods

Site description

Forest floor cores were extracted from five old-growth cedar–hemlock forests near Port McNeill on Vancouver Island, British Columbia (50°38′N, 127°06′W). Cedar–hemlock forests are located within the very wet maritime Coastal Western Hemlock (CWH) biogeoclimatic zone (Green and Klinka 1994) and receive an average annual precipitation of 1,700 mm, 65% of which falls between October and February. The mean annual temperature is 7.9°C with a daily average range from 2.4°C (January) to 13°C (August) (Prescott and Weetman 1994).

Western red cedar dominates the main canopy of cedar–hemlock forests and western hemlock is typically found in the co-dominant, intermediate and suppressed layers. The understory is primarily salal with minor amounts of *Vaccinium* spp., deer fern (*Blechnum spicant* (L.) Roth), bunch berry (*Cornus canadensis* L.), salmonberry (*Rubus spectabilis* Pursh), and moss [*Hylocomium splendens* (Hedw.) B.S.G., *Kindbergia oregana* (Sull.) Ochyra and *Rhytidiadelphus loreus* (Hedw.) Warnst.] (DeMontigny 1992). Thick forest floors classified as humimors (i.e. with well developed H horizons) or lignomors (i.e. with large amounts of decomposing wood in the H horizon) (Green et al. 1993) overlay duric or orthic Humo-Ferric podzols (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).

Test seedling preparation

Intact cores of forest floor were collected from five old-growth cedar–hemlock forests in May 1999. Using root saws, circular cores 15 cm wide and 13 cm deep were cut out of the forest floor and 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), transferred to the horticulture greenhouse at the University of British Columbia and within 1 week, planted with one or two 1-year-old cedar, hemlock or salal plants that had been germinated and grown in cedar–hemlock forest floor. The potted plants were watered as necessary, exposed to daily (24-h) temperature ranges of 10–34°C through the growing season (May–August) and grown for 11–17 months prior to experiment initiation. The plants were overwintered outside from September to April.

Amino acid uptake experiment

To assess the abilities of cedar, hemlock and salal to take up amino acids, acid intact, one of the three solutions, ^{15}N-labelled ammonium chloride (NH_4Cl) (98.6 atom%), ^{15}N,^{13}C-labelled glutamic acid (98.2–98.3 atom%) or control (deionized water), was injected into the forest floor surrounding a single cedar, hemlock or salal plant. The experiment was a completely randomized block design with eight blocks, blocked by establishment time for a total of 72 experimental units. Glutamic acid was chosen as the test amino acid because it provides a conservative estimate of amino acid uptake as it is typically absorbed in smaller quantities relative to other amino acids (Chapin et al. 1993; Kielland 1994) and is in high demand by microbes (Lipson et al. 1999).

During establishment of each block, a total of 60 ml of treatment solution was injected with a stainless steel blunt-ended syringe needle (14G) (Popper, New York) at six equally spaced points around each seedling (10 ml at each point). The 6-inch needle was inserted approximately 3/4 of pot depth (9 cm) into the forest floor, and the solution was released as the needle was withdrawn, to provide homogeneous distributions of the treatments in the forest floor core (Schimel and Chapin 1996). Any solution that leaked from the bottom of the pot after injection was collected and poured over the surface of the forest floor to ensure that the treatment was contained within the core. A total of 0.1178 mmol N (10 μg N g^{-1} forest floor) was applied with the glutamic acid treatment.

The cedar, hemlock and salal seedlings were harvested in the order of injection, 6–7 h after treatment application. Plants were removed from the pots and all loosely adhering forest floor was shaken and rinsed from the root systems and roots. The whole plants were rinsed with deionized water and the roots were soaked twice in 0.5 mM $CaCl_2$ for 5 min to remove any treatment compound adsorbed to root surfaces and present in the apoplastic free-space (Nasholm et al. 1998). Care was taken to remove as much live root as possible. However, because salal has very fine roots (Xiao 1994), typical of the Ericaceae (Read 1991; Ehrenfeld et al. 1992), not all of the salal root system could be recovered. The whole seedlings were then enclosed in aluminum foil, frozen in liquid N_2, and stored at −80°C until they were freeze-dried. Following freeze-drying, the seedlings were weighed and ground to a fine powder in a Wiley mill followed by a Fritsch ball mill and the ground samples were analyzed for total N, C, ^{15}N and ^{13}C by a PDZ Europa Scientific Integra combustion-continuous flow mass spectrometry carbon–nitrogen analyzer (Cheshire, UK) at the Stable Isotope Facility at the University of California, Davis.

To isolate the fraction of nutrients recently absorbed by the plants, a second set of analyses were conducted on the whole-plant samples (Nasholm et al. 1998; Nordin et al. 2001). Thirty-five milligrams of each ground sample were extracted with 3 ml of 10 mM phosphate buffer (pH 8.0) (Nasholm et al. 1998) and centrifuged at 4,200 rpm for 10 min. The supernatants were decanted and stored in the fridge and the extraction process was repeated on the centrifuged sample pellets. The two supernatants from each sample were combined and the 6 ml extract was then frozen and stored at −80°C until being reduced to between 50 and 150 μl in a reduced pressure evaporator (Savant Speed Vac). The extracts were then pipetted into 8×5 mm tin capsules, dried at 70°C, sealed and analyzed for total N, C, ^{15}N and ^{13}C at the Stable Isotope Facility.

Organic N uptake experiment

To assess the abilities of cedar, hemlock and salal to utilize N from a variety of organic and inorganic N sources, one of six ^{15}N-labelled solutions, calcium nitrate [$Ca(NO_3)_2$] (98.3 atom%), ammonium sulphate [$(NH_4)_2SO_4$] (98.5 atom%), glutamic acid (98.5 atom%), plant protein (30.21 atom%), plant protein–tannin complex (40.43 atom %) and control (deionized water) were injected into the forest floor around cedar, hemlock or salal plants using the same methods and treatment application rates as for the amino acid uptake experiment. However, the pots with two seedlings were used in this experiment, the protein and protein–tannin treatments were only partially soluble and were mixed into a suspension before being applied, and because of an error in the certificate of analysis, 0.1604 mmol N (14 μg N g^{-1} forest floor) was applied in the $Ca(NO_3)_2$ treatments. The experiment was established as a completely randomized block design with seven blocks, blocked by establishment time, for a total of 126 experimental units.

The $Ca(NO_3)_2$, $(NH_4)_2SO_4$ and glutamic acid compounds were purchased from Cambridge Isotope Laboratories and the protein and protein–tannin complex were made in our laboratory at the University of British Columbia. To produce protein powder, barley was grown and watered with ^{15}N-enriched nutrient solution for 2 weeks and the soluble protein fraction was extracted from the shoots of the plants according to methods outlined by Gegenheimer (1990). The protein solution was dialyzed to produce a ^{15}N-enriched treatment of soluble proteins greater than 3,500 molecular weight, and 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 high protein purity (data not shown).

The protein–tannin complex was produced with a portion of the prepared ^{15}N-enriched protein and condensed tannins from salal (provided by Dr. Caroline Preston at the Pacific Forestry Centre in Victoria, British Columbia) 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 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 min before being centrifuged at 6,000 rpm for 10 min. The precipitated protein–tannin pellets were freeze-dried. Both the protein and protein–tannin treatments were

stored below 0°C, and samples of each were analyzed for N and ^{15}N content at the Stable Isotope Facility.

The plants were harvested from the cores 1, 3, 7 and 20 days after treatment injection and in the order in which they were injected. The plants were removed from the pots, all loosely adhering forest floor was shaken off the root systems, the whole plants were rinsed thoroughly with deionized water and roots soaked in 0.5 mM $CaCl_2$, as described for the amino acid uptake experiment. The two seedlings from each pot were combined and dried to constant weight at 70°C and ground to a fine powder in a Wiley mill followed by a Fritsch ball mill. Ground samples were analyzed for total N and ^{15}N contents at the Stable Isotope Facility.

Calculations

For both experiments, using the ^{15}N results from the ground tissue analyses, total amounts of treatment N assimilated by the seedlings were calculated using:

$$F = T(A_S - A_B)/A_F$$

where F is the total weight of the N derived from the treatment; T is the total weight of N in the plant sample; A_S is the atom% excess ^{15}N in the plant sample; A_B is the atom% excess ^{15}N in the control plant samples; and A_F is the atom% excess ^{15}N in the treatment applied. Atom% excess is defined as atom% ^{15}N in the sample minus the atom% ^{15}N in the control (deionized water) plants (Powlson and Barraclough 1993).

Mycorrhizal assessment

Cedar, hemlock and salal respectively share VA, ecto- and ericoid mycorrhizal associations. To confirm that the plants used in the experiments were mycorrhizal, the roots of eight potted seedlings of each species were assessed for the presence of mycorrhizal fungi. The plants were removed from the pots, rinsed with tap water and the roots were stored in FAA (90% formalin, 5% acetic acid and 5% ethanol) solution until they were examined. Cedar and hemlock roots were cleared and stained according to modified methods outlined by Kormanik and McGraw (1984). Briefly, the roots were autoclaved (30 min at 15 PSI) in 10% potassium hydroxide (KOH) (w/v), bleached in an hydrogen peroxide solution (30 ml 10% hydrogen peroxide (H_2O_2), 3 ml ammonium hydroxide (NH_4OH) and 567 ml water) for 30–90 min and stained with a trypan blue solution (lactic acid: glycerol: distilled water at a 1:1:1 ratio with 0.1% trypan blue). The salal roots did not require clearing or staining. All roots were examined under dissecting or compound microscopes to determine the presence of fungal structures in or on the roots.

Statistical analyses

To confirm that a proportion of the glutamic acid was absorbed intact, differences between ^{13}C in the phosphate buffer extracts from control, glutamic acid and NH_4^+ treated plants were determined using analysis of variance (ANOVA), general linear model (GLM) procedure. These analyses were followed by pairwise *t*-test comparisons of the least square means, and the alpha level was adjusted for the number of comparisons using Bonferroni's adjustment (Neter et al. 1996).

Analysis of variance (GLM procedure) and an alpha level of 0.05 were also used to determine differences in the amounts of treatment N absorbed by cedar, hemlock and salal in NH_4^+ and glutamic acid treatments.

Differences in the amounts of $Ca(NO_3)_2$, $(NH_4)_2SO_4$, glutamic acid, protein, protein–tannin treatment N taken up by cedar, hemlock and salal at each harvest time were determined using ANOVA (GLM procedure) followed by pairwise *t*-test comparisons as outlined above. Because the total amount of N added differed between NO_3^- and the other treatments, N uptake was calculated as proportion assimilated (% of applied). The data were 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. SAS was used for all analyses (SAS 1993).

Results

Amino acid uptake experiment

The extractable fractions from cedar, hemlock and salal receiving the glutamic acid treatments had significantly higher amounts of ^{13}C than in the other treatments (Fig. 1). ^{13}C levels in the control and NH_4^+-treated plants were not different. The ^{13}C enrichment in the glutamic acid-treated plants strongly suggests that all three species took up a portion of the amino acid intact (Miller and Bowman 2003). As previously discussed by Nasholm et al. (2001), it is not likely that the enrichment was due to photosynthetic fixation of respired $^{13}CO_2$ because control, NH_4^+ and glutamic acid-treated plants were randomly arranged in the greenhouse and ^{13}C levels were not elevated in the control and NH_4^+-treated plants.

From the data collected 6 h following treatment application, it was not possible to accurately estimate the amounts of glutamic acid absorbed intact by cedar, hemlock and salal. To increase the sensitivity to enriched levels of ^{13}C, the extractable fractions of the whole plants were analyzed, however the measured ^{13}C enrichments were still too low and were probably heavily diluted by endogenous C (Nasholm and Persson 2001; Nordin et al. 2001; Miller and Bowman 2003; Persson et al. 2003). Analyzing the extractable fraction from the roots alone

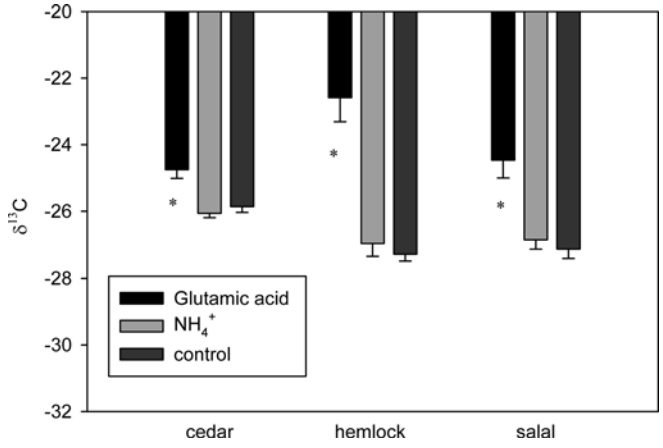

Fig. 1 $\delta^{13}C$ in the extractable fractions of cedar, hemlock and salal 6 h following the injection of $^{15}NH_4^+$, ^{15}N,^{13}C-labelled glutamic acid or deionized water (*control*) in the amino acid uptake experiment. Means ± standard error of the means (SEM) are reported. *Asterisks* represent significantly different $\delta^{13}C$ means within a species based on ANOVA (GLM procedure) ($P<0.05$, n=7–8)

may have provided clearer results (Nasholm et al. 1998,2000; Nasholm and Persson 2001). In addition, the recently assimilated ^{13}C-labelled glutamic acid may have been metabolized within the plant and converted into α-ketoglutarate, an intermediate in the *tri*-carboxylic acid cycle performed in the mitochondria of plant cells (Taiz and Zeiger 1991). During this cycle, a main pathway for the metabolism of glutamic acid, α-ketoglutarate is decarboxylated and two CO_2 are lost, barring re-fixation. During a preliminary experiment, a decline in the ratio of excess ^{13}C: ^{15}N assimilated by hemlock over the course of 24 h was found, supporting the metabolism and loss of $^{13}CO_2$ from the plant tissues (data not shown). Estimates of uptake are therefore derived from the ^{15}N results. Based on the excess ^{15}N concentrations in cedar, hemlock and salal, all three species absorbed significantly larger amounts of NH_4^+ than glutamic acid (Fig. 2).

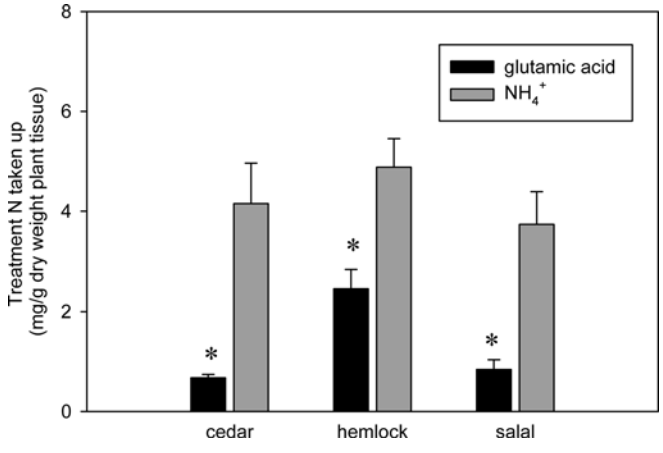

Fig. 2 Excess ^{15}N in whole-plant tissues of cedar, hemlock and 6 h following the injection of $^{15}NH_4^+$, ^{15}N,^{13}C-labelled glutamic acid in the amino acid uptake experiment. Means ± SEM are reported. *Asterisks* represent significantly different ^{15}N means within a species based on ANOVA (GLM procedure) ($P<0.05$, n=8)

Organic N uptake experiment

Although cedar, hemlock and salal took up different amounts of N from the five treatments, the three species showed similar patterns of N uptake. During the first 24 h, significantly larger amounts of N from the inorganic N treatments (NO_3^- and NH_4^+) were absorbed than N from the organic treatments (Fig. 3). The only exception was hemlock, which absorbed similar amounts of N from NO_3^- and glutamic acid sources. There were no significant differences between the amounts of glutamic acid, protein and protein–tannin N absorbed by cedar. Hemlock and salal took up larger amounts of N from the glutamic acid than from the protein–tannin treatments.

Similar trends were seen for the remainder of the experiment. The amounts of NO_3^- and NH_4^+ N absorbed by cedar, hemlock and salal were always significantly greater than the amounts of N taken up from the organic N treatments. After day 1, more NO_3^- was absorbed by cedar, followed by NH_4^+ and N from the organic compounds (Fig. 4a,b). There were no significant differences between the amounts of treatment N taken up by cedar in the organic N treatments, but during the 20-day period larger amounts of glutamic acid and protein N were absorbed than protein–tannin N. For the full duration of the experiment, salal also took up larger amounts of NO_3^- but differences in the amounts of N absorbed in the NO_3^- and NH_4^+ treatments were only significant on day 3 (Fig. 5a,b). The proportions of treatment N absorbed by salal from the organic N compounds did not differ, except on day 3 when N uptake from the glutamic acid treatments was greater than that from protein and protein–tannin treatments. In general, the N from the glutamic acid and protein treatments also increased in availability to salal relative to protein–tannin N during the 20 days. There were no differences in the amounts of treatment N absorbed by hemlock in the NO_3^- and NH_4^+ treatments, but larger amounts of NH_4^+ were taken up by hemlock up

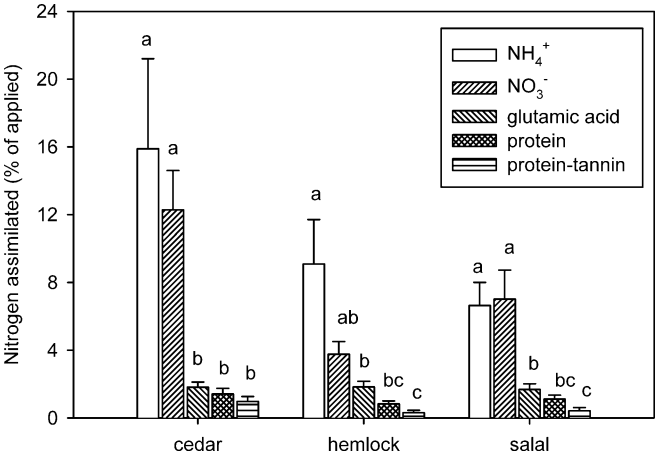

Fig. 3 Nitrogen uptake by cedar, hemlock and salal during the first 24 h of the organic N uptake experiment. Values are means ± SEM. *Different letters* indicate significantly different treatment means within a species based on ANOVA (GLM procedure) ($P<0.05$, n=5–7)

to day 7, after which more NO_3^- was absorbed (Fig. 6a,b). On days 3 and 7, significantly larger amounts of glutamic acid than protein–tannin N were taken up by hemlock. In general, very small amounts of N from the protein–tannin treatment were absorbed by cedar, hemlock and salal. Less than 2% of the N applied in this form was taken up by the plants during the 20-day experiment. All cedar, hemlock and salal root samples were mycorrhizal.

Discussion

Based on the enriched ^{13}C levels in the plants from the glutamic acid treatments in the amino acid uptake experiment, cedar, hemlock and salal growing in forest floor from cedar–hemlock forests are able to absorb amino acids intact. Similar abilities have been shown by plants growing in boreal (Nasholm et al. 1998; Nordin et al. 2001; Persson et al. 2003), alpine (Lipson and Monson 1998; Miller and Bowman 2003), and agricultural (Nasholm et al. 2000, 2001) systems, but all these studies

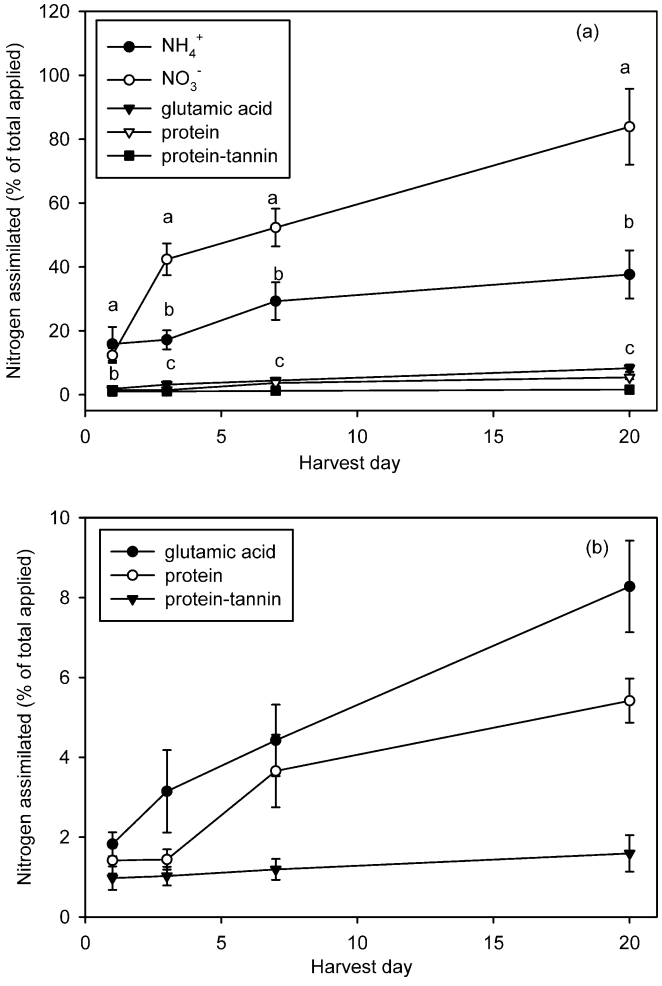

Fig. 4a, b Nitrogen absorbed by cedar during the organic N uptake experiment in all treatments (**a**) and in the organic N treatments only (**b**). Values are means ± SEM. *Different letters* indicate significantly different treatment means within a harvest day based on ANOVA (GLM procedure) ($P<0.05$, $n=5–7$)

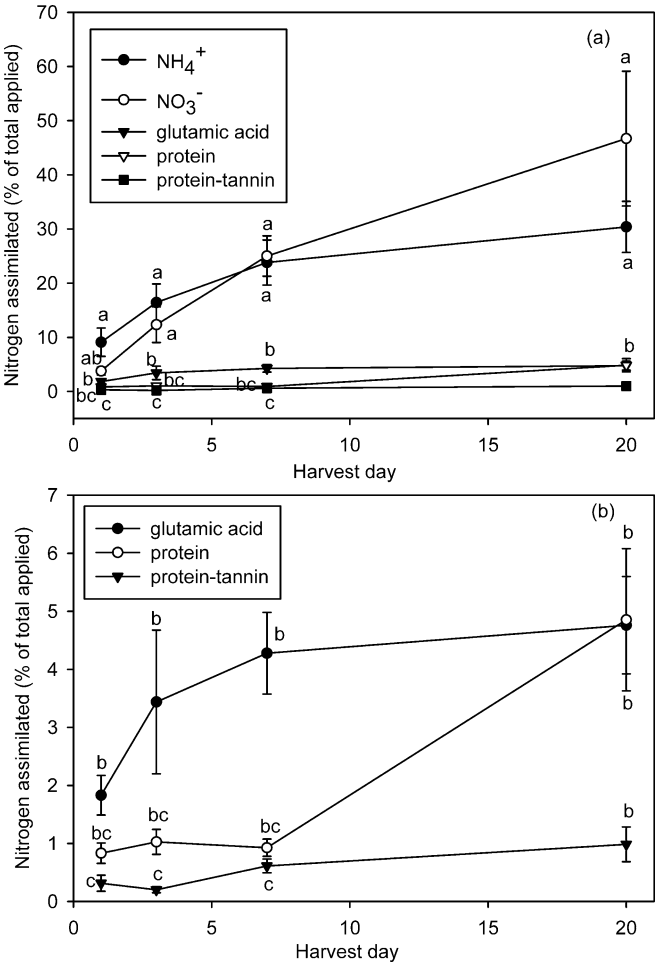

Fig. 5a, b Nitrogen absorbed by hemlock during the *organic N uptake experiment* in all treatments (**a**) and in the organic N treatments only (**b**). Values are means ± SEM. *Different letters* indicate significantly different treatment means within a harvest day based on ANOVA (GLM procedure) ($P<0.05$, $n=5–7$)

used glycine as the test amino acid. Lipson et al. (1999), however, compared the uptake of glycine and glutamic acid by the alpine sedge *Kobresia myosuroides* and found there to be greater competition between the plants and soil microbes for glutamic acid. Therefore, demonstrating the capacities of cedar, hemlock and salal to absorb this amino acid in the face of competition with microbes suggests that the three species are able to effectively compete for amino acid N in cedar–hemlock forest floor, and that amino acids should be considered an available source of N in these forests. Water-extractable amino acid concentrations in the F and H layers of cedar–hemlock forest floors accounted for 0.03 kg N ha^{-1} (5% of KCl-extracted mineral N) (Hannam and Prescott 2003).

According to the results from the organic N uptake experiment cedar, hemlock and salal growing in cedar–hemlock forest floor appear to show similar patterns of N uptake from NO_3^-, NH_4^+, glutamic acid, protein and tannin–protein sources. All three species absorbed large amounts of the applied NO_3^- and NH_4^+ and small proportions of N from the organic N treatments. Up to

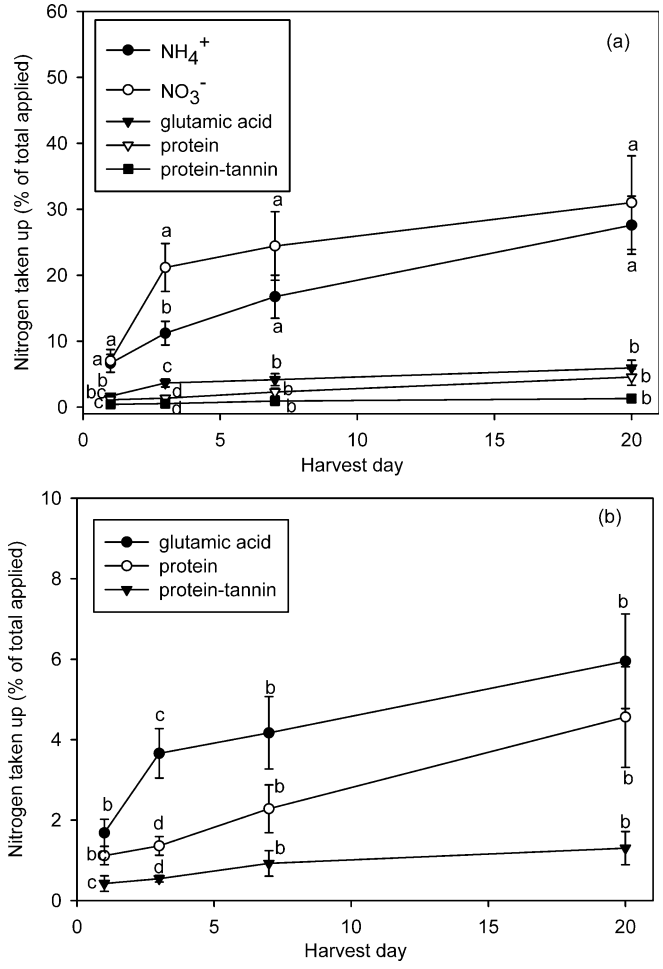

Fig. 6a, b Nitrogen absorbed by salal during the organic N uptake experiment in all the treatments (**a**) and in the organic N treatments (**b**). Values are means ± SEM. *Different letters* indicate significantly different treatment means within a harvest day based on ANOVA (GLM procedure) ($P<0.05$, $n=5–7$)

15% of the N from the organic treatments was absorbed during the first 24 h, and after 20 days, only 5–8, 5, and 1–2% of the N had been taken up from the applied glutamic acid, protein and tannin–protein, respectively. Because the cedar, hemlock and salal plants were all growing in cedar–hemlock forest floor, we assumed treatment dilution by background levels of the respective N compound to be similar for the three species, allowing for an accurate comparison of N uptake patterns.

It must be recognized, however, that it was not possible to determine the amounts of applied glutamic acid, protein and protein–tannin absorbed intact or in an organic form during the 20-day experiment. The compounds were only labeled with ^{15}N, and therefore may have been transformed prior to uptake. Unlike the NO_3^-, NH_4^+, glutamic acid treatments, large organic compounds such as the applied protein and protein–tannin compounds require cleavage by exoenzymes produced by mycorrhizal fungi prior to absorption (Bajwa et al. 1985; Leake and Read 1990; Bending and Read 1996; Chalot and Brun 1998). Therefore, all these compounds were at least broken down into peptides or amino acids prior to absorption. It is also likely that at least a portion of the organic N compounds were mineralized by saprophytic soil organisms prior to plant uptake, with the degree of mineralization increasing over the 20 days. However, it is beyond the scope of this study to determine if and the degree to which such transformations occurred. We also could not ascertain if the amount of treatment compound mineralization in the forest floors differed between species. Notwithstanding, the patterns of N utilization over the 20-day period were similar for cedar, hemlock and salal indicating that irrespective of the exact mechanism, the different inorganic and organic treatment compounds were of similar importance in the nutrition of the three species growing in cedar–hemlock forest floors.

Our study is the first (to our knowledge) that allows a test of the hypothesis of Northup et al. (1995a,b) that plant tissues with high tannin concentrations are an adaptation to nutrient-poor sites and provide the plants on these sites greater access to N. This hypothesis is based on the principle that protein–tannin complexes are formed and precipitate in the soil. Plants through their ericoid mycorrhizae are able to mobilize the complexed proteins (Bending and Read 1996) and access N that would otherwise be lost through leaching or competition with other organisms. The results from our study, however, indicate that tannin-bound proteins were not a large source of N to cedar, hemlock or salal in cedar–hemlock forest floors as a maximum of 2% of N from the added protein–tannin complex was absorbed. Salal is ericoid mycorrhizal, and so was expected to utilize the largest proportion of the complex organic N treatments. During the first 24 h, however, the protein–tannin complex was a more significant source of N for cedar than for salal. Similar amounts of glutamic acid, protein and protein–tannin N were taken up by cedar. Condensed tannins account for 21 and 18% by weight of salal foliage and roots, respectively (Preston 1999), so according to the hypothesis of Northup et al. (1995a,b), salal would be expected to absorb more N from tannin–protein sources. This was not the case in our study.

The similarities in the patterns of N uptake by cedar, hemlock and salal in the organic N uptake experiment also do not support the hypothesis that plant species co-existing in N-poor systems partition the forms of N absorbed from the soil. Although plant growth in cedar–hemlock forests is N limited, as indicated by fertilization studies (Weetman et al. 1989; Bennett et al. 2003), cedar, hemlock and salal showed similar patterns of N uptake; all three species absorbed large proportions of the inorganic N and relatively small quantities of N from the glutamic acid, protein and protein–tannin treatments. The findings from other studies also do not support the partitioning of N uptake by N-form hypothesis. Persson et al. (2003) examined the abilities of VA, ecto- and ericoid mycorrhizal plants growing together in a boreal ecosystem to utilize NH_4^+, NO_3^-, glycine, arginine and peptides over the course of 64 days. The three species, regardless of mycorrhizal association mobilized similar amounts of N from the different N sources. Similarly Nordin et al.

(2001) found the uptake of NH_4^+, NO_3^- and glycine by plant species within communities along a productivity gradient in a boreal forest to be similar. Uptake patterns between communities, however did differ between systems and were correlated with soil N concentrations.

In conclusion, cedar, hemlock and salal growing in cedar–hemlock forest floor appear to be able to absorb amino acids intact. All three species also utilized similar amounts of N from NO_3^-, NH_4^+, glutamic acid, protein and protein–tannin sources. Thus in these cedar–hemlock systems where the majority of N is in an organic form, cedar, hemlock and salal all have a limited ability to access and utilize N from a variety of organic N forms. However, none of the species had distinct patterns of N uptake. This may be an example of the N uptake abilities of plants being modified by the availabilities of N forms on site (Nordin et al. 2001); in this case, all species present on these N-poor sites are able to utilize several N forms.

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References

Abuzinadah RA, Read DJ (1986) The role of proteins in the nitrogen nutrition of ectomycorrhizal plants III. Protein utilization by *Betula*, *Picea* and *Pinus* in mycorrhizal association with *Hebeloma crustuliniforme*. New Phytol 103:507–514

Bajwa R, Read DJ (1985) The biology of mycorrhiza in the Ericaceae. IX. Peptides as nitrogen sources for the ericoid endophyte and for mycorrhizal and non-mycorrhizal plants. New Phytol 101:459–467

Bending GD, Read DJ (1996) Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. Soil Biol Biochem 28:1603–1612

Bennett JN, Andrew B, Prescott CE (2002) Vertical fine root distributions of western red cedar, western hemlock and salal in cedar–hemlock forests on northern Vancouver Island. Can J For Res 32:1208–1216

Bennett JN, Prescott CE, Barker JE, Blevins DP, Blevins LL (2003) Long-term improvement in productivity and nutrient availability following fertilization and vegetation control on a cedar–hemlock cutover. Can J For Res 33:1516–1524

Bradford MM (1976) A rapid and sensitive method for the estimation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Chalot M, Brun A (1998) Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. FEMS Microbiol Rev 22:21–44

Chapin FS III, Moilanen L, Kielland K (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature 361:150–153

DeMontigny L (1992) An investigation into the factors contributing to the growth-check of conifer regeneration on northern Vancouver Island. Dissertation, University of British Columbia

Ehrenfeld JG, Kaldor E, Parmelee RW (1992) Vertical distribution of roots along a soil toposequence in the New Jersey Pinelands. Can J For Res 22:1929–1936

Finlay RD, Frostegard A, Sonnerfeldt A-M (1992) Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex Loud. New Phytol 120:105–115

Gegenheimer P (1990) Preparation of extracts from plants. In: Deutscher MP (ed) Methods of enzymology. A guide to protein purification, vol 182. Academic, New York, pp 174–193

Green RN, Klinka K (1994) A field guide to site identification and interpretation for the Vancouver forest region. Crown, Victoria

Green RN, Trowbridge KL, Klinka K (1993) Toward a taxonomic classification of humus forms. For Sci Monogr 29 [suppl Sci 39]

Griffiths RP, Caldwell BA (1991) Mycorrhizal mat communities in forest soils. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International, Wallingford, pp 98–105

Hannam KD, Prescott CE (2003) Soluble organic nitrogen in forests and adjacent clearcuts in British Columbia, Canada. Can J For Res 33:1709–1718

Kerley SJ, Read DJ (1995) The biology of mycorrhiza in the Ericaceae: XVIII. Chitin degradation by *Hymenoscyphus ericae* and transfer of chitin-nitrogen to the host plant. New Phytol 131:369–375

Kielland K (1994) Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. Ecology 75:2373–2383

Kormanik PP, McGraw A-C (1984) Quantification of Vesicular–Arbuscular mycorrhizae in plant roots. In: Schenck NC (ed) Methods and principles of mycorrhizal research. The American Phytopathological Society, USA, pp 37–45

Leake JR, Read DJ (1990) Proteinase activity in mycorrhizal fungi: I. The effect of extracellular pH on the production and activity of proteinase by ericoid endophytes from soils of contrasted pH. New Phytol 115:243–250

Lewis T (1985) Ecosystems of Quatsino Tree-Farm License (TFL 6). Internal report. Western Forest Products, British Columbia

Lewis JA, Starkey RL (1968) Vegetable tannins, their decomposition and effects on decomposition of some organic compounds. Soil Sci 106:241–247

Lipson DA, Monson RK (1998) Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events. Oecologia 113:406–414

Lipson DA, Raab TK, Schmidt SK, Monson RK (1999) Variation in competitive abilities of plants and microbes for specific amino acids. Biol Fertil Soils 29:257–261

Lipson DA, Raab TK, Schmidt SK, Monson RK (2001) An empirical model of amino acid transformations in an alpine soil. Soil Biol Biochem 33:189–198

Miller AE, Bowman WD (2003) Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. Plant Soil 250:283–292

Nadelhoffer K, Shaver G, Fry B, Giblin A, Johnson L, McKane R (1996) ^{15}N natural abundances and N use by tundra plants. Oecologia 107:386–394

Nasholm T, Persson J (2001) Plant acquisition of organic nitrogen in boreal forests. Physiol Plant 111:419–426

Nasholm T, Ekblad A, Nordin A, Giesler R, Hogberg M, Hogberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392:914–916

Nasholm T, Huss-Danell K, Hogberg P (2000) Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81:1155–1161

Nasholm T, Huss-Danell K, Hogberg P (2001) Uptake of glycine by field grown wheat. New Phytol 150:59–63

Neter J, Kutner MH, Nachtsheim CJ, Wasserman W (1996) Applied linear statistical models, 4th edn. McGraw-Hill, Chicago

Nordin A, Hogberg P, Nasholm T (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. Oecologia 129:125–132

Northup RR, Dahlgren RA, Zengshou Y (1995a) Intraspecific variation of conifer phenolic concentration on an marine terrace soil acidity gradient; a new interpretation. Plant Soil 171:255–262

Northup RR, Zengshou Y, Dahlgren RA, Vogt KA (1995b) Polyphenol control of nitrogen release from pine litter. Nature 377:227–229

Persson J, Hogberg P, Ekblad A, Hogberg M, Nordgren A, Nasholm T (2003) Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field. Oecologia 137:252–257

Powlson DS, Barraclough D (1993) Mineralization and assimilation in soil-plant systems. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques. Academic, San Diego, pp 209–242

Prescott CE, Weetman GF (1994) Salal Cedar Hemlock integrated research program: a synthesis. Faculty of Forestry, University of British Columbia, Vancouver

Preston CM (1999) Condensed tannins of salal (*Gaultheria shallon* Pursh.): a contributing factor to seedling "growth check" on northern Vancouver Island. In: Gross GG, Hemingway RW, Yoshida T (eds) Plant polyphenols 2: chemistry, biology, pharmacology, ecology. Basic life series, vol 66. Kluwer / Plenum, New York, pp 825–841

Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–390

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, vol 3. A lab manual. 2nd edn. Cold Spring Harbour Laboratory, New York

SAS Institute (1993) SAS/SYSTAT User's Guide. Rel. 6.07. SAS Institute, N.C.

Schimel JP, Chapin FSI (1996) Tundra plant uptake of amino acid and NH_4 nitrogen in situ: plants compete well for amino acid N. Ecology 77:2142–2147

Schmidt S, Stewart GR (1997) Waterlogging and fire impacts on nitrogen availability and utilization in a subtropical wet heathland (wallum). Plant Cell Environ 20:1231–1241

Schulze E-D, Chapin FSI, Gebauer G (1994) Nitrogen nutrition and isotope differences among life forms at the northern treeline of Alaska. Oecologia 100:406–412

Stewart GR, Pate JS, Unkovich M (1993) Characteristics of inorganic nitrogen assimilation of plants in fire-prone Mediterranean-type vegetation. Plant Cell Environ 16:351–363

Taiz L, Zeiger E (1991) Plant physiology. Benjamin/Cummings, Redwood City

Turnbull MH, Goodall R, Stewart GR (1995) The impact of mycorrhizal colonization upon nitrogen source utilization and metabolism in seedlings of *Eucalyptus grandis* Hill ex Maiden and *Eucalyptus maculate* Hook. Plant Cell Environ 18:1386–1394

Weetman GF, Fournier R, Barker J, Schnorbus-Panozzo E (1989) Foliar analysis and response of fertilized chlorotic western hemlock and western red cedar reproduction on salal-dominated cedar–hemlock cutovers on Vancouver Island. Can J For Res 19:1512–1520

Xiao G-P (1994) The role of root-associated fungi in the dominance of *Gaultheria shallon*. Dissertation, Department of Soil Science, University of British Columbia, Vancouver