

Intact amino acid uptake by northern hardwood and conifer trees

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Abstract Empirical and modeling studies of the N cycle in temperate forests of eastern North America have focused on the mechanisms regulating the production of inorganic N, and assumed that only inorganic forms of N are available for plant growth. Recent isotope studies in field conditions suggest that amino acid capture is a widespread ecological phenomenon, although northern temperate forests have yet to be studied. We quantified fine root biomass and applied tracer-level quantities of U- $^{13}\text{C}_2$ - ^{15}N -glycine, $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ in two stands, one dominated by sugar maple and white ash, the other dominated by red oak, beech, and hemlock, to assess the importance of amino acids to the N nutrition of northeastern US forests. Significant enrichment of ^{13}C in fine roots 2 and 5 h following tracer application indicated intact glycine uptake in both stands. Glycine accounted for up to 77% of total N uptake in the oak–beech–hemlock stand, a stand that produces recalcitrant litter, cycles N slowly and has a thick, amino

acid-rich organic horizon. By contrast, glycine accounted for only 20% of total N uptake in the sugar maple and white ash stand, a stand characterized by labile litter and rapid rates of amino acid production and turnover resulting in high rates of mineralization and nitrification. This study shows that amino acid uptake is an important process occurring in two widespread, northeastern US temperate forest types with widely differing rates of N cycling.

Keywords Amino acid · Nitrogen · Temperate forest · ^{15}N · ^{13}C

Introduction

Research on the nitrogen (N) cycle of temperate forests has traditionally focused on the mechanisms regulating the production and turnover of inorganic N (Binkley et al. 1986; Marschner 1995; Matson and Vitousek 1981; Nadelhoffer et al. 1985; Parton and Rasmussen 1994). This long-standing focus reflects the importance of inorganic N to the N nutrition of temperate forest species (Lajtha 1994; Nadelhoffer et al. 1984; Templer and Dawson 2004; Zerihun and Bassirirad 2001), an observation substantiated by strong correlations between the net rate of inorganic N production and forest productivity (Pastor et al. 1984; Reich et al. 1997).

With the development of isotopic methods, studies in arctic, alpine, and boreal ecosystems have applied double-labeled ($^{13}\text{C}/^{15}\text{N}$) amino acids in situ and found intact amino acid uptake in mycorrhizal and non-mycorrhizal plants in direct competition with soil microbes (McFarland et al. 2002; Näsholm et al. 1998; Nordin et al. 2001; Persson et al. 2003). This research suggests that in cold regions where slow rates of decomposition result in low supply rates

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of inorganic N, plants take up a significant fraction of their annual N requirement in the form of organic N (Kielland et al. 2006). By extension it has been argued that organic N uptake should be substantially reduced in warmer climates where decomposition is faster and microbial populations mineralize organic N more rapidly (Schimel and Bennett 2004). However, recent field studies conducted under varying climate and land use (Harrison et al. 2007; Hofmockel et al. 2007; Näsholm et al. 2000; Rains and Bledsoe 2007; Schmidt and Stewart 1999; Turnbull et al. 1996; Warren and Adams 2007) suggest that amino acid capture is a widespread ecological phenomenon. Although organic N is increasingly recognized as a major contributor to plant nutrition in many ecosystems, data for northeastern temperate forests are still lacking.

In previous research, we studied two widely distributed forest types in northeastern North America—a sugar maple and white ash forest type, and a red oak, beech and hemlock forest type. We found that N cycling was strongly influenced by the species composition of these stands because of individualistic variations in the litter chemistry (Finzi and Canham 1998; Finzi et al. 1998a, b; Talbot and Finzi 2008), and that amino acids serve as important substrates for mineralization and nitrification (Finzi and Berthrong 2005). Moreover, we found that surface organic horizons had among the highest concentrations of extractable amino acids ever measured (Berthrong and Finzi 2006), suggesting that soil horizon development may affect amino acid uptake. Finally, an excised-root study showed that the dominant tree species in these northern hardwood and mixed-hardwood conifer forests took up NH_4^+ significantly more rapidly than glycine, and in turn, that glycine was taken up more rapidly than NO_3^- (Finzi and Berthrong 2005). While this pattern was consistent between forest types, sugar maple- and white ash-dominated stands had significantly greater rates of inorganic N uptake than glycine uptake compared to the oak–beech–hemlock-dominated forests.

Although excised root studies may provide insights into the form of N taken up by plants growing in different ecosystems (Chapin et al. 2002), field studies are required to validate lab results, because of the many-fold factors affecting organic N cycling and availability in the soil. These factors include the composition and activity of the microbial community (Chalot and Brun 1998; Hodge et al. 2000), the chemistry of soil organic matter (Talbot and Finzi 2008), the mobility and concentration of different N forms (Owen and Jones 2001) and the mass and distribution of fine roots (Chapin et al. 2002). In situ experiments that include the full suite of plant–microbe–soil interactions are therefore required to assess amino acid uptake by trees in different forest types.

The primary objective of this study was to use in situ methods to quantify the importance of amino acid uptake

relative to NH_4^+ and NO_3^- uptake in two dominant forest types throughout northeastern North America. The secondary objective was to identify the factors responsible for variability in the form of N taken up from the soil. The northern hardwood and hardwood-conifer forests we studied here are well characterized in terms of species effects on soil processes (Finzi and Canham 1998; Finzi et al. 1998a, b; Talbot and Finzi 2008), and rates of N cycling including amino acid cycling (Berthrong and Finzi 2006; Finzi and Berthrong 2005). By working in these well-characterized systems, we were able to link variability in amino acid and inorganic N uptake to variability in the production and turnover of N in the soil in two contrasting forest–soil systems. To meet our research objective, we quantified the depth distribution of fine root biomass, the concentration of inorganic and amino acid N at each site, and applied double-labeled ($\text{U-}^{13}\text{C}_2/^{15}\text{N}$) glycine, and ^{15}N -enriched NH_4Cl or KNO_3 into the organic horizon and top 15 cm of mineral soil. We then measured the concentration of ^{13}C and/or ^{15}N in fine roots 2 and 5 h after tracer application.

Materials and methods

Study sites and soil sampling

This research was conducted in two forest types common to the northeastern US. Trees in the oak–beech–hemlock stand grow on glacial till overlying mica-schist bedrock. Because of the production of recalcitrant litter, this forest type is characterized by the development of a thick organic horizon above the mineral soil horizon (Berthrong and Finzi 2006). The concentration of amino acids in the organic horizon is nearly an order of magnitude higher than that in the top 15 cm of mineral soil (Berthrong and Finzi 2006). Gross and net rates of mineralization and nitrification are low (Finzi et al. 1998b; Finzi and Berthrong 2005). Trees in the sugar maple–white ash forest grow on glacial till overlying dolomitic bedrock. Rapid rates of litter decomposition and the occurrence of earthworms result in no surface organic horizon in these stands. By contrast to the oak–beech–hemlock stand, rates of amino acid production and turnover are significantly higher in the sugar maple–white ash stand, as are rates of gross and net mineralization and nitrification (Finzi and Berthrong 2005). Additional site and soil properties are presented in Table 1.

At each research site, we located four replicate 20×20 -m plots. We quantified fine root biomass (<2 mm) in each plot in July 2005. The organic horizon was sampled as a 10×20 -cm monolith. The mineral soil was sampled with a 5-cm diameter soil bulk density sampler to a depth of 60 cm in 15-cm increments.

Table 1 Selected properties of each study site (mean \pm 1 SE)

Property	Research site	
	Oak–beech–hemlock	Sugar maple–ash
Basal area (m ² ha ⁻¹)	44.8 (4.0)	40.0 (3.6)
Percent of basal area by species	<i>Fagus grandifolia</i> , 28% <i>Tsuga canadensis</i> , 23% <i>Quercus rubra</i> , 22% <i>Acer rubrum</i> , 21%	<i>Fraxinus americana</i> , 42% <i>Acer saccharum</i> , 27% <i>Carya ovata</i> , 9% <i>Populus grandidentata</i> , 9%
Soil texture		
% Sand	44.7 (3.2)	46.6 (1.6)
% Silt*	45.1 (1.7)	35.2 (1.5)
% Clay*	10.2 (2.8)	18.3 (1.1)
pH		
O Horizon		
0–15 cm*	3.76 (0.13)	5.69 (0.10)
Organic horizon mass (g m ⁻²)	4265 (490)	–
Bulk density (g cm ⁻³)		
0–15 cm*	0.72 (0.04)	0.95 (0.05)
15–30 cm*	0.99 (0.03)	1.11 (0.06)
Total C (g m ⁻²)		
O Horizon	1607 (185)	–
0–15 cm*	6199 (433)	5782 (509)
Total N (g m ⁻²)		
O Horizon	81 (9)	–
0–15 cm*	348 (24)	494 (44)
Extractable N pools ($\mu\text{g g soil}^{-1}$) ^a		
Organic horizon		
Amino acid N	49.4 (3.94)	–
NH ₄ ⁺ -N	8.6 (1.00)	–
NO ₃ ⁻ -N	1.1 (0.14)	–
Mineral soil (0–15 cm depth)		
Amino acid N*	3.5 (0.36)	2.3 (0.27)
NH ₄ ⁺ -N	1.3 (0.10)	1.2 (0.13)
NO ₃ ⁻ -N*	0.40 (0.08)	1.6 (0.23)

* $P < 0.05$ (significant differences between sites)

^a Each value is the mean of seven sample dates, including the results from this study and from Berthrong and Finzi (2006)

Immediately after coring, the samples were weighed and then sieved through 2-mm mesh in the field. The concentration of amino acid N, NH₄⁺, and NO₃⁻ in each sample was extracted in 150 ml of 2 M KCl from root-free subsamples of the mineral soil horizon (50 g) and O horizon (25 g; Shepherd et al. 2001). The remainder of the sieved soil sample was transported back to the laboratory on ice, and quantitatively root picked. All the roots were then carefully washed and dried at 60°C to determine the total fine root biomass.

The concentration of NH₄⁺-N in each sample was measured by the phenolate method on an autoanalyzer

(Lachat Quickchem 8000; Zellweger Analytics, Milwaukee, Wis.). The concentration of NO₃⁻-N was measured by the cadmium reduction method (Lachat Quickchem 8000). The concentration of amino acid N in each sample was measured by reaction with ninhydrin (Rosen 1957), and comparing the optical absorbance of the samples at 570 nm relative to a leucine standard curve, correcting for interference by NH₄⁺ (Berthrong and Finzi 2006).

In August 2005 we conducted an isotope-labeling experiment in the field. Within each plot, four randomly selected sampling points received an injection of either double-labeled (U-¹³C₂/¹⁵N) 98% atom-enriched glycine,

98% atom-enriched $^{15}\text{NH}_4\text{Cl}$, 98% atom-enriched K^{15}NO_3 , or water (control). The O-horizon was injected from its boundary with the mineral soil horizon upwards at 50 points within a 10×20 -cm sampling grid. Each point received an 0.8-ml aliquot of labeling solution. The total volume of labeling solution applied to the O-horizon was 40 ml. The mineral soil horizon was injected to a depth of 15 cm using a coring-injection grid, made of a 20×20 -cm piece of Lexan (modified from McFarland et al. 2002). The coring grid consisted of a single, centralized 5.5-cm, outside-diameter hole through which a soil corer could fit. The coring grid was fastened to the soil by four no. 16 galvanized nails. The injection template was the 5.5-cm-diameter piece of Lexan cut from the coring grids with 13 separate injection points. Two milliliters of labeling solution was delivered at each injection point using a syringe inserted at 15-cm depth. As the needle was removed from the soil, the syringe was progressively emptied.

To avoid any fertilization effect, we added the ^{13}C and ^{15}N at tracer levels, increasing the concentration of the background pool of N no more than 10% and enriching to ~ 2.0 atom% ^{15}N . In the O horizon, the total quantity of ^{15}N applied was 0.54, 0.21, and 0.03 $\mu\text{g } ^{15}\text{N g soil}^{-1}$ for the glycine, NH_4^+ and NO_3^- treatments, respectively. In the mineral soil horizon, the total quantity of ^{15}N applied was 0.11, 0.07, and 0.05 $\mu\text{g } ^{15}\text{N g soil}^{-1}$ for the glycine, NH_4^+ and NO_3^- treatments, respectively. The quantity of ^{13}C applied in the glycine treatment was 0.94 and 0.19 $\mu\text{g } ^{13}\text{C g soil}^{-1}$ in the organic and the mineral soil horizons, respectively. Given the rapid turnover time of amino acids in soil (Finzi and Berthrong 2005; Jones and Hodge 1999), we sampled 2 and 5 h following isotope addition with the expectation that these time intervals would allow us to observe intact uptake if it indeed occurred.

Immediately after coring, the samples were weighed, and then sieved through 2-mm mesh in the field. The fine roots were removed from the sieve, rinsed in water for 3 min, rinsed in 1 mmol l^{-1} KCl for 3 min to remove adsorbed ^{15}N , and frozen in liquid N to prevent further metabolism of the amino acids. These frozen samples were returned to the laboratory, oven dried at 60°C , weighed and ground under liquid nitrogen with a mortar and pestle. The remainder of each soil sample was refrigerated, brought back to Boston University, and thereafter all the roots and rocks were removed by hand, oven dried and weighed. Total fine root biomass was calculated as the sum of the root biomass frozen in liquid nitrogen and the remaining fine root biomass of the soil core. The bulk density of each horizon was calculated after determining the water content and the mass of roots and rocks in each sample.

Given that the glycine treatment contained very little ^{13}C relative to background C pools in the root (Bennett and Prescott 2004; Nordin et al. 2001), we extracted the soluble

C fraction within the root (i.e., amino acids, peptides, proteins) and measured the recovery of ^{13}C in this fraction to determine whether intact glycine uptake had occurred (Näsholm et al. 1998). In brief, the soluble C fraction was extracted as follows: 70 mg of dried ground-root samples was weighed in 15-ml centrifuge tubes, extracted with 1 ml of 10 mM phosphate buffer (pH 8), and centrifuged at 3,700 r.p.m. for 20 min. The supernatant was decanted and stored, and the extraction process was repeated with an additional 1 ml of buffer. The two supernatants were combined, filtered, and the total volume was reduced to 100 μl in a reduced pressure evaporator (Savant Speed Vac). The reduced extracts were pipetted into 9×10 -mm tin capsules containing two Whatman GF/D filter punches that had been ashed previously at 510°C for 5 h. The sample then was placed in a desiccator overnight. All the samples were sent for analysis of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, %N, and %C on a mass spectrometer (Europa Integra, Cheshire, UK) at the University of California, Davis.

Under the assumption that the spacing of the injection points within the injection grid allowed for the diffusion of the labeling solutions throughout the entire 5-cm-diameter \times 15-cm-depth volume of soil with no tracer loss, we used the isotope mixing model presented in (McKane et al. 2002) to calculate the uptake of N per unit of root mass (U_{N} ; $\mu\text{g N g root}^{-1} \text{ h}^{-1}$) from the soil. The mass-specific rate of N uptake depends upon knowing the rate at which each labeled form of N was taken up from the soil, and isotope dilution of each form due to variably sized pools of N in the soil. The uptake of ^{15}N after 2 and 5 h ($U_{15\text{N}}$; $\mu\text{g } ^{15}\text{N g root}^{-1} \text{ h}^{-1}$) was calculated as the product of the N content of fine roots and the atom% ^{15}N excess of the bulk fine root sample. For each soil horizon, the concentration of ^{15}N applied ($C_{15\text{N label}}$; $\mu\text{g } ^{15}\text{N g soil}^{-1}$) was calculated by dividing the total quantity of ^{15}N applied (volume applied in $\text{ml} \times$ concentration of labeling solution in $\mu\text{g } ^{15}\text{N ml}^{-1}$) by the weight of each soil core (g soil). The dilution of the isotope was accounted for by dividing the concentration of each N form in the soil ($C_{\text{available N}}$; $\mu\text{g N g soil}^{-1}$), by the concentration of the applied label ($C_{15\text{N label}}$; $\mu\text{g } ^{15}\text{N g soil}^{-1}$). When tracer quantities of label are applied, there is, in theory, no perturbation of the system, and the ratio $^{15}\text{N}/^{14}\text{N}$ of the N taken up by trees is assumed to be the same as the $^{15}\text{N}/^{14}\text{N}$ ratio of available N in the soil. Hence the mass-specific rate of N uptake for each form was calculated according to:

$$U_{\text{N}} = U_{15\text{N}} \times \frac{C_{\text{available N}}}{C_{15\text{N label}}}$$

To calculate the total quantity of N taken up from the soil for each form separately, we multiplied the mass-specific rate of N uptake (i.e., U_{N}) for each form by the biomass of the fine roots in each plot. The percent

contribution of each N form to total N uptake was calculated as the total quantity of a particular form taken up from the soil divided by the uptake rate of all forms combined. This calculation is valid under the assumption that: (1) the uptake of different forms was unaffected by the addition of the label, which is likely in this case given the low levels of tracer application; (2) that there was no difference in the transfer of the different N forms out of the roots toward the shoot over the 2- and 5-h time frame; and (3) that glycine was representative of the total amino acid pool extracted by 2 M KCl, and that all amino acids were equally taken up by trees.

Statistical analyses

In order to account for any correlation between depths at given locations, a mixed model was used to test for the effect of forest type and soil depth on the biomass of fine roots and the concentration of amino acid N, NH_4^+ , and NO_3^- in the soil, with site, depth and site \times depth as fixed effects, and sampling plot as a random effect. To test for the effect of tracer form (glycine, NH_4^+ , NO_3^-), forest type and sampling time on the recovery of ^{13}C in the soluble C fraction and ^{15}N in bulk fine roots, the uptake of different N forms from the soil, and the total quantity of N taken up from the soil, we used a mixed model with tracer form and time as fixed effects, and tracer nested in plot as a random effect. Because the sugar maple–white ash stand had no O horizon the forest type variable in the isotope-labeling experiment had three levels combining both site and soil horizon information (i.e., sugar maple–white ash mineral soil, oak–beech–hemlock organic horizon, and oak–beech–hemlock mineral soil). Both data sets were analyzed in SAS (version 9; SAS Institute, Cary, N.C.) with the MIXED

procedure. The Tukey's adjustment was used for comparisons of least square means at the $P < 0.05$ level. We log-transformed the data when they did not conform to assumptions of normality and homogeneity of variance.

Results

Fine root biomass was higher ($P < 0.05$) in the oak–beech–hemlock stand than in the sugar maple–white ash stand (Fig. 1a). Fine root biomass decreased with depth in the soil ($P < 0.0001$), but the decrease was significantly greater ($P = 0.01$) in the sugar maple–white ash forest. Amino acid and inorganic N concentrations were highest in the O horizon and decreased with depth ($P < 0.0001$; Fig. 1b, c). Amino acid concentrations were higher in the top 15 cm of mineral soil of the oak–beech–hemlock stand than the sugar maple–white ash stand ($P < 0.05$), whereas the opposite was true for inorganic N ($P = 0.001$, Table 1). The total quantity of amino acid N to 60-cm depth was slightly higher in the oak–beech–hemlock stand than in the sugar maple–white ash stand, but this result was not statistically significant (Fig. 1b). The total quantity of inorganic N was higher in the sugar maple–white ash stand ($P < 0.05$; Fig. 1c).

Excess ^{13}C was not detected in bulk-root samples (data not shown); the label was masked by the large concentration of C in root tissues. By contrast, the soluble C pool in the fine roots was significantly enriched in ^{13}C at both sites ($P < 0.05$) and all horizons 2 h following tracer application (Fig. 2). After 5 h, the soluble C pool was enriched in ^{13}C in the top 15 cm of mineral soil but not in the O horizon (Fig. 2). ^{15}N was readily detected in bulk, fine root samples. ^{15}N enrichment was significantly higher 2 and 5 h

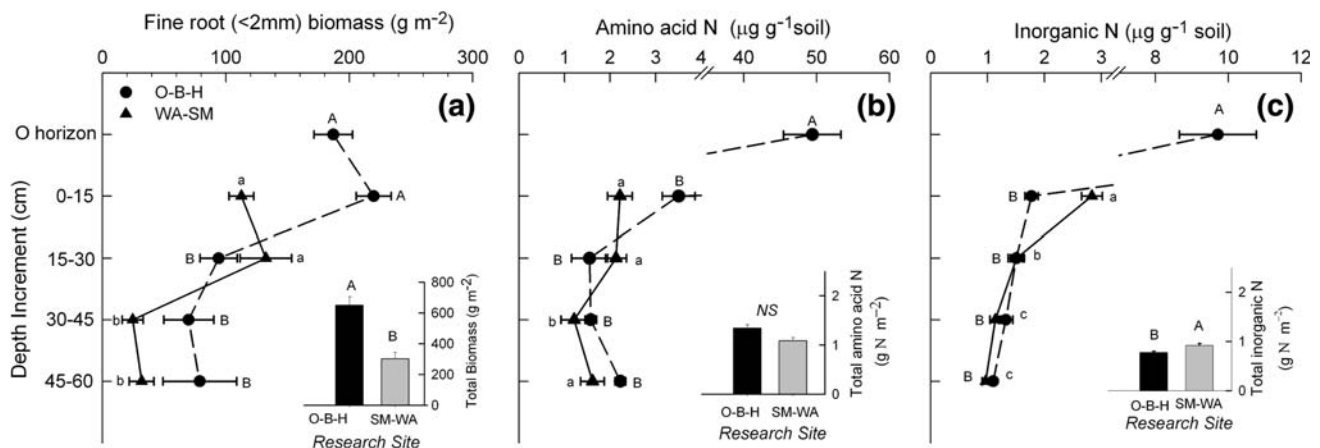


Fig. 1 Depth distribution of (a) fine roots, (b) amino acid N concentrations, and (c) inorganic N concentrations in the different horizons of the oak–beech–hemlock (O–B–H) and white ash–sugar maple (WA–SM) stands. Different capital letters indicate significant

($P < 0.05$) differences among depths in the O–B–H stand, and different lower-case letters indicate significant ($P < 0.05$) differences in the WA–SM stand. Bar charts plot total fine root biomass (a), and the quantity of amino acid N (b) and inorganic N (c) in the profile

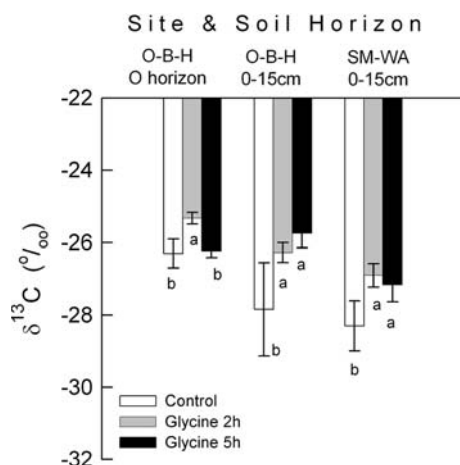


Fig. 2 Mean (± 1 SE) $\delta^{13}\text{C}$ in the soluble C fraction of the fine roots in the glycine treatment 2 and 5 h following tracer application in the organic horizon at the O-B-H stand and in the mineral soil horizons at the O-B-H and WA-SM stands. Different capital letters indicate a significant ($P < 0.05$) difference in tracer enrichment within a particular site and horizon. For abbreviations, see Fig. 1

following the application of glycine ($P < 0.05$) and NH_4^+ ($P < 0.01$) tracers at both sites in both horizons (Fig. 3a, b). The application of $^{15}\text{NO}_3^-$ only increased ^{15}N enrichment of the fine roots in the sugar maple–white ash stand ($P = 0.01$; Fig. 3c).

In the O horizon of the oak–beech–hemlock forest, mass-specific rates of glycine uptake ($\mu\text{g N g root}^{-1} \text{h}^{-1}$) were significantly higher than NH_4^+ uptake rates which, in turn, were significantly greater than NO_3^- uptake rates ($P < 0.001$; Fig. 4a). In the top 15 cm of mineral soil, mass-specific rates of glycine and NH_4^+ uptake were comparable but both were significantly greater than NO_3^- uptake ($P < 0.01$; Fig. 4b). By contrast, in the sugar maple–white ash stand mass-specific rates of NO_3^- uptake

($P < 0.01$) were significantly greater than glycine uptake rates, with mass-specific NH_4^+ uptake rates intermediate between these two forms ($P < 0.05$; Fig. 4c). In general, there was a decrease in the mass-specific rate of N uptake for all forms between the 2- and the 5-h sampling.

Averaging across the 2- and 5-h sampling times, glycine accounted for 77% of total N uptake ($\mu\text{g N m}^{-2} \text{h}^{-1}$) from the O horizon of the oak–beech–hemlock stand, with NH_4^+ and NO_3^- accounting for 22 and 1%, respectively (Fig. 4d–f). In the mineral soil of the oak–beech–hemlock stand, glycine and NH_4^+ accounted for 48 and 45% of total N uptake, respectively, while NO_3^- uptake only accounted for 7% of total N uptake. In the mineral soil of the sugar maple–white ash stand, NO_3^- accounted for 47% of total N uptake whereas NH_4^+ and glycine accounted for 33 and 20% of total N uptake, respectively (Fig. 4d–f). Total N uptake was significantly higher ($P < 0.05$) 2 h after labeling than after 5 h for each form of N (Fig. 4d–f).

Discussion

The objective of this study was to quantify the importance of amino acid uptake relative to NH_4^+ and NO_3^- uptake in two hardwood–conifer forests differing in rates of N cycling. Canopy trees at both research sites took up the amino acid glycine in direct competition with the microbial community (Figs. 2, 3, 4). The total quantity of glycine taken up in the slow N cycling oak–beech–hemlock stand was significantly greater than that taken up in the fast N cycling sugar maple–white ash stand, particularly in the surface organic horizon where glycine accounted for $\sim 77\%$ of total N uptake (Fig. 4). In the sugar maple–white ash stand NO_3^- and NH_4^+ dominated uptake with glycine accounting for only 20% of total N uptake (Fig. 4).

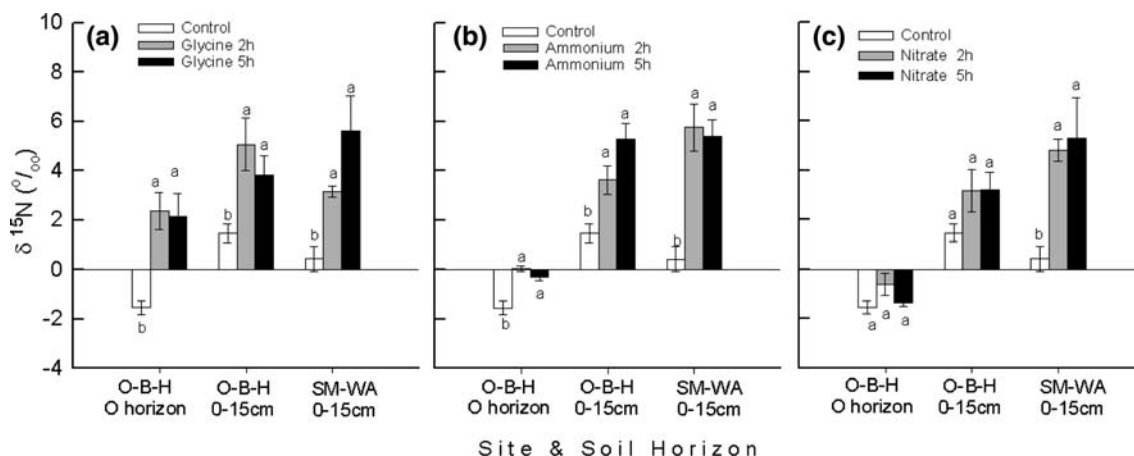


Fig. 3 Mean (± 1 SE) $\delta^{15}\text{N}$ in bulk fine roots of the (a) glycine, (b) NH_4^+ , and (c) NO_3^- treatments 2 and 5 h following tracer application at both sites. Different capital letters indicate a significant ($P < 0.05$)

difference in tracer enrichment within a particular site and horizon. For abbreviations, see Fig. 1

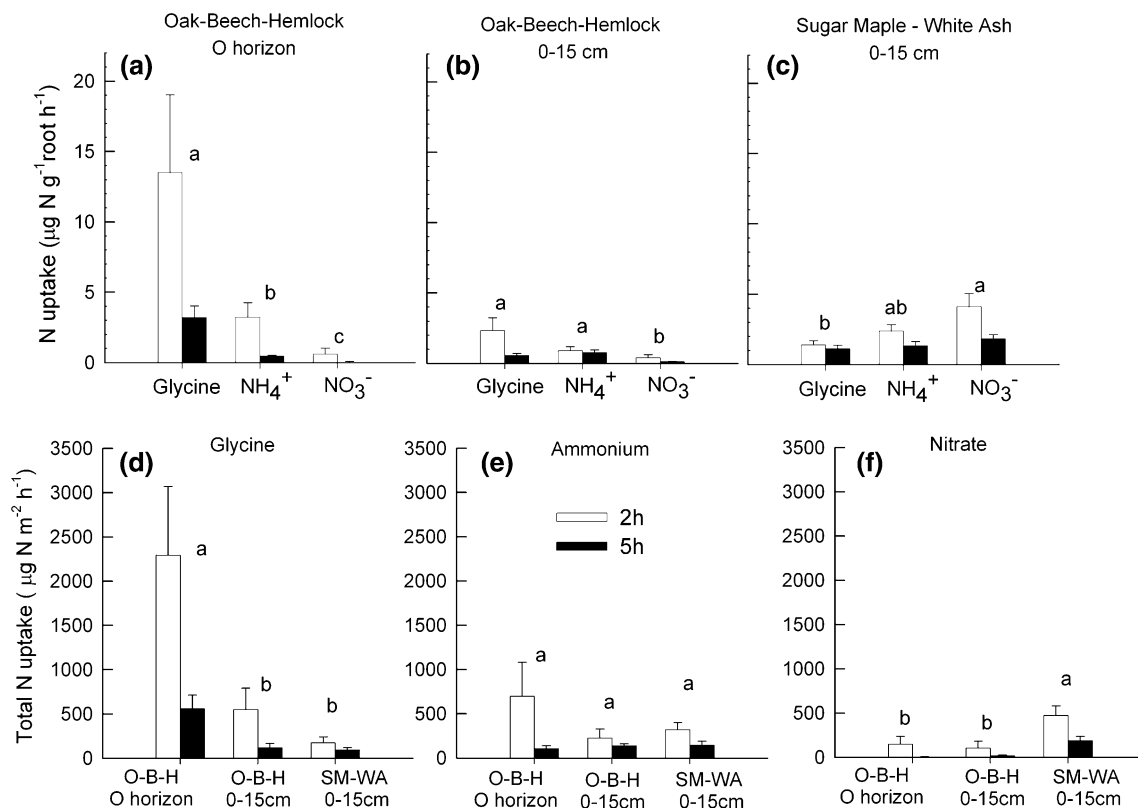


Fig. 4 The mass-specific rate of N uptake ($\mu\text{g N g}^{-1} \text{root h}^{-1} \pm 1$ SE) as glycine, NH_4^+ , and NO_3^- in the (a) organic horizon of the O-B-H stand, and in the top 15 cm of mineral soil in the (b) O-B-H and (c) WA-SM stands, in addition to the total quantity of N taken up from the soil ($\mu\text{g N m}^{-2} \text{h}^{-1} \pm 1$ SE) as (d) glycine, (e)

NH_4^+ and (f) NO_3^- 2 and 5 h following tracer addition. **a–c** Bars with *different letters* indicate a significant ($P < 0.05$) difference in the rate of N uptake among forms. **d–f** Bars with *different letters* indicate differences in the total quantity of each N form taken up across sites and soil horizons. For abbreviations, see Fig. 1

The results presented here suggest that soil horizon development may affect organic N uptake in temperate forests, and if so, implies an important link between the chemistry of tree species litter and the form of N taken up from the soil (Northup et al. 1995). The oak–beech–hemlock stand is characterized by a relatively thick O horizon that is not present in the sugar maple–white ash stand. Oak, beech, and hemlock trees produce litter with significantly higher lignin content and higher C-to-N ratios than sugar maple and white ash trees (Aber and Melillo 1982; Finzi and Canham 1998). As a consequence, leaf litter decomposition is slow and litter accumulates above the mineral soil horizon. The O horizon in this stand also has very high concentrations of extractable amino acids (Berthrong and Finzi 2006; Fig. 1c), thereby maximizing the potential for amino acid uptake (Jones et al. 2005b). In the sugar maple–white ash stand by contrast, leaf litter and soil organic matter decomposition are significantly more rapid (Talbot and Finzi 2008), as are rates of mineralization and nitrification (Finzi and Berthrong 2005), both of which result in high concentrations of inorganic N in the mineral soil horizon and the apparent dominance of inorganic N uptake (Fig. 4f). Whether soil horizon development is important to

organic N uptake in all temperate forest soils is currently unknown. Additional work on organic N uptake in other sites will be necessary to confirm the observations presented here.

To quantify in situ uptake from two functionally distinct soil horizons, we had to make three assumptions. First, we labeled soil cores assuming no loss of the tracer, a reasonable assumption given the spacing within the injection grids and the small volume of water associated with tracer application. Since we did not measure tracer recovery in the different soil solution N pools, however, we cannot exclude the possibility of some loss. If so, the tracer dilution associated with loss will result in an underestimation of actual uptake rates (i.e., $\text{C}_{15\text{N}}$ label would be smaller if losses occurred). This is most likely to be the case for NO_3^- which is mobile in soil water, and unlikely for NH_4^+ which is rapidly sorbed by soil components (Owen and Jones 2001). Second, we applied the tracer to a small volume of soil that prevented us from recovering ^{13}C and/or ^{15}N in bolewood and foliage. In the absence of measurements of tracer recovery in other parts of the tree, we assumed that there was no difference in the translocation of the three different N forms out of the roots. Two recent

studies show that the transport of NO_3^- out of roots is faster than either amino acid or NH_4^+ transfer (Persson et al. 2006; Schmidt and Stewart 1999). Consequently, the actual quantity of NO_3^- taken up could be greater than that measured (i.e., $U_{15\text{N}}$ for NO_3^-). Because the relative contributions of glycine, NH_4^+ and NO_3^- uptake depend upon the total quantity of N taken up (i.e., the sum of total glycine, NH_4^+ and NO_3^- uptake), greater losses and faster translocation of NO_3^- out of roots may overestimate the relative contribution of glycine and NH_4^+ . Third, we chose the single amino acid glycine as a tracer for all free amino acids extracted by 2 M KCl, mainly because glycine is in general present in large quantities in soils and easily taken up by plants (Lipson and Näsholm 2001). However, many amino acids are not as available to plants as glycine because of sorption to soil (i.e., amino acids with net positive charge; Jones and Hodge 1999) and because microbes prefer other organic sources of N owing to the low C content of glycine (Schimel and Bennett 2004). Despite these assumptions and methodological limitations, the substantial differences between stands and soil horizons in the form of N uptake suggests that small to moderate bias in our estimate of uptake is unlikely to alter the main conclusion that organic matter chemistry and soil horizon development play important roles in regulating the contribution of glycine to N uptake in temperate forests.

In an attempt to create ecologically realistic conditions, we applied tracer quantities of 98 at.% ^{13}C and/or ^{15}N -enriched forms to the soil system, increasing background pools of N by 1–11%. The advantage to applying tracer-quantities of N is avoiding any fertilization effect that could artificially increase plant capture of amino acid N or saturate microbial uptake thereby leaving more amino acid N for plant uptake (Jones et al. 2005a). Accordingly, the increase in background pools of N in this study are substantially lower than that applied in most studies with enrichments ranging from 20% to greater than 1000% (Harrison et al. 2007; Kielland et al. 2006; McFarland et al. 2002; Näsholm et al. 1998; Nordin et al. 2004; Persson et al. 2003). The drawback to this approach is that the ^{13}C label in the amino acid labeling study was easily swamped by the background ^{13}C in the root.

We found significantly elevated concentrations of ^{13}C in the soluble C fraction of the fine roots in the glycine-treated samples at both sites, and in both horizons after 2 h, and at both sites after 5 h in the mineral soil horizon (Fig. 2). Typically, intact uptake is documented by observing a similar ratio of ^{13}C -excess to ^{15}N -excess in the roots and in the labeled compound (i.e., 2:1 for glycine; Näsholm et al. 1998). A slope with a ratio different than 2:1 would indicate some transformation of glycine before or after its uptake by the roots. The ^{13}C -excess to ^{15}N -excess ratio of the soluble C pool in this study ranged from 2 to 19, much

wider than the expected 2:1 ratio, indicating that the soluble C extraction did not recover all the excess ^{15}N in the root. The soluble-C-pool- ^{13}C -excess to bulk-root- ^{15}N -excess ratio ranged from 0.6 to 3.8, much closer to the expected 2:1 ratio, and similar to that observed in other studies (e.g., McFarland et al. 2002; Nordin et al. 2001), but depends on two different ways of measuring isotopes in the roots (i.e., soluble ^{13}C , bulk root ^{15}N). Losses of ^{13}C occur as a result of root and microbial metabolism (Näsholm et al. 1998; Schmidt and Stewart 1999) where two molecules of glycine are rapidly decarboxylated and converted to serine. The translocation of serine from roots to shoots can also affect the ^{13}C recovery in roots (Schmidt and Stewart 1999). Furthermore, some of the glycine that is taken up by microbes can be deaminated resulting in the release of ^{15}N to the soil solution and the retention or respiration of ^{13}C (Barraclough 1997; Finzi and Berthrong 2005). Alone, or in combination, these processes could have affected isotope recovery in our experiment, but they would tend to decrease the ratio of ^{13}C -excess to ^{15}N -excess in roots, not widen the ratio as was observed here. Despite the uncertainty in the ^{13}C -to- ^{15}N ratio of the root, the significant enrichment of ^{13}C in the soluble C pool provides strong support for intact amino acid uptake; there is no other mechanism that we are aware of that could cause such an enrichment of ^{13}C in the root.

In previous research, we found that excised roots of the tree species at these sites took up significantly more NH_4^+ than glycine, and significantly more glycine than NO_3^- (Finzi and Berthrong 2005). In contrast to the excised-root study, there was substantially more glycine and NO_3^- uptake observed in the field. In particular, NO_3^- uptake dominated all other forms of N uptake in the soil in the sugar maple–white ash stand (Fig. 4a), whereas glycine dominated N uptake in the oak–beech–hemlock stand (Fig. 4c). The difference in the form of N capture between the field and lab study may reflect the mobility of the different N forms in the soil. In a temperate grassland soil, Owen and Jones (2001) found that NO_3^- diffused through soil water more rapidly ($0.282 \text{ cm}^2 \text{ day}^{-1}$) than either glycine ($0.0078 \text{ cm}^2 \text{ day}^{-1}$) or NH_4^+ ($0.0023 \text{ cm}^2 \text{ day}^{-1}$), because glycine and NH_4^+ tended to be sorbed by negatively charged clay particles and soil organic matter (Barber 1995). Consistent with ion mobility as a factor controlling N uptake, we found that the slope of the relationship between N uptake and the concentration of N in the soil was significantly greater for NO_3^- (slope = 658.7, $r^2 = 0.35$, $P < 0.01$, $n = 24$) than for glycine (slope = 69.3, $r^2 = 0.56$, $P < 0.0001$, $n = 24$). The steeper slope for NO_3^- indicates that uptake is more strongly concentration dependent, presumably because sorption in the soil has little effect on the mobility of this ion. By contrast, the smaller slope for glycine suggests that sorption decreases

glycine availability in the soil following depolymerization. Importantly, there was no correlation between NH_4^+ uptake and the concentration of NH_4^+ in the soil, suggesting strong physicochemical control over NH_4^+ availability in temperate forest soils that are known to have a high cation exchange capacity. The interactive effects of production and sorption on the form of N taken up from the soil are an important avenue for future research (Lipson et al. 2001).

Mycorrhizal fungi play a critical role in transferring soil-derived N to plants in return for photoassimilates (Read and Perez-Moreno 2003). To date, much of the emphasis on the capture of organic N has focused on ectomycorrhizal (ECM) fungi because of their known proteolytic capability (Chalot and Brun 1998) and their transfer of N to host plants in culture studies (Abuzinadah and Read 1989; Leake and Read 1990). In contrast to many ECM fungi, arbuscular-mycorrhizal (AM) fungi lack proteolytic capacity, and they are thought to be more important in the capture and transfer of inorganic nutrients to host plants (Smith and Read 1997). In this study, we found intact amino acid uptake in stands dominated by trees associating with both ECM fungi (oak, beech, and hemlock) and AM fungi (sugar maple and white ash), although uptake in the AM trees was much lower (Fig. 4). Several species of grasses and shrubs that associate with AM fungi have also been shown to take up intact amino acids (Näsholm et al. 2000; Nordin et al. 2001; Persson et al. 2003; Rains and Bledsoe 2007). Although these results suggest that AM fungi might play a role in organic N uptake, there is currently no information available to determine which part of the root, the arbuscule within the root or the arbuscule-free portion of the root, was involved in organic N capture.

Research conducted in the northeastern US has figured prominently in the development of the classic model for soil N cycling in which microbial activity controls N availability and plants take up only inorganic N (e.g., Melillo 1981; Nadelhoffer et al. 1985). Positive correlations between the rate of net N mineralization and net primary production, the flux of N in litterfall, the rate of N uptake from the soil, and N-use efficiency have reinforced the idea that inorganic N alone accurately reflects N availability and the form of N uptake (Nadelhoffer et al. 1985; Pastor et al. 1984; Reich et al. 1997). Based on these observations, biogeochemical models have been parameterized with an inorganic N economy for the N nutrition of trees (Aber et al. 1997; Comins and McMurtrie 1993; Melillo et al. 1993; Parton and Rasmussen 1994; Rastetter et al. 1997). This study shows that temperate forest trees take up the intact amino acid glycine, in direct competition with the microbial community, with variability in the degree of uptake resulting from differences in root biomass

(Fig. 1), rate of production (Finzi and Berthrong 2005) soil-horizon development (Fig. 4), and ion mobility. To accurately characterize the availability of a key, growth-limiting resource in northern hardwood-conifer forests, future empirical and modeling studies should be reformulated to allow organic N uptake from the soil.

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