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ECOSYSTEM ECOLOGY - ORIGINAL PAPER

# Amino acid uptake by temperate tree species characteristic of low- and high-fertility habitats

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**Abstract** The relationship between inorganic nitrogen (N) cycling and plant productivity is well established. However, recent research has demonstrated the ability of plants to take up low molecular weight organic N compounds (i.e., amino acids) at rates that often rival those of inorganic N forms. In this study, we hypothesize that temperate forest tree species characteristic of low-fertility habitats will prefer amino acids over species characteristic of high-fertility habitats. We measured the uptake of $^{15}$N-labeled amino acids (glycine, glutamine, arginine, serine), ammonium ($NH_4^+$), and nitrate ($NO_3^-$) by four tree species that commonly occur in eastern North America, where their abundances have been correlated with inorganic N availability. Specific uptake rates of amino acids were largely similar for all tree species; however, high-fertility species took up $NH_4^+$ at rates more than double those of low-fertility species, rendering amino acid N relatively more important to the N nutrition of low-fertility species. Low-fertility species acquired over four times more total N from arginine compared to $NH_4^+$ and $NO_3^-$; high-fertility species acquired the most N from $NH_4^+$. Arginine had the highest uptake rates of any amino acid by all species; there were no significant differences in uptake rates of the remaining amino acids. Our results support the idea that the dominant species in a particular habitat are those best able to utilize the most available N resources.

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## Introduction

Nitrogen (N) is a fundamental component of plant tissues, and is often considered to be the nutrient that most limits plant growth. As a result, there has been considerable research across a wide range of terrestrial ecosystems that has been devoted to studying how this element is made available to plants. Until relatively recently, the majority of this work has focused on the cycling of inorganic forms of N (i.e., ammonium and nitrate), assuming that they represented the form most available to plants (Pastor et al. 1984; Zak et al. 1986, 1989). However, more recent research has demonstrated the ability of plants to take up low molecular weight organic N compounds, primarily free amino acids, at rates that often rival those of ammonium ($NH_4^+$) and nitrate ($NO_3^-$) (Kielland 1994; Schimel and Chapin 1996; Näsholm et al. 2000; Näsholm and Persson 2001; Thornton and Robinson 2005). Therefore, organic N is a potentially important pool of plant-available N that has largely been overlooked in previous studies relating soil N cycling with plant productivity.

In an attempt to incorporate organic N into conceptual models of soil N cycling, Schimel and Bennett (2004) proposed that the predominant form of plant-available N would shift from organic N to $NH_4^+$-N to $NO_3^-$-N as the overall N cycling rate of a habitat increased. This new model emphasizes the microbial depolymerization of large organic polymers into bioavailable, N-containing monomers (e.g., amino acids, amino sugars) as the key process in the soil N cycle, rather than N mineralization. The shift in emphasis away from N mineralization assumes that plants

can compete successfully with microbes for organic N when mineralization is constrained. This pattern has largely been born out in high latitude/altitude ecosystems where cold temperatures limit N mineralization and organic matter accumulates in soils (Kielland 1994, 1995; Schimel and Chapin 1996; Raab et al. 1999; Nordin et al. 2001; Öhlund and Näsholm 2001). In these systems, free amino acids dominate pools of potentially available N in habitats where inorganic N is scarce (Kielland 1994, 1995; Raab et al. 1999; Nordin et al. 2001). Moreover, there are numerous studies documenting the ability of boreal, arctic, and alpine plants to take up free amino acids (Kielland 1994; Schimel and Chapin 1996; Näsholm et al. 1998; Raab et al. 1999). Interestingly, there is evidence of covariation between the availability of amino acid versus inorganic N in soil and the physiology of the dominant plant species in these cold climate ecosystems. For example, the dominance of different plant species in tussock tundra habitats was related to their ability to utilize the largest pool of available N in a particular habitat, be it inorganic or organic (McKane et al. 2002). Nordin et al. (2001) found that plant species growing in a low-fertility habitat along a boreal forest N-fertility gradient had 30% of their total N uptake fulfilled by glycine, whereas in the high-fertility habitat, glycine uptake was only 10% of total plant N uptake. Correspondingly, the largest N pool at the low-fertility site comprised amino acid N (always at least 70% of the total N pool), whereas amino acid N was never more than 19% of the total N at the high-fertility site. This trend was reversed with $NO_3^-$, where both plant uptake of $NO_3^-$ and pool sizes were largest at the high-fertility site. The authors speculated that the change in plant species composition across the gradient might be a function of particular species' ability to acquire different forms of N.

There has been less work in temperate ecosystems investigating the availability of amino acids across the landscape and the role these compounds may play in plant N nutrition. However, there is growing evidence that amino acid pools are relatively more abundant in temperate forests where N mineralization rates are low (Finzi and Berthrong 2005; Gallet-Budynek et al. 2009; Rothstein 2009), providing a pool of potentially plant-available N in what were previously considered low-fertility habitats. Also, a few studies have shown that temperate trees have the ability to use amino acid N (Bennett and Prescott 2004; Hofmockel et al. 2007; Warren and Adams 2007). In an attempt to link plant nutrition with patterns of amino acid versus inorganic N availability, Finzi and Berthrong (2005); Gallet-Budynek et al. (2009) compared root uptake of $^{15}N$-labeled amino acids among several forest ecosystems and found, in general, that roots from sites with slower rates of N mineralization had a higher $^{15}N$ content than those from more fertile sites. Neither study identified the roots to species, though, so it is unclear how different species of plants varied in their use of amino acid versus inorganic forms of N. However, oak–beech–hemlock forests dominated the low-fertility sites of both studies, while sugar maple–white ash forest dominated the high-fertility sites, suggesting that the shift in uptake rates of glycine by roots was at least partly driven by species replacement.

A large number of the studies evaluating plant uptake of amino acids have used glycine as their "test" amino acid due to its low molecular weight, relative ease of mobility in the soil compared to other amino acids (Owen and Jones 2001), and poor substrate quality for microbial growth (Lipson et al. 1999). However, soluble N pools in the soil contain a wide variety of different amino acids with varying abundances and molecular structures (Kielland 1995; Yu et al. 2002; Rothstein 2009). While glycine is often one of the dominant free amino acids in field soil solutions, ranging from approximately 5 to 20% of total free amino acids depending on the ecosystem (Kielland 1995; Rothstein 2009), other amino acids can occur in equal or greater concentrations (Senwo and Tabatabai 1998; Raab et al. 1999), suggesting that glycine uptake potential alone does not necessarily represent plant access to amino acid N pools. Plants have been shown to take up a variety of other amino acids (Kielland 1994; Öhlund and Näsholm 2001; Persson and Näsholm 2001), sometimes to a greater extent than glycine (Weigelt et al. 2005), suggesting that studies evaluating amino acid uptake should use multiple amino acids when trying to determine whether or not amino acid N is important for plant nutrition.

The objective of this study was to test the hypothesis that temperate deciduous forest species that are characteristic of low-fertility sites would exhibit a greater preference for amino acids than for species characteristic of high-fertility sites. We used tree species that commonly occur in eastern North American forests, where it has been well documented that tree species composition is correlated with patterns of inorganic N pools in repeatable assemblages across the landscape (Host et al. 1988; Zak et al. 1986, 1989; Finzi et al. 1998). These predictable associations make this region an ideal study area to investigate whether or not amino acid N availability might also influence tree species dynamics, especially since recent studies have shown that gradients of free amino acids in soil run opposite to those for inorganic N (Gallet-Budynek et al. 2009; Rothstein 2009). Our research compared the uptakes of four amino acids, $NH_4^+$, and $NO_3^-$ by seedlings of red oak (*Quercus rubra*), American beech (*Fagus grandifolia*), white ash (*Fraxinus americana*), and black cherry (*Prunus serotina*). We hypothesized that red oak and American beech would take up amino acids to a greater extent than black cherry and white ash because the former two species are most often found in habitats with relatively

slow rates of N mineralization and abundant pools of amino acids (Host et al. 1988; Zak et al. 1986; Rothstein 2009). Conversely, we hypothesized that black cherry and white ash would prefer inorganic forms of N because they are most abundant in habitats with rapid ammonification and nitrification as well as smaller pools of free amino acids. Finally, we hypothesized that the distribution of N within seedlings would depend on the form in which it was taken up. Specifically, we hypothesized that $NO_3^-$-N would most likely be found in leaves due to its ability to bypass assimilation into organic molecules in the roots (Andrews 1986); that $NH_4^+$-N would predominantly reside in roots; and that the distribution of amino acid-derived N would vary depending on the metabolic roles of individual amino acids.

## Methods

### Plant establishment

In order to determine whether or not there were differences in how northern hardwood trees use amino acids versus inorganic N forms, we germinated seeds of red oak, American beech, white ash, and black cherry obtained from F.W. Schumacher Co., Inc., Sandwich, MA, USA (41.759°N, 70.494°W), and supplied them with $^{15}$N-enriched substrates. The seeds were soaked overnight in tap water and stratified at 5°C for 30–90 days, depending on the species' requirements. Prior to sowing, all seeds were surface sterilized in a 10% sodium hypochlorite solution for 30 s, rinsed in deionized (DI) water, and allowed to air dry. The seeds were sown in a peat, vermiculite, and perlite growth medium (Faford #2) in 10 × 36 cm pots; the potting medium contained a starter supply of nutrients, including inorganic N. All pots were placed in a greenhouse at natural light levels. The average high temperature in the greenhouse over the course of the experiment was 26.5°C, while the average low temperature was 18.3°C. Two weeks after germination, each germinant began receiving 250 ml of a fertilizer solution containing a cocktail of 0.6 mM arginine, 2.5 mM glycine, 2.5 mM serine, and 1.3 mM glutamine twice per week for approximately 19–21 weeks (depending on the species); amino acid concentrations were varied so that each supplied an equimolar amount of N to the fertilizer solution. We used amino acids in our fertilizer solution to ensure that amino acid uptake by our seedlings would not be limited by a predisposition towards inorganic N forms (Henry and Jefferies 2003). The macronutrient concentrations in the solution followed a modified Hoagland's solution (Hoagland and Arnon 1938): 2.0 mM $CaSO_4$, 1.0 mM $Ca(H_2PO_4)_2$, 2.5 mM $K_2SO_4$, 2.0 mM $MgSO_4$. The micronutrient makeup was: 46.3 μM $H_3BO_3$, 9.0 μM $MnSO_4{\cdot}H_2O$, 0.8 μM $ZnSO_4{\cdot}7H_2O$, 0.3 μM $CuSO_4{\cdot}5H_2O$, 0.02 μM $(NH_4)Mo_7O_{24}{\cdot}4H_2O$, 12.0 μM Fe EDTA. At the time of our labeling experiment, the seedling biomass averaged 7.17 g for red oak (range: 2.96–14.97), 2.45 g for American beech (range: 1.56–3.50), 1.93 g for white ash (range: 0.46–6.86), and 10.40 g for black cherry (range: 3.78–15.95).

### Plant labeling

To conduct our labeling experiment, the tree seedlings were carefully removed from the potting medium, and their roots were gently washed with DI water; a visual inspection of the roots after washing suggested that mycorrhizal infection was either absent or limited in scope. Once clean, the roots were immediately sterilized by submersion in a 10% sodium hypochlorite solution for 30 s (Reissinger et al. 2001), followed by a rinse in a 0.5 mM $CaCl_2$ solution and a second rinse in reverse osmosis water. We wanted to measure the specific uptake of N when there was competition between inorganic and amino acid N to capture any inhibitory effects of one N species on another that might influence plant uptake (Thornton and Robinson 2005). Therefore, each seedling's root system was submerged in 750 mL of a solution containing 300 μM $L^{-1}$ of ammonium nitrate ($NH_4NO_3$), 300 μM $L^{-1}$ of one of four amino acids (i.e., glycine, serine, glutamine, or arginine), and 0.5 mM of $CaCl_2$ (to maintain membrane integrity), although only one N species was enriched in $^{15}$N per treatment (Table 1). We selected the 300 μM $L^{-1}$ concentration for our labeled substrates to ensure that N uptake by the seedlings would not be substrate limited over the course of our labeling exercise (Henry and Jefferies 2003). We used the L-isomer instead of the D-isomer of each amino acid since they are more prevalent in soils (Lipson and Näsholm 2001). In most cases, each treatment per species had 5 replicate seedlings, although the glutamine and $NO_3^-$ treatments for American beech and the $NO_3^-$ treatment for black cherry only had 4; white ash had only 4 replicates in each treatment due to lower seedling survival. We included an additional treatment of universally labeled glycine (U-$^{13}C_2$, $^{15}$N-glycine) in order to verify that our experimental setup would allow for the uptake of intact amino acids by the tree species selected; plant tissues enriched in $^{13}$C would indicate that the C skeleton of the amino acid was taken up concomitant with the N group (Näsholm et al. 1998). The seedlings were suspended for 50 min in aerated solutions that had been adjusted to pH 5.5 (a typical surface-soil pH for northern hardwood forests in our region) with 0.1 N hydrochloric acid or sodium hydroxide as appropriate. The control solution contained only $CaCl_2$.

After labeling, each seedling's root system was soaked in a 5.0 mM $CaCl_2$ solution for 5 min to remove any $^{15}$N

**Table 1** Labeling solution compositions and the chemical formulae of the labeled N forms. The labeled N forms in each solution are highlighted in bold

| Solution number | Solution name | Inorganic N | Organic N | Molecular formula |
|---|---|---|---|---|
| 1 | Ammonium | $^{15}\mathbf{NH_4}NO_3$ (98% $^{15}$N) | Glycine | $NH_4$ |
| 2 | Nitrate | $NH_4^{15}\mathbf{NO_3}$ (98% $^{15}$N) | Glycine | $NO_3$ |
| 3 | Glycine | $NH_4NO_3$ | **$^{15}$N-glycine** (98% $^{15}$N) | $C_2H_5NO_2$ |
| 4 | Glutamine | $NH_4NO_3$ | **$^{15}$N-glutamine** (98% amide $^{15}$N) | $C_5H_{10}N_2O_3$ |
| 5 | Arginine | $NH_4NO_3$ | **$^{15}$N-arginine** (98% guanido $^{15}$N) | $C_6H_{14}N_4O_2$ |
| 6 | Serine | $NH_4NO_3$ | **$^{15}$N-serine** (98% $^{15}$N) | $C_3H_7NO_3$ |
| 7 | Dual-glycine | $NH_4NO_3$ | **U-$^{13}C_2$, $^{15}$N-glycine** (98% $^{13}$C, $^{15}$N) | $C_2H_5NO_2$ |

adsorbed to the exterior of the roots (Persson and Näsholm 2001). The seedling roots were rinsed thoroughly in reverse osmosis water before the plant was separated into above- and belowground organs, frozen in liquid nitrogen, and stored frozen until further processing. Tissues were later oven dried at 65°C for 48 h (dual-labeled glycine plants were lyophilized) and separated into fine and coarse roots, shoots, and leaves. Dried plant material was weighed, ground first with a mortar and pestle then a ball mill, and analyzed for $^{15}$N and $^{13}$C content on a Europa Integra continuous flow isotope ratio mass spectrometer at the Stable Isotope Facility, University of California at Davis.

### Calculations and statistical analyses

The atom% excess of plant organs was determined by subtracting the mean $^{15}$N and $^{13}$C abundances of unlabeled plants from those supplied with enriched compounds. We determined the quantity of tracer $^{15}$N or $^{13}$C in each organ by multiplying the atom% excess by the total moles of N or C. These values were summed for each plant and expressed on a $g^{-1}$ of fine root $h^{-1}$ basis to account for variations in plant size and root:shoot ratios. To examine the partitioning of $^{15}$N throughout the seedlings, we calculated the percentage of $^{15}$N tracer found in different plant organs (leaves, stems, coarse roots, fine roots) as the amount of tracer $^{15}$N in each tissue divided by the whole plant $^{15}$N tracer and multiplied by 100.

We used general linear models to determine if there were significant differences in the uptake rates of the four amino acids, $NH_4^+$, and $NO_3^-$ within a tree species. The models consisted of specific uptake rate as the dependent variable and the labeled N species as the independent variable. We used pairwise *t* tests with a Bonferroni correction to compare differences in uptake rates between specific N species. The specific uptake rates for all tree species were log transformed to meet assumptions of normality. We used analysis of covariance (ANCOVA) to determine whether or not there were different distribution patterns of $^{15}$N tracer in fine roots and leaves across our treatments within each tree species. An ANCOVA approach allowed us to account for the influence of total plant $^{15}$N uptake on the $^{15}$N tracer found in specific organs (Atchley et al. 1976). We focused on fine roots and leaves because they were the most likely to capture differences in the metabolism and partitioning of our substrates. The amount of $^{15}$N tracer (μg) in each organ was the independent variable, the N form was the main effect, and the total plant $^{15}$N tracer (μg) was the covariate. We initially modeled our data with an interaction term between N form and total plant $^{15}$N tracer, but it was never significant; therefore, our final models contained only main effects. $^{15}$N tracer of specific organs and total plant $^{15}$N tracer were log transformed when appropriate to ensure a normal distribution of residuals. Finally, we compared the $\delta^{13}$C of fine roots in tree seedlings labeled with U-$^{13}C_2$,$^{15}$N-glycine to our unlabeled controls using *t* tests within each species. For black cherry, we used a Welch's *t* test because our dual-labeled and unlabeled seedlings had unequal variances. We used linear regressions within each tree species to determine whether plants fed U-$^{13}C_2$, $^{15}$N-glycine took up the amino acid intact (Näsholm et al. 2000). Our models consisted of excess $^{15}$N (per gram of fine root tissue) as the independent variable and excess $^{13}$C (per gram of fine root tissue) as the dependent variable. All of our analyses were accepted as significant at $\alpha = 0.05$ and were conducted using R statistical software (R Development Core Team 2008).

## Results

All tree species examined were able to take up the full complement of N forms offered, although there were significant differences in specific uptake rates depending on the N form. Red oak and American beech took up $NH_4^+$ and arginine at significantly higher rates than the remaining N forms (ANOVA, oak: $F_{5,24} = 21.39$, $P < 0.001$; beech: $F_{5,22} = 26.08$, $P < 0.001$; Fig. 1a). There were no significant differences in specific uptake rates among glutamine, serine, glycine, or $NO_3^-$. White ash and black cherry had significantly faster specific uptake rates for $NH_4^+$ compared to any other N form (ANOVA, ash: $F_{5,18} = 22.30$, $P < 0.001$; cherry: $F_{5,23} = 22.32$, $P < 0.001$). There were

no significant differences in specific uptake rates among the amino acids or $NO_3^-$ for black cherry, although white ash took up arginine significantly faster than $NO_3^-$. Overall, our high-fertility species had specific uptake rates for $NH_4^+$ that were over 5 times faster than for our low fertility species. When we evaluated the total amount of N contributed by each $^{15}$N-enriched N form to our seedlings, arginine provided red oak and American beech with significantly more N than any other N form (ANOVA, red oak: $F_{5,24} = 55.82$, $P < 0.001$; beech: $F_{5,22} = 56.68$, $P < 0.001$; Fig. 1b). $NH_4^+$-N still provided white ash and black cherry with their greatest source of N, although the contrast between $NH_4^+$ and arginine was not statistically significant for black cherry (ANOVA, ash: $F_{5,18} = 129.24$, $P < 0.001$; cherry: $F_{5,23} = 26.61$, $P < 0.001$). Among the remaining amino acids, glutamine-N supplied our seedlings with the most N, although the differences were not always significant. Across all tree species, the average specific uptake rates decreased as the molecular weight increased for glycine, serine, and glutamine, but then increased to the highest specific uptake rates for arginine, the heaviest amino acid (Fig. 2).

The distribution of $^{15}$N between fine roots and leaves varied significantly across our treatments; however, not all species demonstrated the same $^{15}$N distribution patterns. There were no significant differences in leaf $^{15}$N across all N forms for red oak (ANCOVA, $F_{5,23} = 0.42$, $P = 0.83$) or American beech (ANCOVA, $F_{5,21} = 0.22$, $P = 0.95$), and only 11 and 3% of total $^{15}$N were found in the leaves of these species, respectively (Fig. 3a, b). However, white ash and black cherry had significantly more $^{15}$N in leaves from the $^{15}NO_3^-$ treatment compared to most other treatments (ANCOVA, ash: $F_{5,17} = 4.73$, $P = 0.01$; cherry: $F_{5,22} = 13.40$, $P < 0.001$), with an average of 38 and 33% of total $^{15}$N found in leaves, respectively (Fig. 3c, d). In fine roots, the $^{15}$N-arginine and $^{15}NH_4^+$ treatments had significantly

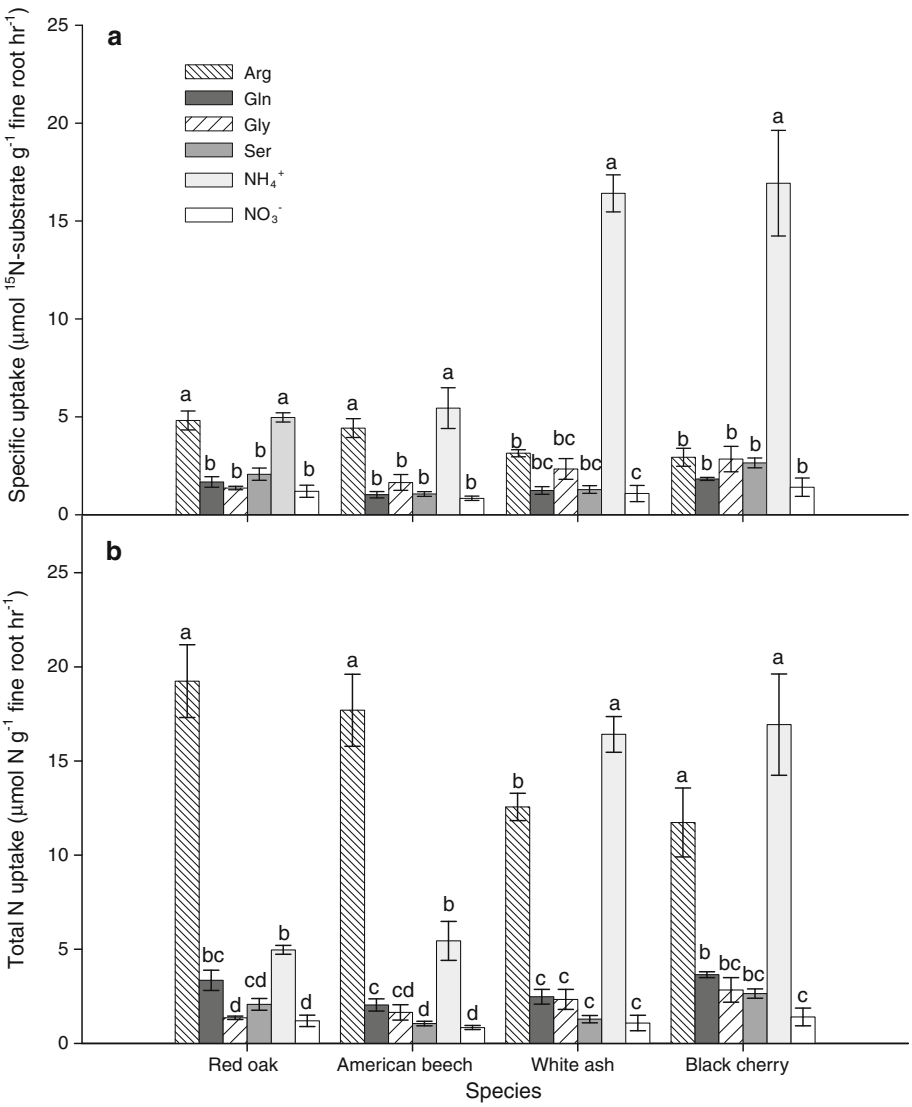


**Fig. 1** **a** Specific uptake rates (mean ± 1SE) and **b** total N uptake rates (mean ± 1SE) of $^{15}$N-enriched amino acids, $^{15}NH_4^+$, and $^{15}NO_3^-$, for red oak (*Quercus rubra*, $n = 30$), American beech (*Fagus grandifolia*, $n = 28$), white ash (*Fraxinus americana*, $n = 24$), and black cherry (*Prunus serotina*, $n = 29$). *Bars with the same letter(s)* are not significantly different from each other. *Arg* Arginine, *Gln* glutamine, *Gly* glycine, *Ser* serine, $NH_4^+$ ammonium, $NO_3^-$ nitrate

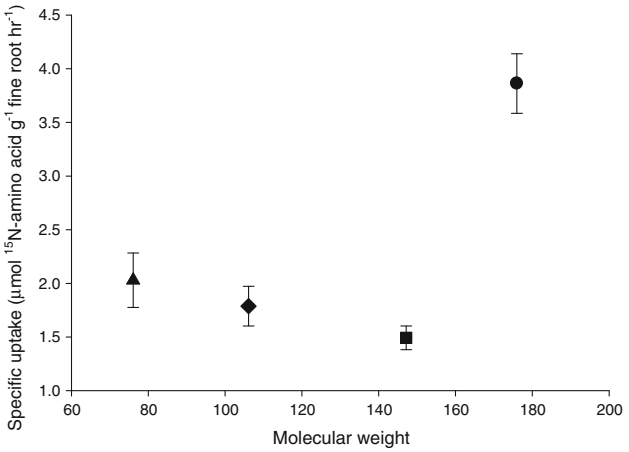


**Fig. 2** Specific uptake rates (mean ± 1SE, $n = 75$) of glycine (*triangle*), serine (*diamond*), glutamine (*square*), and arginine (*circle*) averaged across species as a function of amino acid molecular weight

more $^{15}N$ compared to most other N forms for all species except black cherry, where only the $^{15}NH_4^+$ treatment was significantly higher than the rest (ANCOVA, oak: $F_{5,23} = 31.27$, $P < 0.001$; beech: $F_{5,21} = 113.27$, $P < 0.001$; ash: $F_{5,17} = 36.85$, $P < 0.001$; cherry: $F_{5,22} = 43.76$, $P < 0.001$). The distribution of the $^{15}N$ tracer between above- and belowground organs was the most disparate for the two inorganic N forms we supplied our seedlings: in the $^{15}NO_3^-$ treatment, roughly 20, 16, 62, and 45% of the $^{15}N$ tracer was found in aboveground organs for red oak, American beech, white ash, and black cherry, respectively, while only 3, 5, 9, and 2% of the $^{15}N$ tracer was found in aboveground tissues for the $^{15}NH_4^+$ treatment, respectively. The percentages of $^{15}N$ in aboveground organs from the amino acid treatments were generally intermediate between the $^{15}NO_3^-$ and $^{15}NH_4^+$ treatments and decreased in the following order: $^{15}N$-glutamine > $^{15}N$-glycine = $^{15}N$-serine > $^{15}N$-arginine in all tree species.

The fine roots of red oak, American beech, white ash, and black cherry seedlings supplied with U-$^{13}C_2$, $^{15}N$-glycine were significantly enriched in $^{13}C$ compared to unlabeled seedlings (oak: $t = 4.08$, $df = 11$, $P < 0.01$; beech: $t = 5.42$, $df = 12$, $P < 0.01$; ash: $t = 5.05$, $df = 10$, $P < 0.01$; cherry: $t = 7.00$, $df = 9.58$, $P < 0.01$). Linear regressions of $^{15}N$ and $^{13}C$ for whole plants were not significant (data not shown). However, when the $^{15}N$ content of fine roots was regressed against $^{13}C$ content of fine roots, red oak ($t = 3.69$, $df = 6$, $P < 0.05$) and American beech ($t = 4.53$, $df = 8$, $P < 0.01$) had significant linear relationships that corresponded to a minimum of 78 and 51% of the glycine taken up intact, respectively (Fig. 4a, b). White ash and black cherry had weak linear relationships between $^{15}N$ and $^{13}C$ content (ash: $t = 1.52$, $df = 5$, $P = 0.19$; cherry: $t = 1.33$, $df = 7$, $P = 0.23$; Fig. 4c, d).

## Discussion

In this study, we evaluated the role amino acids played in the N nutrition of temperate hardwood tree species

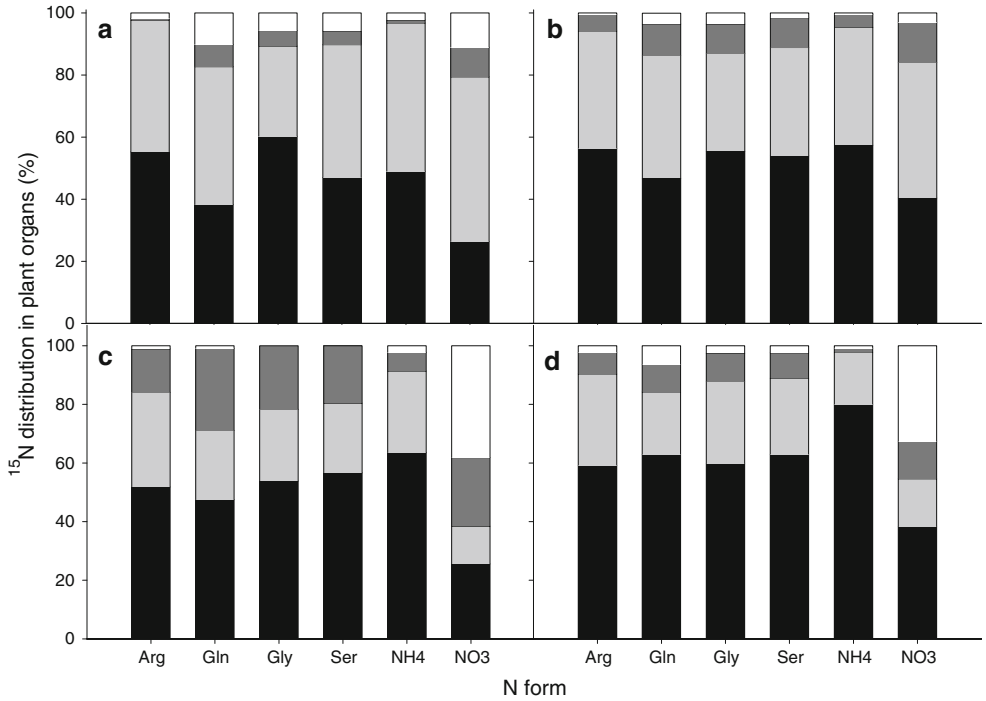


**Fig. 3** The proportion of $^{15}N$ tracer found in coarse roots (*black bars*), fine roots (*light gray bars*), stems (*dark gray bars*), and leaves (*white bars*) for each N-form treatment within a species. **a** Red oak, **b** American beech, **c** white ash, and **d** black cherry. See Fig. 1 for abbreviations

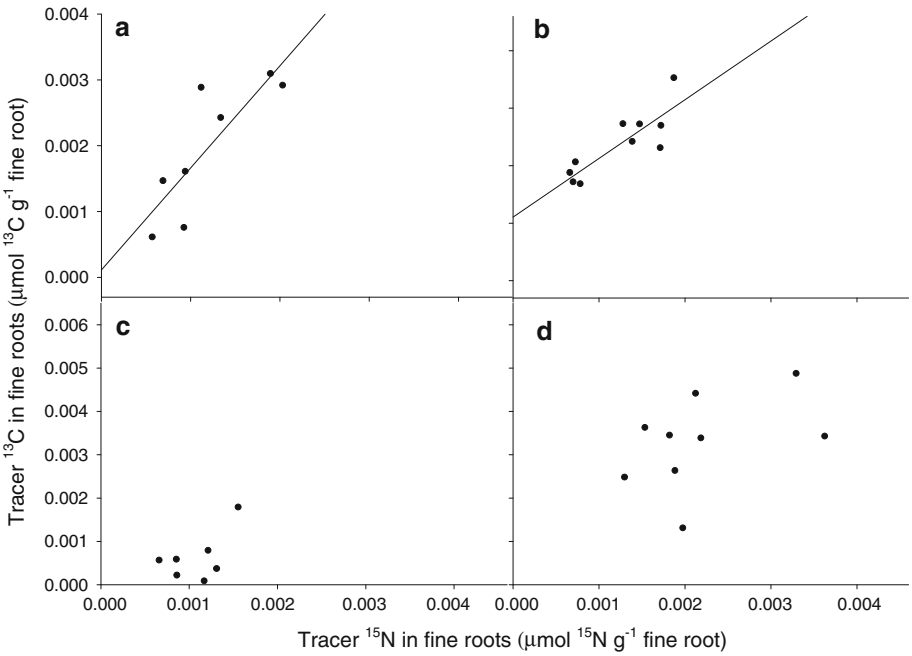


**Fig. 4** Linear regressions of fine root tracer $^{15}$N on fine root tracer $^{13}$C for **a** red oak ($y = 1.55x + 0.0001$, $r^2 = 0.70$, $n = 8$), **b** American beech ($y = 1.02x + 0.0011$, $r^2 = 0.72$, $n = 10$), **c** white ash, and **d** black cherry. Regressions for white ash and black cherry were not statistically significant

characteristic of habitats with either low or high inorganic N availability. We hypothesized that the uptake of amino acids by each tree species would reflect the N fertility of the habitats in which they were typically found, with low-fertility species (i.e., red oak and American beech) taking up amino acid N at greater rates than high-fertility species (i.e., white ash and black cherry), which would prefer inorganic N. Contrary to our hypothesis, specific uptake rates of amino acids were largely similar for all tree species (Fig. 1a). However, patterns of $NH_4^+$ uptake were consistent with our hypothesis; high-fertility species took up $NH_4^+$ at rates more than double those of the low-fertility species. The disparity between the rates of $NH_4^+$ uptake between our low- and high-fertility species rendered amino acid N relatively more important to the N nutrition of low-fertility species. Amino acid uptake was between 27–97% (red oak) and 19–81% (American beech) of their respective $NH_4^+$ uptake rates for the low-fertility species, whereas it was between 8–19% (white ash) and 11–17% (black cherry) for high-fertility species. When we evaluated the total amount of N taken up from each N form, our results provided additional evidence supporting a greater role for amino acids in the N nutrition of low-fertility species. Red oak and American beech acquired over four times more N from arginine compared to $NH_4^+$ and $NO_3^-$; white ash and black cherry acquired the most N from the $NH_4^+$ treatment, although there was no significant difference between arginine-N and $NH_4^+$-N for white ash (Fig. 1b). Additionally, glutamine supplied an equal amount of N to red oak as $NH_4^+$. All of our tree species were supplied with identical labeling solutions within a treatment, so our results reflect inherent species differences rather than uptake patterns based on the availability of substrate. Therefore, the similar patterns of N-form uptake between species from low inorganic N habitats and similar patterns from those in high inorganic N habitats suggest they were physiologically adapted to access the more prevalent pool of plant-available N in their respective habitats, in agreement with studies from other ecosystems (McKane et al. 2002; Nordin et al. 2001; Weigelt et al. 2005). These patterns of N-form uptake by our trees support Schimel and Bennett's (2004) model of N biogeochemistry, which predicts that plants will be more likely to access amino acid pools in habitats where N mineralization is low and free amino acid pools are high. While this model has largely been based on data from cold climates, our study suggests it is also applicable to temperate ecosystems. The ability of the low-fertility species to take up amino acids (Figs. 1, 4), combined with the presence of large pools of amino acids in low-fertility stands (Gallet-Budynek et al. 2009; Rothstein 2009), suggest that some habitats may not be as depleted in plant-available N as previously thought based solely on measures of inorganic N. In Northern Michigan, free amino acid N comprised 31% of the total pool of plant-available N in forest stands where red oak and American beech dominate, providing an N source that is generally lacking in stands where N mineralization rates are high (free amino acid N = 2% of total N pool in high-fertility stands; Rothstein 2009). Conversely, N mineralization rates in stands where white ash and black cherry occur are close to 127.8 µg N g$^{-1}$: roughly two and a half times faster than stands where red oak and American beech are prevalent (Zak et al. 1989). The greater availability of $NH_4^+$ in these stands, and the paucity of amino acids, would make $NH_4^+$

a more available source of N for white ash and black cherry to access.

For three of the four amino acids we supplied to our seedlings, there was a decreasing trend of uptake rate with amino acid molecular weight (Fig. 2). This relationship was realized among the neutral amino acids with glycine uptake > serine uptake > glutamine uptake. However, arginine, a basic amino acid, was taken up at the highest rates by all tree species, despite being the heaviest amino acid. The basic nature of arginine may have contributed to its distinct uptake pattern in two ways: first, the positive charge on arginine's side chain could have increased its attraction to negatively charged root surfaces (Haynes 1980) compared to the other amino acids, thereby facilitating its uptake. This mechanism may also explain why $NH_4^+$ was taken up at such high rates by all tree species. Second, different genes regulate membrane transporters for basic amino acids than neutral amino acids (Tanner and Caspari 1996; Näsholm and Persson 2001), with the lysine histidine transporter 1 (LHT1) and amino acid permease 1 (AAP1) responsible for neutral and acidic amino acid transport, and amino acid permease 5 (AAP5) responsible for basic amino acid transport (reviewed by Näsholm et al. 2009). Membrane transporters for neutral amino acids have broad substrate affinities that synchronously regulate the transport of amino acids across membranes (Näsholm and Persson 2001), which may explain the nonsignificant differences in the specific uptake rates of glycine, serine, and glutamine (Fig. 1). In this instance, bioenergetics would support lighter molecules being preferentially transferred across membranes, resulting in higher specific uptake rates for smaller molecules, in agreement with our average uptake pattern for neutral amino acids (Fig. 2). This interpretation is consistent with the results of Harrison et al. (2007), who found that low molecular weight compounds were taken up at greater rates by grassland plants due to their relative ease of transport across cellular membranes.

The relatively high specific uptake rates of arginine by all species, combined with the high N content of arginine (i.e., 4 N atoms per molecule), suggest it may be an important source of amino acid N for plants. However, most studies evaluating plant uptake of amino acids use glycine to determine whether or not amino acid N is important to plant nutrition (i.e., Näsholm et al. 2000; Weigelt et al. 2005; Finzi and Berthrong 2005; Gallet-Budynek et al. 2009). Based on our results, glycine uptake rates alone could underestimate the overall importance of amino acid N to plants. All of our tree species had the highest specific uptake rates for arginine, sometimes taking it up 3.5 times faster than glycine (i.e., red oak; Fig. 1a). Also, the total amount of N contributed to our trees by arginine was 4–14 times greater than the amount of N supplied by glycine (Fig. 1b). Persson and Näsholm (2001) also found significantly higher rates of arginine uptake compared to glycine by *Pinus sylvestris* in an experiment similar to ours. Whether or not the patterns we found between glycine and arginine uptake are realized in the field is unclear. In a natural environment, trees must compete for amino acids with microorganisms and abiotic soil sorption processes (Jones and Hodge 1999; Rothstein 2010). Glycine diffuses relatively easily through soil to root surfaces because of its low molecular weight and neutral charge (Owen and Jones 2001); it is also a poor substrate for microbial growth (Lipson et al. 1999), making it an ideal amino acid for plant uptake. Arginine, however, has slower diffusion rates due to its relatively high molecular weight and positive charge, which increases its propensity to sorb to negatively charged soil colloids (Owen and Jones 2001; Weigelt et al. 2005), although Öhlund and Näsholm (2001) suggested that this same phenomenon might reduce potential losses. Arginine is also readily mineralized by a variety of microorganisms in forest soil (Alef and Kleiner 1986; Lin and Brookes 1999). These characteristics may reduce plant access to arginine in the field in favor of glycine.

The uptake rates of amino acids and inorganic N forms measured in our study represent gross uptakes by our tree species and do not account for the potential efflux of these compounds from roots. As a result, the uptake rates found in this study may overestimate the importance of an N form in plant N nutrition if efflux rates are high. In particular, glycine and serine have demonstrated relatively high rates of efflux compared to influx in several agriculturally important plant species (Lesuffleur et al. 2007). Plant species vary in their tendency to exude different N forms (Kronzucker et al. 2003, Lesuffleur et al. 2007), suggesting that the different uptake patterns of N forms across our species may change when efflux is considered. However, in a literature review that addressed this question, Näsholm et al. (2009) found that the efflux of amino acids did not significantly detract from conclusions of amino acid absorption by plants in gross labeling studies.

At the end of our labeling period, the distribution of $^{15}N$ in our seedlings followed our predictions, with all species partitioning the most $^{15}N$ into aboveground organs in the $^{15}NO_3^-$ treatment, the least in the $^{15}NH_4^+$ treatment, and intermediary levels in the $^{15}N$-amino acid treatments (Fig. 3). Other studies evaluating plant uptake of inorganic and amino acid N-forms have also found a greater distribution of $NO_3$-N in leaves compared to $NH_4$-N and amino acid N, citing a faster transport of $NO_3^-$ out of roots compared to amino acids or $NH_4^+$ (Persson et al. 2006). $NO_3^-$ can be transported directly from roots to other plant organs before being assimilated into organic compounds via nitrate reductase and the glutamine synthetase/glutamate synthase (GS/GOGAT) system (Andrews 1986). Conversely, $NH_4^+$

must be assimilated in roots via the GS/GOGAT system into glutamine before it can be transported throughout a plant.

In contrast to $NH_4^+$ and $NO_3^-$, amino acids are already in a form that is immediately usable by plants and therefore are not necessarily metabolized into other compounds before being transported within a plant. For example, glutamine acts as a vector for transporting N assimilated in roots to N sinks, such as leaves, and is prevalent in phloem and xylem (Lam et al. 1996; Miller and Cramer 2004). Its primary role in N transport from roots to aboveground organs was evident in our data by the relatively high percentage of $^{15}N$ in aboveground organs in this treatment for all tree species. Arginine, however, is primarily a storage amino acid (Rosnitschek-Schimmel 1985; Staswick 1994), which may explain the prevalence of $^{15}N$ in roots and its near absence in leaves in this treatment compared with the others. Additionally, our labeling period occurred in late summer, when the seedlings may have begun storing reserves for their winter dormancy, also contributing to the predominance of arginine in belowground organs. Glycine and serine were the most similar of the amino acids we examined—both being small, hydrophilic molecules with low C:N (glycine, 2:1; serine, 3:1). They are involved in similar metabolic processes and are readily interconvertible (Cossins and Sinha 1966), which may explain their similar $^{15}N$ distributions in all species. Our study was limited to examining only short-term distribution patterns of N forms within seedlings. Therefore, it is unclear whether or not the differences we found in N-form distribution will be maintained over longer periods of time.

Although the overall distribution patterns of $^{15}N$ in our seedlings from the various N forms were similar for all species, there were distinct differences in the proportion of $^{15}N$ in above- versus belowground organs from the $^{15}NO_3^-$ treatment between our species from high- versus low-N habitats. White ash and black cherry had approximately 62 and 45% of $^{15}N$ from the $NO_3^-$ treatment in aboveground organs, respectively, compared to only 20% for red oak and 16% for American beech (Fig. 3). Plant species from high-N environments tend to assimilate most of their $NO_3^-$ in aboveground organs, while those from low-N environments assimilate $NO_3^-$ in belowground organs (Andrews 1986), in agreement with our results. These differences would likely be greater in the field, where $NO_3^-$ assimilation would also be dependent on substrate availability; greater external $NO_3^-$ concentrations can result in an increase in shoot $NO_3^-$ assimilation (Gebauer et al. 1988).

Because our experiment was conducted with only $^{15}N$-enriched substrates, we cannot conclusively rule out the possibility that amino acids were mineralized prior to plant uptake (and therefore not taken up as intact molecules). However, based on several lines of evidence, we feel confident that our data represent intact amino acid uptake. First, we took efforts with our experimental design to limit opportunities for microbial mineralization to occur; prior to labeling with $^{15}N$-enriched substrates, the roots of all tree seedlings were thoroughly washed with reverse osmosis water to remove any attached potting medium, and they were then sterilized with bleach to reduce the microbial population adhering to roots. We also used a hydroponic method to supply seedlings with labeled compounds, so we did not contend with the potential mineralization of amino acids that might have occurred if we had labeled plants grown in soils. Second, the different patterns of $^{15}N$ distribution in our trees for the amino acids compared to $NH_4^+$ suggests our amino acids were not mineralized to $NH_4^+$ prior to uptake (Fig. 3). If $^{15}N$ uptake in our amino acid treatments was dominated by uptake of mineralized $^{15}NH_4^+$, we would have expected there to be no significant differences in $^{15}N$ distribution patterns in fine roots between the amino acids and $^{15}NH_4^+$. Similarly, $NH_4^+$-specific uptake rates were significantly different than those of the amino acids we examined in most cases (Fig. 1a). Again, if our amino acids were mineralized prior to uptake we would have expected there to be no significant differences in specific uptake rates of the different substrates. Third, other studies that have used amino acids enriched in both $^{13}C$ and $^{15}N$ have documented the uptake of arginine (Öhlund and Näsholm 2001; Persson and Näsholm 2001), glycine (Nordin et al. 2001; Rains and Bledsoe 2007), and serine (Weigelt et al. 2005; Harrison et al. 2007) as intact molecules, demonstrating that it is physiologically possible for plants to do so. Finally, we tested whether glycine was taken up intact on a subset of our seedlings by supplying them with universally labeled glycine (U-$^{13}C_2$, $^{15}N$-glycine) and using regressions of $^{13}C$ excess on $^{15}N$ excess in fine roots to determine the minimum proportion of glycine taken up intact (Näsholm et al. 1998, 2000; Öhlund and Näsholm 2001). This method compared the slope of the regression with the ratio of C:N in the amino acid of interest (2:1 in the case of glycine), with 100% uptake occurring when the C:N ratio of the amino acid was equal to the slope of the regression line (Näsholm et al. 1998). All of our tree species had slopes of less than 2, suggesting that some amount of C was lost either prior to uptake via mineralization or nitrification (Quastel and Scholefield 1949) or after uptake by the metabolism of glycine, which results in the loss of C to $CO_2$ (Näsholm et al. 1998). Because our experimental design sought to limit microbial influences, we assume that most C was lost by plant respiration. Only red oak and American beech had statistically significant regressions, which suggested that roughly 78% and 51% of glycine were taken up intact by our seedlings, respectively (Fig. 4). We were unable to determine the fraction of glycine taken up intact by white ash and black cherry for two possible reasons. First, white ash had the

lowest level of $^{13}C$ excess of any of our species, which can make detecting the amount of amino acid taken up intact difficult (Näsholm and Persson 2001). Second, black cherry had the highest variation in levels of $^{13}C$ excess of any of our species (SE = 0.79 compared to 0.10 for oak, 0.03 for beech, and 0.04 for ash), which may have obscured our ability to detect a linear relationship between $^{13}C$ and $^{15}N$ excess. However, the fine roots of all our species were significantly enriched in $^{13}C$ compared to control plants, suggesting that some portion of glycine was taken up intact by all our species. Based on the above reasons, we conclude that $^{15}N$ uptake in the amino acid treatments was dominated by intact amino acid uptake by our trees.

## Conclusion

The results of our study demonstrate the ability of four temperate tree species to take up amino acids in direct competition with inorganic N. We also demonstrated that trees from low-fertility habitats acquired more N from amino acids compared to inorganic forms. These results support the idea that the dominant species in a particular habitat are those best able to utilize the N resources most available, be they inorganic or organic (McKane et al. 2002). All of the amino acids we investigated are commonly found in soil amino acid pools in a variety of ecosystems (Kielland 1995; Nordin et al. 2001; Senwo and Tabatabai 1998), including hardwood forests in eastern North America, where our tree species occur (Rothstein 2009). Therefore, it is plausible that free amino acids could provide a source of N to trees in this region that has previously been overlooked, especially in low-fertility habitats. Evaluating amino acid uptake by red oak, American beech, white ash, and black cherry in the field is the next logical step towards elucidating the role amino acid N plays in the N nutrition of these temperate trees.

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