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Cross-Ecosystem Comparisons of In Situ Plant Uptake of Amino Acid-N and NH_4^+

Jack W. McFarland,[1]* Roger W. Ruess,[2] Knut Kielland,[2] Kurt Pregitzer,[3] Ronald Hendrick,[4] and Michael Allen[5]

[1]*Department of Biology and Wildlife, University of Alaska, PO Box 757000, Fairbanks, Alaska 99775-7000, USA;* [2]*Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775, USA;* [3]*Department of Natural Resources and Environmental Science, University of Nevada, Reno, Nevada 89557, USA;* [4]*Warnell School of Forest Resources, University of Georgia, Athens 30602, Georgia, USA;* [5]*College of Natural and Agricultural Sciences, University of California, Riverside, California 92521, USA*

Abstract

Plant and microbial use of nitrogen (N) can be simultaneously mutualistic and competitive, particularly in ecosystems dominated by mycorrhizal fungi. Our goal was to quantify plant uptake of organic and inorganic N across a broad latitudinal gradient of forest ecosystems that varied with respect to overstory taxon, edaphic characteristics, and dominant mycorrhizal association. Using ^{13}C and ^{15}N, we observed in situ the cycling dynamics of NH_4^+ and glycine through various soil pools and fine roots over 14 days. Recovery of ^{15}N as soil N varied with respect to N form, forest type, and sampling period; however, there were similarities in the cycling dynamics of glycine and NH_4^+ among all forest types. Microbial immobilization of ^{15}N was immediately apparent for both treatments and represented the largest sink (~25%) for ^{15}N among extractable soil N pools during the first 24 h. In contrast, fine roots were a relatively small sink (<10%) for both N forms, but fine root ^{13}C enrichment indicated that plants in all forest types absorbed glycine intact, suggesting that plants and microbes effectively target the same labile soil N pools. Relative uptake of amino acid-N versus NH_4^+ varied significantly among sites and approximately half of this variation was explained by mycorrhizal association. Estimates of plant uptake of amino acid-N relative to NH_4^+ were 3× higher in ectomycorrhizal-dominated stands (1.6 ± 0.2) than arbuscular mycorrhizae-dominated stands (0.5 ± 0.1). We conclude that free amino acids are an important component of the N economy in all stands studied; however, in these natural environments plant uptake of organic N relative to inorganic N is explained as much by mycorrhizal association as by the availability of N forms per se.

Key words: glycine; ammonium; ^{13}C ^{15}N; microbial biomass N; organic N uptake; ectomycorrhizae; arbuscular mycorrhizae; boreal forest ecosystem; temperate forest ecosystem.

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**Corresponding author; e-mail:* jwmcfarland@alaska.edu

Introduction

The majority of soil nitrogen (N) is closely associated with soil organic matter (SOM) (Schulten and Schnitzer 1998), and in many forested ecosystems the mean residence time for SOM fractions may vary from several years to several thousand years

(Stevenson and Cole 1999). The slow turnover of this N pool has been cited as a major constraint to plant N supply. However, the operational definition for organic N is broad, encompassing both labile and recalcitrant forms. Polymeric molecules are often too large to cross membranes, but monomeric forms of organic N, for example, amino acids and amino sugars, are readily absorbed by plants (Schobert and others 1988; Kielland 1994; Raab and others 1999; Weigelt and others 2003; Finzi and Berthrong 2005). Though these N forms often represent only a small proportion of total soil organic N, the turnover of these substrates has important implications for plant–microbial competition for N, effectively functioning as an alternative pathway for plant N supply in some ecosystems (Chapin 1995; Kaye and Hart 1997).

Measures of amino acid turnover reveal that free amino acid (FAA) cycling rates are rapid across a variety of terrestrial ecosystems (Jones 1999; Vinolas and others 2001; Jones and others 2004; Kielland and others 2006) and this has important implications with regard to plant N supply among these divergent landscapes. For example, in the boreal forest, laboratory incubations of late successional black spruce soils from interior Alaska have demonstrated that gross rates of N flux throughout the soil FAA pool are substantially greater than gross rates of N mineralization (Jones and Kielland 2002). Correspondingly, plants in black spruce ecosystems absorb amino acids to a much greater extent than ammonium, roughly in proportion to their availability in the soil (Kielland and others 2006). Production and soil concentrations of FAA can also be high in temperate ecosystems (Berthrong and Finzi 2006). Residence times for FAA in some temperate forest soils are less than 2 h (McFarland and others 2010), further supporting the notion that the cycling dynamics of other labile N forms may be as significant to the N economy of terrestrial plants as the turnover of soluble inorganic N.

Our incomplete understanding of the mechanisms controlling turnover and subsequent plant uptake of soil organic N is a major obstacle in modeling the N cycling dynamics of terrestrial forest ecosystems. This recognition has led to a revision of the traditional view of soil N cycling, which emphasized the importance of net N mineralization in regulating plant N availability (Schimel and Bennett 2004). Under the ''new paradigm'' of N cycling, plant preference for N reflects the relative dominance of a particular N form, for example, NO_3^-, NH_4^+, or FAA-N, which is in turn mediated by microbial activity. In extremely N-limited environments, organic N forms can dominate labile pools (Weintraub and Schimel 2005), and it is likely that plants and microorganisms rely heavily on monomers of organic N, for example, amino acids, to satisfy N requirements (Nordin and others 2004). Because soil microorganisms have higher substrate affinities and larger surface-to-volume ratios than roots, plant N uptake under these conditions is considered to be ''opportunistic'' as soluble organic N diffuses past the fine root complex from N-rich to N-poor microsites. At the other end of the N availability gradient, high rates of N mineralization coupled with a relatively homogenous resource distribution, reduces microbial competition for N; nitrifiers flourish in the presence of excess NH_4^+, and plants rely primarily on inorganic N forms.

Conceptually elegant, this model of terrestrial N cycling has not fully evolved to encompass the role of plant and associated symbionts in mediating N bioavailability. Plant–microbial interactions for N can be simultaneously mutualistic and competitive, particularly in forest ecosystems where the microbial community structure is dominated by fungi. For example, in some floodplain forests of interior Alaska, it is estimated that 85–90% of soil microbial biomass is fungal (Flanagan and van Cleve 1983) and over 60% of fungal richness is represented by root-associated taxa (D.L. Taylor personal communication). How much fungal biomass is actually in symbiosis with plants remains uncertain for many forest ecosystems, but from at least one account we know that extramatrical mycelia of ectomycorrhizal (EM) fungi can contribute up to one-third of the soil microbial biomass (Högberg and Högberg 2002). This has several important implications with regard to plant nutrition as there is some evidence that organic matter quality, mineral N turnover, the type of mycorrhizal association, and organic N uptake by plants are intercorrelated across a broad range of tropical, temperate, and boreal forests (Allen and others 1995).

Expanding on Moser's (1967) global map of forest mycorrhizal types, Read (1983) proposed a geographical distribution of mycorrhizal associations based on their use of organic N forms. This hypothesis was based on observations that soils high in surface organic matter have ecto- and ericoid-mycorrhizae, whereas environments with low accumulation of surface organic matter are dominated by arbuscular mycorrhizae (AM). Direct uptake of labile organic N has been demonstrated for many plant species across a range of biomes irrespective of mycorrhizal association (Chapin and

others 1993; Kielland 1997; Näsholm and others 1998; Raab and others 1999; Schmidt and Stewart 1999; Streeter and others 2000; Näsholm and others 2000; Henry and Jefferies 2003; Nordin and others 2004; Xu and others 2004; Finzi and Berthrong 2005). However, ericoid and some EM fungal associations are physiologically capable of directly hydrolyzing certain components of SOM and transferring the resultant N to the host plant (Bajwa and Read 1985; Finlay and others 1992). Work in stands of *Pinus muricata* in northern California suggests a coevolved strategy for N acquisition whereby exoenzyme production by EM symbionts facilitates host access to recalcitrant soil organic N derived from its own litter (Northrup and others 1995). In contrast, AM associations appear to have a more limited ability to decompose organic matter (Read 1991; but see Hodge and others 2001). Still, AM fungi possess permeases for amino acid uptake (Cappellazzo and others 2008), and recent experiments of plant amino acid uptake indicate that both AM plants and some non-mycorrhizal plants absorb amino acids under conditions where soil concentrations of FAA are relatively high (Näsholm and others 1998; Raab and others 1996; Hawkins and others 2000). Whereas the importance of mycorrhizal associations in nutrient acquisition for vascular plants is well established (Allen 1991), the aforementioned studies pose several challenging questions regarding N cycling dynamics in terrestrial ecosystems. To what extent is plant potential for DON uptake realized in the field? Do mycorrhizal types differ in their capacity to sequester organic N for their host? If not, what are the principal factors controlling plant N source selectivity in terrestrial forests? These linkages between plant N uptake and mycorrhizal type have never been explicitly tested across ecosystems that vary in climate, SOM quality, or dominant forest species.

The objective of this project was to evaluate plant uptake of organic and inorganic N in forest ecosystems across a broad latitudinal gradient of forest ecosystems. We hypothesized that plant preference for FAA across this range of forest ecosystems was inversely correlated with rates of inorganic N mineralization. Thus, in N-limited boreal ecosystems where low soil temperatures constrain N mineralization rates, we predicted that FAA would represent a greater proportion of the annual N requirements of vascular plants. Second, we hypothesized that mycorrhizal association plays a major role in determining the ratio of organic to inorganic N uptake among ecosystem types. As EM-dominated soil communities generally exhibit more extensive hyphal networks and a greater capacity to degrade complex soil organic N than AM-dominated communities (Smith and Read 1997), we predicted that plant species from predominantly EM colonized stands would have higher uptake of organic relative to inorganic N forms in the field.

Methods and Materials

Study Sites

We chose a series of forest ecosystems that differed in climate, soil type, overstory taxon, and dominant mycorrhizal association (EM versus AM), including stands from three regions in North America: southern temperate, northern temperate, and boreal. In the southeastern United States, we sampled from two deciduous hardwood stands: AM-dominated tulip poplar (*Liriodendron tulipifera*) at the USDA Forest Service Coweeta Research Station in North Carolina and EM-dominated white oak (*Quercus alba*) at the B. F. Grant Experimental Forest in central Georgia. In northern Michigan, we sampled in an AM-dominated sugar maple (*Acer saccharum*) stand near the Ford Forestry Center, as well as an EM-dominated red pine (*Pinus resinosa*) plantation just outside Houghton. Our boreal site was an EM-dominated white spruce (*Picea glauca*) stand located within the floodplain portion of the Bonanza Creek Experimental Forest near Fairbanks, AK. Two of these sites, Bonanza Creek and Coweeta belong to the Long-Term Ecological Research (LTER) network, whereas the B.F. Grant and upper Michigan stands include areas where studies on carbon (C) and N cycling dynamics have been conducted for several decades. Select site and soil properties are presented in Table 1; specific characteristics for these stands are described in detail elsewhere (Pregitzer and others 2002; McFarland and others 2010).

Field Sampling

Turnover processes for labile N can be rapid (Davidson and others 1990; Jones and Kielland 2002). Ideally, these processes are probably best measured in intact systems where plant–microbial interactions during N acquisition can be evaluated with undisturbed mycorrhizal hyphal networks. This community structure is nearly impossible to recreate under controlled environmental conditions. However, previous experiments in floodplain willow, balsam poplar, and black spruce communities along a primary successional gradient in interior Alaska, have demonstrated the effectiveness of

Table 1. Select Site and Soil Properties of Forest Ecosystems

Parameter	Site				
	Tulip poplar	White oak	Sugar maple	Red pine	White spruce
Latitude	35° 4′ N	33° 25′ N	46° 39′ N	47° 6′ N	64° 41′ N
Dominant mycorrhizal association	AM	EM	AM	EM	EM
Percent mycorrhizal infection[1]	30	>90	42	>90	>90
Percent overstory	85	68	92	100	98
Mean annual temperature (°C)	12.7	16.5	3.8	3.8	−3.3
Mean annual precipitation (mm)	1816	1263	841	883	287
Soil classification	Humic Hapludult	Typic-rhodic Hapludult	Typic Haplorthod	Entic Haplorthod	Typic Cryofluvent
Soil texture	Sandy loam	Clay loam	Sandy loam	Sandy loam	Organic to alluvial silt
DIN (μg N g^{-1})	3.24 ± 0.21[a]	2.67 ± 0.36[a]	3.50 ± 0.29[a]	1.13 ± 0.11[b]	5.50 ± 0.50[a]
DON (μg N g^{-1})	23.8 ± 1.6[a]	24.7 ± 1.2[a]	14.0 ± 1.8[b]	13.6 ± 0.9[b]	42.6 ± 4.0[c]
FAA-N (μg N g^{-1})	0.57 ± 0.09[c]	1.30 ± 0.39[c]	0.97 ± 0.14[c]	3.25 ± 0.69[bc]	6.45 ± 1.20[ab]
Soil moisture (g H_2O 100 g^{-1})[2]	45.4 ± 1.3	24.1 ± 1.3	28.0 ± 1.7	4.6 ± 0.2	50.3 ± 1.5
Fine root mass (g m^{-2})[3]	237.8 ± 12.7[c]	323.9 ± 14.3[b]	323.3 ± 11.0[b]	275.0 ± 14.2[bc]	350.0 ± 14.9[a]
Fine root N (mg N g^{-1})	16.5	7.6	12.3	11.4	8.7
Average core dry mass (g)	202 ± 4	230 ± 4	264 ± 5	285 ± 4	90 ± 2
Soil temperature (°C)[4]	17.0	21.5	13.8	15.7	9.3

AM arbuscular mycorrhizae; EM ectomycorrhizae; DIN dissolved inorganic N; DON dissolved organic N; FAA-N free amino acid-N. Extractable N pools are reported as μgN g^{-1} dry soil.
[1]Percent mycorrhizal infection is the number of healthy AM or EM tips per total tips counted. Dead tips or tips that were older or not easily distinguishable as mycorrhizal were not counted (Lansing unpublished data).
[2]Soil moisture content was calculated from subsamples collected for determination of FAA-N.
[3]Fine root mass estimates are reported on an areal basis to a soil depth of 12 cm.
[4]Soil temperatures represent daily averages calculated from hourly measurements collected during the first 24 h of the tracer experiment.
Soil and fine root data are mean ± SE, n = 6–9. Letters denote significant differences ($P \leq 0.05$) between sites.

using a multiple isotope (^{13}C, ^{15}N) tracer approach (McFarland and others 2002; Kielland and others 2007), to quantify plant and microbial uptake of NH_4^+ and amino acid-N in situ. Therefore, despite caveats in interpreting overall patterns of recovery (see ''Discussion'' section), we employed our ^{15}N tracer design in the field to quantify plant and microbial uptake of NH_4^+ and amino acid-N.

The field component for our project was conducted in July 1999 for white spruce and from June to July 2000 for the remaining forest types. Our sampling protocol was nearly identical to that of previous work conducted in floodplain balsam poplar stands of interior Alaska (McFarland and others 2002). Briefly, our system for applying treatments and collecting soil cores consisted of two components, an injection grid, and a coring grid. These grids measured 80 cm × 15 cm and were constructed of lexan sheets, which were flexible enough to mold to the surface of the forest floor. Injections grids consisted of six identical templates with 37 holes each. These grids were held in position by four buried steel pins, which made it easy to align the two grids over the area being treated (see McFarland and others 2002 for visualization of grid design).

Using a randomized complete block design, we established three replicate injection areas within a 9-m^2 subplot. For the temperate forest stands, this design was replicated six times ($n = 6$) along a transect so that a minimum of 30 m separated each subplot. In Alaska, our experiment was conducted within three mature white spruce stands scattered along a 10-km stretch of the Tanana River. Within each stand, we established three subplots ($n = 9$) separated by a minimum of 20 m. For all forest types, the three grids in each subplot were injected with one of two treatment solutions or a control (distilled water). Treatment solutions were (1) $(^{15}NH_4^+)_2SO_4$ plus U-[^{13}C]-glycine or

(2) $(NH_4^+)_2SO_4$ plus U-[^{13}C][^{15}N]-glycine. Unlabeled ammonium was added to the dually labeled glycine treatment to mirror any fertilization effect brought about by the addition of glycine to the ^{15}N-labeled ammonium treatment. Soils were injected to a depth of 10 cm beginning at the L–F interface and cored to 12 cm to account for any vertical leaching of the label and to stay well within the zone for fine root development (Hendrick and Pregitzer 1996; Ruess and others 2006). We removed all leaf litter prior to injection, but added it back following treatment application to maintain moisture and temperature constancy in the soil environment around the injection area. Total injection volume was 37 ml ($\sim$1 ml cm^{-2} for each injection area along our treatment grid), which delivered approximately 0.39 g ^{13}C m^{-2} and 0.22 g ^{15}N m^{-2} for both labeled solutions. Injecting a complete subplot with all treatments required approximately 20 min. Cores within each grid were harvested sequentially at 45 min, 2, 12, and 24 h, and 7 and 14 days.

Our coring grids consisted of six holes large enough to allow a soil corer with an inside diameter of 5.5 cm to pass through unobstructed (McFarland and others 2002). Harvest periods were randomized within a grid. For each core, the areal extent of our injections at 1 cm resolution was twice that of the area harvested. As the center of each soil core removed matched exactly the center of the respective treatment area, we were able to remove a soil core with a theoretically known amount of added label (600 μg ^{15}N and 1,040 μg ^{13}C). In addition, as part of a companion experiment investigating turnover rates for glycine in situ, we monitored $^{13}CO_2$ evolution in the headspace above injection areas treated with $(^{15}NH_4^+)_2SO_4$ plus U-[^{13}C]-glycine. These data are discussed elsewhere (McFarland and others 2010); however, we present some results from that study (see ''Discussion'' section) as corroborating evidence for cross-ecosystem patterns of FAA-N utilization by soil microbes.

To minimize the effect of microbial activity following harvest, initial processing for each soil core was conducted on site. Briefly, each core was split vertically into two equal halves. One half was used for sorting and freezing roots for ^{13}C and ^{15}N analysis. The other half was used for ^{13}C and ^{15}N analysis of (1) total soil C and N, (2) extractable dissolved inorganic N (DIN) and dissolved organic N (DON), and (3) microbial biomass N (MBN). Soils were sieved in the field through a 2.36-mm (#8) screen. Samples for determination of DIN were extracted in the field with 0.5 M K_2SO_4, gravity filtered through 0.7 μm glass fiber discs and treated with 5 ppm phenyl mercuric acetate to inhibit microbial growth during storage. Samples for determining MBN were fumigated in the field with ethanol-free chloroform for 24 h in a modified pressure cooker, transported to a laboratory under vacuum, and then extracted in 250 ml glass beakers with 0.5 M K_2SO_4 (Brookes and others 1985). All extractions were conducted on a shaker table at 80 rev min^{-1} for 1 h. After initial processing, all root and soil samples were frozen with liquid N in the field, and transported on ice to laboratory facilities at the University of Alaska, Fairbanks (white spruce stands) or stored at −80°C for several days (temperate stands) prior to overnight shipment to Fairbanks, Alaska.

We collected additional soil cores (n = 15) to a depth of 12 cm within each forest type for determination of amino acid-N concentrations. Sampling depth was chosen to coincide with cores harvested to trace the fate of our ^{15}N additions. Cores were transported to our laboratory facility on ice and processed within 4 h of collection. Each core was gently hand-mixed and sieved (2.36 mm mesh) to remove rocks, large roots, and as many small roots as possible. We subsampled the coarsely homogenized soils for determination of gravimetric moisture content and extraction with 75 ml distilled water (15 min at 150 rev min^{-1}). Soil extracts were vacuum-filtered through a 0.2 μm cellulose acetate filter (Corning Inc, Corning, New York, USA) and stored frozen in 2 ml sterile polyethylene vials until analysis. We quantified FAA-N in soil extracts fluorometrically using a Biotek FL600 Fluorescence and Absorbance Microplate Reader (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) with excitation and emission wavelengths set to 340 and 450 nm, respectively. Details concerning derivatization are presented in Jones and others (2002). Each sample was run in quadruplicate and the results are reported as μg amino acid-N per g dry soil.

Laboratory Analyses

Root samples were thawed and hand sorted to remove any residual organic matter. We separated roots into two size classes. Roots over 1 mm diameter were classified as coarse, whereas those 1 mm or less were classified as fine with the exception of tulip poplar, which had much thicker fine roots than the other forest types (see below). The threshold for fine root classification in tulip poplar was set at 2 mm. Fine root samples were freeze-dried and subsequently powdered using a modified roller mill to ensure complete homogenization. Soil moisture content was determined by

drying subsamples at 60°C for 48 h, and subsamples were ground to a powder using the same roller mill design. Both roots and soils were analyzed for C, N, ^{13}C, and ^{15}N using a Europa Scientific continuous flow mass spectrometer (PDZ Europa, Inc.). All isotope values obtained for root and soil C and N were normalized using standards derived from NBS-19 or IAEA ammonium sulfate, respectively.

Fumigated and nonfumigated soil extracts were digested using a modified micro-Kjeldahl procedure (Bremner and Mulvaney 1982). All soil extracts were analyzed for available NH_4^+ and NO_3^- (DIN) by flow injection colorimetry, and total Kjeldahl N (TKN) using a Technicon autoanalyzer (Whitledge and others 1981). DON was calculated as the difference between TKN and DIN in unfumigated extracts, whereas MBN was calculated as the difference in TKN between fumigated and unfumigated digests. All data for extractable N pools are expressed as μg N per g dry soil. We did not use a conversion factor to correct the extraction efficiency of N (K_N) for microbial biomass determinations. A conversion factor is highly dependent on edaphic characteristics within each forest type, including microbial composition, and thus likely to be quite variable among the stands used in this study. Moreover, a conversion factor could overrepresent the amount of label sequestered within microbial biomass, as freshly assimilated N is more chloroform labile than the more recalcitrant components of MBN.

Subsamples from all three soluble N pools (DIN, DON, and MBN) were diffused in sealed glass containers to determine ^{15}N content as described by Khan and others (1997). For undigested soil extracts, this procedure entailed pipetting 10 ml of the sample into a one-pint (~500 ml) Mason jar. Two quartz filter discs (Whatman QM-A) were placed on stainless steel holders attached to the lid of each Mason jar and acidified with 10 μl of 0.5 M H_2SO_4. Five acid-washed glass beads were added to the jar along with 0.2 g of Devarda's alloy to reduce and collect nitrate-^{15}N. To bring the sample N concentration up to a detectable range, we spiked each container with 50 μl of a 100 ppm $(^{14}NH_4)_2SO_4$ solution (0.366% ^{15}N). We added approximately 0.2 g of MgO to each container just prior to sealing the unit and heating it to 45°C for 8 h. Adding MgO increases the pH of the extraction solution causing all NH_4^+ in solution to volatilize and collect on the acidified discs. The protocol for digested samples follows a similar procedure with the exception that 10 ml of 10 M NaOH is used in lieu of MgO and the addition of Devarda's alloy is not necessary. For all pools, standards with a known atom% ^{15}N were analyzed along with the samples to evaluate diffusion efficiency.

Calculations and Statistical Analyses

We report all isotope values for soil and root C and N pools as percent recovery of added label. Recovery of isotopic labels was calculated for individual cores by multiplying pool size (μg C or N g^{-1} dry root or soil) by the respective ^{13}C or ^{15}N atom% enrichment (APE) and dividing by the amount of label added. APE was determined by subtracting the atom% ^{13}C or ^{15}N of control cores from the atom% ^{13}C or ^{15}N of treated cores. Control values were averaged within stands prior to use in estimating isotopic enrichment. To economize our analyses, we randomly selected one control core from each subplot within a stand ($n = 6$). For each core, the areal extent of our injections (59.7 cm^2) was approximately 2.5 × the areal extent of the core harvested (23.7 cm^2; total core volume = 285 cm^3); we used this ratio to estimate the amount of isotope (~600 μg ^{15}N and 1,040 μg ^{13}C) applied to each core.

Temporal variation in % label recovery (^{13}C and ^{15}N) within soil and root, and C and N pools was determined with repeated measures analysis of variance (ANOVA) using the GLM procedure (SAS Systems version 9.1, 2003). For each response variable, we included the interaction of treatment and field replicate with sampling period in the ANOVA model. As a significant temporal effect could be due to differences at just one sampling period, we conducted univariate tests to determine at which sampling periods our response variable differed. Specific treatment effects were identified for each sampling period using Tukey's HSD statistic for multiple comparisons of means ($\alpha = 0.05$). When necessary, all variables were log or square-root transformed prior to analysis to meet assumptions of normality and homogeneity of variance. All inferences regarding pool dynamics are made at the stand level. Values throughout are presented as means ± 1 S.E. of untransformed data.

Results

Soil and Root Pools of N

We observed several quantitative and qualitative differences in soils along our latitudinal transect. For instance, though we did not measure soil bulk density, we did calculate the average mass of cores harvested at each site. White spruce cores averaged less than half the mass (90 g) of cores collected

from the temperate biomes (200–300 g), reflecting the high organic matter content in the upper horizons. Values derived from control samples revealed that soluble N pools also differed significantly among sites; however, most of this variation was attributable to comparisons between boreal white spruce and the temperate forest types (Table 1). DIN concentrations were lowest in red pine (1.1 ± 0.1 μg g^{-1}) and highest in white spruce (5.5 ± 0.5 μg g^{-1}), although temperate forest soils generally had higher rates of net mineralization. DON was also lowest in red pine (13.6 ± 0.9 μg g^{-1}) and highest in white spruce (42.6 ± 4.0 μg g^{-1}), with the two southern temperate stands having average DON concentrations that were almost twice those observed in the northern temperate stands. Soil concentrations of FAA-N varied more predictably with latitude, increasing over 10-fold from just over 0.5 μg AA-N g^{-1} in tulip poplar to 6.5 μg AA-N g^{-1} in white spruce. Together, these data indicate that the N additions associated with our treatment applications enhanced the DIN and DON concentrations differently at each site. Though white spruce soils had the highest concentrations of DIN, enhancement of DIN was lowest in sugar maple (68%) due to substantially higher masses for the temperate soil cores. In contrast, we increased DIN concentrations to the greatest degree in red pine (185%) due to relatively low standing stocks of NH_4^+ and NO_3^-. Augmentation of DON was comparatively even across forest types ranging from 10% in white oak to 17% in sugar maple; although, when considering only the FAA fraction of DON, our amino acid amendment represented a 2–5-fold increase in FAA-N for the temperate deciduous stands, but less than a doubling of FAA-N for either coniferous stand.

Live fine root biomass differed significantly among forest types ($F_{4,391} = 10.53$, $P < 0.001$), though most values fell within a relatively narrow range (238.0–350.0 g m^{-2} to 12 cm depth) and did not appear to vary predictably with taxon, mycorrhizal association, or latitude. Anecdotally, we observed differences in specific root length among stands. In most instances, average fine root diameter was considerably less than 1 mm regardless of forest type; tulip poplar was the notable exception in this instance with some fine roots larger than 1 mm.

Partitioning of $^{15}NH_4^+$ and $^{13}C^{15}N$-Glycine in Soil Pools

Total recoveries of the ^{15}N-labeled tracers are summarized for all sampling periods and soil N pools in Table 2. In bulk soil samples (soil from which only roots are removed), recovery of ^{15}N varied significantly among forest types for both NH_4^+ ($F_{4,193} = 16.07$; $P < 0.0001$) and glycine-amended ($F_{4,193} = 24.40$; $P < 0.0001$) cores. Forty-five minutes following injections average recovery of label in bulk soil ranged from 63 ± 3% (sugar maple) to 108 ± 5% (tulip poplar) for the NH_4^+ treatment, and from 60 ± 5% (white spruce) to 89 ± 6% (tulip poplar) for the glycine treatment (Figure 1A). Mean recovery of ^{15}N declined over the next 2 weeks, albeit slightly for some stands (Table 2); however, this trend was significant only for sugar maple (Figure 1B). We observed no significant treatment effect on recovery at any site with the exception of the white spruce stands where total recovery of ^{15}N in NH_4^+-amended cores (73 ± 2%) cores was approximately 13% higher than cores receiving the glycine treatment (60 ± 2%; $F_{5,40} = 3.60$; $P = 0.001$). Still, comparison of treatment differences at each sampling period indicates that treatment effects in white spruce were significant for only two sampling periods (12 and 24 h), largely due to anomalously high recoveries in cores receiving $^{15}NH_4^+$.

Recovery of ^{13}C in bulk soil declined significantly at all sites throughout the experiment ($P < 0.05$; Figure 2). Initial recoveries in the temperate stands ranged from 78 ± 7% in red pine to 90 ± 7% in sugar maple. After 2 weeks, overall recovery of glycine-derived ^{13}C dropped to about 50% for all stands. In boreal white spruce, temporal patterns of ^{13}C recovery resembled those for the temperate stands, but the proportional recovery of label at any sampling period was approximately half that of the temperate stands (Figure 2). This discrepancy between temperate and boreal stands might be attributable to rapid mineralization of glycine label to CO_2 in white spruce soils by C-stressed microorganisms; however, the more likely explanation is a dilution of our label by high background levels of ^{13}C in these organic matter-rich soils (Näsholm and others 1998). We have observed a similar response in floodplain stands of balsam poplar and black spruce where high SOM content and thus high background ^{13}C can mask tracer additions of labeled-C (Kielland and others 2006; McFarland and others 2002).

Recovery of ^{15}N in soluble soil N pools varied with respect to N form, forest type, and sampling period (Figure 3A–E; Table 2), yet we also observed similarities in the cycling dynamics of both N forms among forest types. Recovery of our $^{15}NH_4^+$ label as DIN declined sharply during the initial hours of the experiment at all sites. Less than 1 h following treatment application, we recovered approximately

Table 2. ^{15}N Recovery Within Plant and Soil Pools at Each Sampling Period

Site	N form	Pool	Recovery of added ^{15}N (%)					
			45 min	2 h	12 h	24 h	168 h	336 h
TP	NH_4^+	DIN	87.27 ± 13.76	48.21 ± 10.56	23.07 ± 5.45	24.18 ± 5.24	6.36 ± 1.96	5.59 ± 1.88
		DON	1.12 ± 0.51	2.68 ± 0.9	7.62 ± 5.30	6.93 ± 3.13	1.67 ± 0.43	2.18 ± 0.54
		Microbial N	20.21 ± 7.62	32.25 ± 5.83	35.49 ± 6.81	30.57 ± 8.70	32.17 ± 4.75	22.58 ± 5.15
		Fine root N	0.41 ± 0.05	0.43 ± 0.15	1.68 ± 0.53	2.21 ± 1.25	3.73 ± 0.54	8.18 ± 1.13
		Bulk soil N	108.32 ± 5.29	96.97 ± 11.77	94.17 ± 4.57	101.83 ± 8.13	88.80 ± 10.33	82.58 ± 9.67
	Gly	DIN	11.32 ± 2.68	16.86 ± 4.05	19.84 ± 7.53	12.79 ± 3.67	8.08 ± 1.52	5.85 ± 1.64
		DON	31.26 ± 2.08	15.00 ± 3.34	6.19 ± 1.66	3.74 ± 0.83	3.10 ± 0.40	2.94 ± 0.90
		Microbial N	28.66 ± 7.96	26.88 ± 4.35	28.48 ± 4.76	34.29 ± 3.32	35.17 ± 3.54	22.56 ± 5.08
		Fine Root N	0.28 ± 0.07	0.25 ± 0.06	0.67 ± 0.24	0.45 ± 0.09	3.24 ± 0.72	5.06 ± 1.54
		Bulk soil N	89.01 ± 10.18	104.25 ± 7.79	102.00 ± 10.43	95.64 ± 11.11	87.36 ± 14.31	89.94 ± 5.85
WO	NH_4^+	DIN	55.25 ± 7.52	34.26 ± 12.03	15.43 ± 3.65	12.15 ± 1.86	10.84 ± 2.02	9.53 ± 2.39
		DON	3.02 ± 1.38	5.56 ± 2.68	4.98 ± 2.10	7.59 ± 2.40	8.61 ± 1.48	9.61 ± 3.55
		Microbial N	8.70 ± 4.08	11.77 ± 3.45	27.92 ± 7.05	24.13 ± 5.60	25.24 ± 6.92	13.72 ± 4.96
		Fine root N	0.26 ± 0.04	0.33 ± 0.04	0.76 ± 0.13	0.86 ± 0.14	1.33 ± 0.37	3.42 ± 1.27
		Bulk soil N	91.79 ± 7.99	85.80 ± 10.49	95.84 ± 9.65	80.95 ± 5.06	90.24 ± 10.33	88.63 ± 11.33
	Gly	DIN	7.80 ± 0.83	10.39 ± 3.78	10.12 ± 2.65	10.45 ± 2.16	6.05 ± 2.22	5.94 ± 1.47
		DON	41.20 ± 7.39	41.24 ± 6.92	15.86 ± 1.37	12.47 ± 3.33	8.01 ± 1.86	12.00 ± 5.21
		Microbial N	9.87 ± 4.51	11.60 ± 5.33	29.66 ± 8.65	21.11 ± 4.87	22.95 ± 4.04	16.99 ± 5.46
		Fine root N	0.36 ± 0.09	0.23 ± 0.05	0.35 ± 0.08	0.84 ± 0.17	1.17 ± 0.20	1.55 ± 0.40
		Bulk soil N	85.86 ± 3.58	90.98 ± 11.55	89.49 ± 6.40	92.21 ± 6.32	92.02 ± 4.25	76.80 ± 9.68
SM	NH_4^+	DIN	66.75 ± 7.99	80.11 ± 6.91	49.00 ± 5.48	36.70 ± 6.81	8.52 ± 3.04	3.04 ± 0.94
		DON	2.95 ± 2.05	2.17 ± 1.43	3.88 ± 1.95	16.05 ± 7.58	2.96 ± 1.03	4.10 ± 1.73
		Microbial N	10.76 ± 1.59	13.96 ± 5.30	17.70 ± 7.81	9.24 ± 1.91	13.07 ± 2.50	13.93 ± 2.28
		Fine root N	0.44 ± 0.04	0.80 ± 0.20	1.53 ± 0.21	2.59 ± 0.29	6.08 ± 0.74	7.27 ± 1.59
		Bulk soil N	63.63 ± 2.58	65.35 ± 4.81	70.09 ± 6.94	66.92 ± 7.44	50.96 ± 5.10	40.90 ± 3.38
	Gly	DIN	6.30 + 1.55	8.49 ± 1.67	16.15 ± 4.11	25.52 ± 4.28	6.88 ± 1.78	6.10 ± 0.81
		DON	25.79 ± 3.29	23.51 ± 4.01	15.62 ± 4.20	4.45 ± 1.85	2.19 ± 0.91	5.94 ± 1.77
		Microbial N	23.51 ± 3.63	28.79 ± 2.45	34.54 ± 6.28	19.52 ± 3.64	18.51 ± 5.56	15.14 ± 5.57
		Fine root N	0.59 ± 0.07	0.64 ± 0.10	0.82 ± 0.08	1.39 ± 0.18	4.21 ± 0.20	4.93 ±0.81
		Bulk soil N	63.13 ± 7.02	67.23 ± 11.45	64.16 ± 8.82	71.27 ± 6.74	41.18 ± 6.61	46.71 ± 6.90
RP	NH_4^+	DIN	72.48 ± 6.65	70.69 ± 2.91	28.80 ± 6.10	18.64 ± 4.03	3.39 ± 0.71	1.36 ± 0.78
		DON	5.36 ± 1.19	9.53 ± 2.71	24.46 ± 9.22	23.59 ± 3.96	5.44 ± 2.51	6.74 ± 2.12
		Microbial N	23.06 ± 5.72	18.37 ± 3.97	20.60 ± 3.81	35.84 ± 9.84	28.51 ± 16.43	5.98 ± 1.94
		Fine root N	0.76 ± 0.17	0.61 ± 0.13	1.64 ± 0.52	2.08 ± 0.57	6.68 ± 2.57	5.50 ± 0.91
		Bulk soil N	100.49 ± 14.26	90.55 ± 9.79	63.04 ± 6.56	69.74 ± 8.18	67.95 ± 15.17	68.55 ± 8.11
	Gly	DIN	4.82 ± 1.31	5.74 ± 0.71	11.88 ± 2.11	11.41 ± 2.29	4.91 ± 1.62	2.57 ± 0.40
		DON	59.04 ± 16.20	43.68 ± 9.57	20.95 ± 4.93	15.24 ± 3.02	5.76 ± 2.17	3.01 ± 0.69
		Microbial N	12.64 ± 1.95	31.28 ± 5.54	39.61 ± 4.84	46.91 ± 7.62	28.44 ± 9.51	16.97 ± 3.31
		Fine root N	0.42 ± 0.11	0.64 ± 0.13	0.98 ± 0.14	1.36 ± 0.25	2.28 ± 0.65	4.54 ± 0.88
		Bulk soil N	78.20 ± 12.20	70.24 ± 8.50	76.94 ± 10.54	63.27 ± 10.40	56.36 ± 9.77	55.02 ± 12.22
WS	NH_4^+	DIN	33.39 ± 3.73	19.52 ± 3.56	10.77 ± 2.17	13.99 ± 2.69	2.98 ± 1.01	2.15 ± 0.61
		DON	2.74 ± 1.15	6.65 ± 2.79	8.17 ± 3.09	16.94 ± 6.23	2.88 ± 0.77	2.38 ± 0.61
		Microbial N	9.17 ± 3.54	11.24 ± 2.82	15.77 ± 4.58	11.13 ± 5.08	8.56 ± 2.90	4.60 ± 1.81
		Fine root N	0.55 ± 0.12	0.60 ± 0.11	1.25 ± 0.23	1.22 ± 0.17	2.08 ± 0.34	1.89 ± 0.28
		Bulk soil N	80.13 ± 6.25	73.16 ± 6.52	68.06 ± 3.04	86.02 ± 3.58	67.41 ± 6.84	64.46 ± 5.71
	Gly	DIN	20.25 ± 2.58	12.66 ± 2.69	12.79 ± 1.75	11.63 ± 1.95	2.61 ± 0.51	3.09 ± 0.79
		DON	34.12 ± 3.98	14.23 ± 2.54	5.01 ± 1.58	2.70 ± 0.94	1.73 ± 0.32	1.35 ± 0.45
		Microbial N	4.94 ± 2.53	8.05 ± 4.01	13.13 ± 2.50	11.29 ± 2.54	6.51 ± 2.48	4.31 ± 2.43
		Fine root N	0.26 ± 0.05	0.43 ± 0.07	0.71 ± 0.11	0.85 ± 0.11	2.02 ± 0.38	1.63 ± 0.12
		Bulk soil N	60.43 ± 5.04	63.11 ± 4.91	55.62 ± 2.46	66.68 ± 4.44	63.18 ± 6.78	53.70 ± 4.52

Values are percentage of added label.
TP tulip poplar; WO white oak; SM sugar maple; RP red pine; WS white spruce; Gly glycine.
Mean ± SE, n = 6–9.

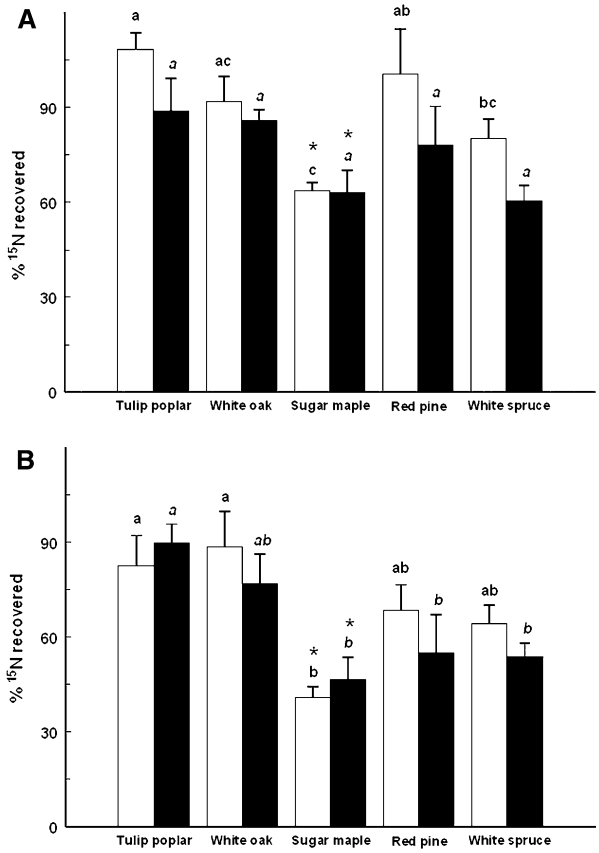

Figure 1. Percent recovery of added ^{15}N in bulk soil N for cores receiving NH_4^+ (*white bar*) and glycine (*black bar*) treatments at **A** 45 min and **B** 14 days following injection. *Letters above the bars* indicate differences ($P \leq 0.05$) among sites within NH_4^+ (*normal text*) and glycine (*italic*) treated cores. Symbols (*) denote differences ($P \leq 0.05$) in recovery between sampling periods within treatment and forest type. *Bars* are mean ± SE, n = 6–9.

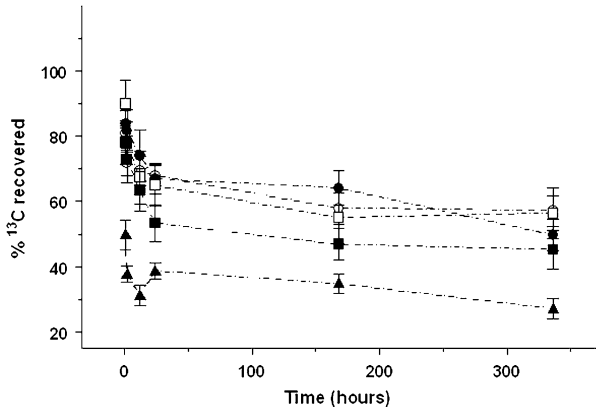

Figure 2. Time-dependent recovery of added ^{13}C in bulk soil pooled across treatments within a forest type. Symbols: *open circle*, tulip poplar; *closed circle*, white oak; *open square*, sugar maple; *closed square*, red pine; and *closed triangle*, white spruce. Mean ± SE, n = 12–18.

60% (averaged across sites) of $^{15}NH_4^+$ label as DIN. This value declined to 20% at 24 h, and to 4% at 2 weeks following injection. This pattern was mirrored by a significant enrichment of the DON pool at all sites, suggesting rapid assimilation and subsequent release of $^{15}NH_4^+$ as organic N by the microbial biomass. Recovery of $^{15}NH_4^+$ as DON-^{15}N increased from 3% at 45 min to 14% at 24 h before declining to near background levels at the conclusion of the 14-day experiment. In glycine-treated cores, we found a similar decline in the recovery of label as DON throughout the experiment. Recovery of ^{15}N-glycine as DON-^{15}N averaged 38% across all sites at the first sampling period, less than 10% at 24 h, and only 5% at the final sampling period. Some of the glycine ^{15}N label was quickly mineralized as we noticed a significant enrichment of the DIN pool at the first sampling period. Average recovery of glycine label in the DIN pool peaked between 12 and 24 h before declining to less than 6% of total ^{15}N addition 2 weeks later.

Immobilization of ^{15}N label by the microbial biomass was apparent for both treatments within the first sampling period and represented the largest sink for ^{15}N among extractable soil N pools (Table 2). In cores receiving $^{15}NH_4^+$, recovery of ^{15}N averaged 14% across all stands at the first sampling period. Recovery peaked 1 day later when microbial immobilization accounted for 21% of the added $^{15}NH_4^+$ before declining to about 11% at 14 days (Figure 3). The amount of ^{15}N recovered in MBN for glycine-amended cores closely mirrored that of cores treated with NH_4^+. Average recovery increased from 15% at 45 min to 25% at 24 h before declining to 14% at 14 days. With the exception of sugar maple (Figure 3C), we observed no significant time-dependent differences in MBN recovery between treatments for any stand type, indicating that both N forms represent a labile N source for microbial assimilation. Total recovery of ^{15}N in the microbial N pool was significantly higher for glycine than NH_4^+ in sugar maple soils ($F_{5,25} = 10.34$; $P = 0.024$). However, this effect disappeared within 12 h after treatment application as ^{15}N recoveries among cores receiving different treatments varied more or less in concert for subsequent sampling periods.

Plant N Uptake In Situ

Net accumulation of ^{15}N in fine roots increased throughout the 2-week experiment for both treatments. After 14 days, average recovery in fine roots ranged from 1.9 ± 0.3% (white spruce) to 8.2 ± 1.1% (tulip poplar) for the NH_4^+ treatment,

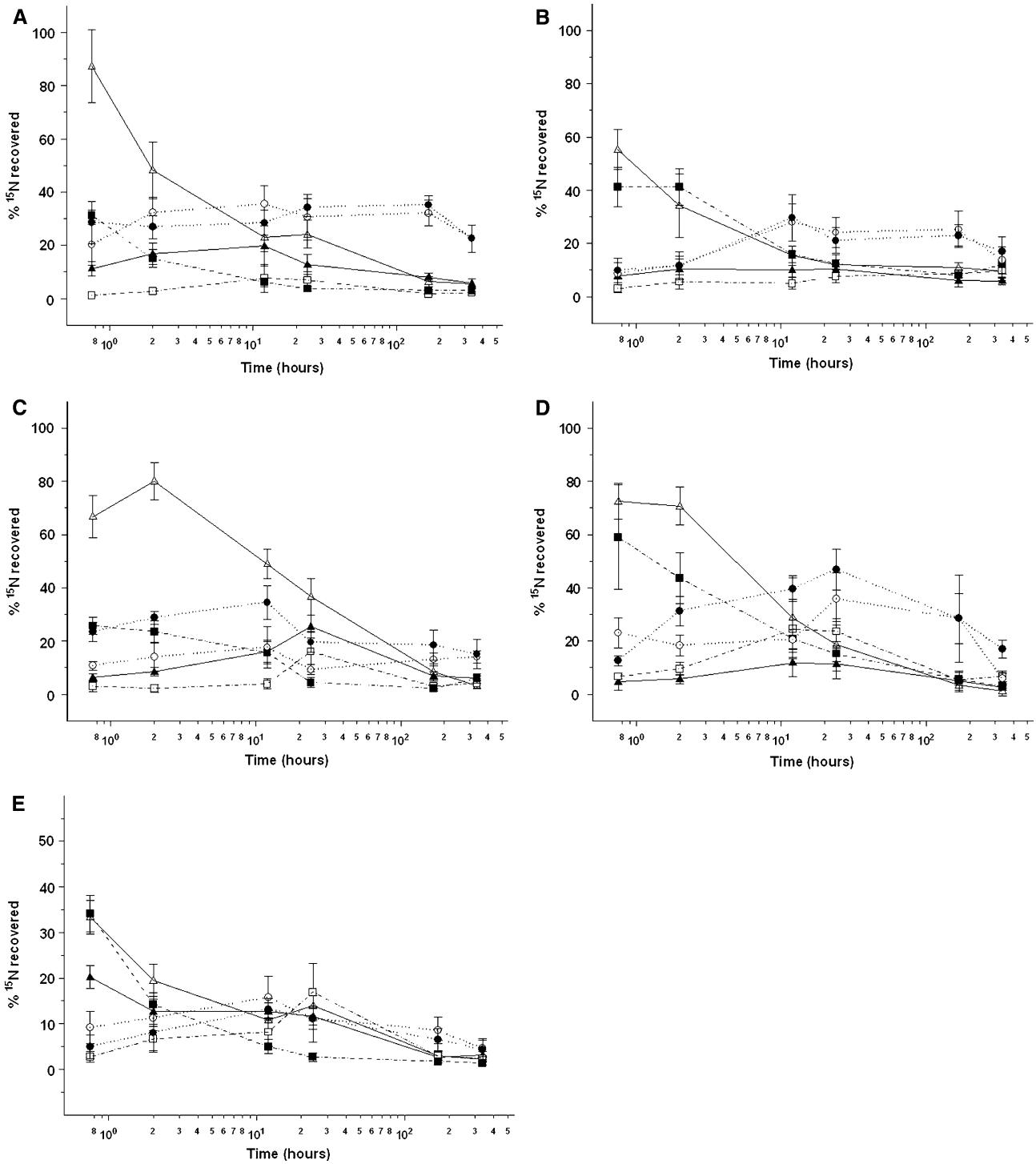

Figure 3. Percent recovery of added ^{15}N within DIN, DON, or microbial N pools against time for **A** tulip poplar; **B** white oak; **C** sugar maple; **D** red pine; and **E** white spruce. Symbols are as follows: *closed circle*, microbial N pool; *closed triangle*, DIN pool; and *closed square*, DON pool. *Open* and *solid symbols* represent ammonium and doubly labeled glycine treatments, respectively. Data are means ± SE.

and from 1.6 ± 0.1% (white spruce) to 5.1 ± 1.5% (tulip poplar) for the glycine treatment. For all stands and sampling periods, ^{15}N recovery in fine roots was higher for cores receiving $^{15}NH_4^+$; however, the significance of this effect was dependent on stand type and sampling period. For example,

12 h into the experiment, root uptake of $^{15}NH_4^+$ was significantly higher than that of ^{15}N-glycine for all temperate deciduous stands, but not for either of the conifers (Figure 4A). Two weeks later, we observed a treatment effect only in tulip poplar fine roots (Figure 4B). The fact that glycine-^{15}N and $^{15}NH_4^+$ were not taken up by fine roots at the same rate in some stands signifies differences either in plant physiological capacity for uptake, or availability of the two N forms, or both (see ''Discussion'' section).

We observed enrichment of fine root ^{13}C above background levels for all forest types at the first sampling period suggesting that a portion of our glycine label was taken up intact. However, in contrast to fine root N, recovery of ^{13}C in fine roots was in most instances less than 1% of additions and demonstrated no significant increase beyond the first few sampling periods. This suggests a substantial fraction of the glycine label was not absorbed intact, but rather much of the ^{15}N sequestered by fine roots was derived from microbial turnover of glycine. To test this idea, we regressed molar excess ^{13}C against molar excess ^{15}N in fine roots from each core receiving the doubly labeled glycine treatment. To expand our comparison, we included data from three balsam poplar stands along the Tanana River in interior Alaska (McFarland and others 2002). Slopes generated from these regressions indicate a decrease in fine root ^{13}C enrichment relative to ^{15}N enrichment throughout the experimental period for all forest types (Figure 5), confirming our assumption that direct uptake of glycine by fine roots was limited to the initial 2 h following treatment application. It is worth noting, however, that root concentrations of either tracer are subject to fluctuation as ^{15}N is translocated to aboveground sinks and ^{13}C is respired from an active fine root biomass. This could result in an over- or underestimation of intact glycine uptake during the initial sampling periods.

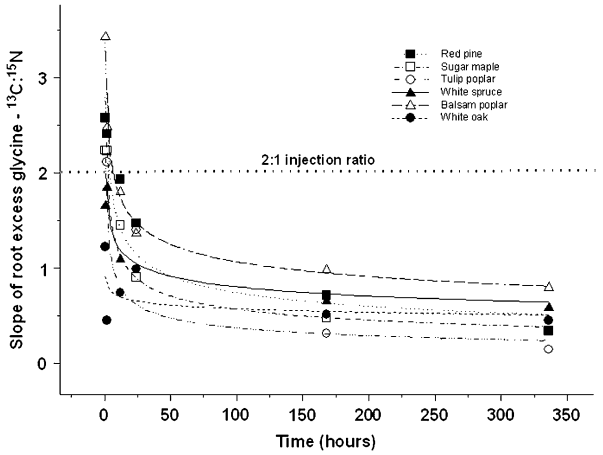

Figure 5. Time-dependent plot of slopes generated from linear regression of molar excess ^{13}C:excess ^{15}N in fine roots of all forest types from the glycine treatment. *Horizontal line* represents the 2:1 injection ratio of C:N administered with the doubly labeled glycine treatment.

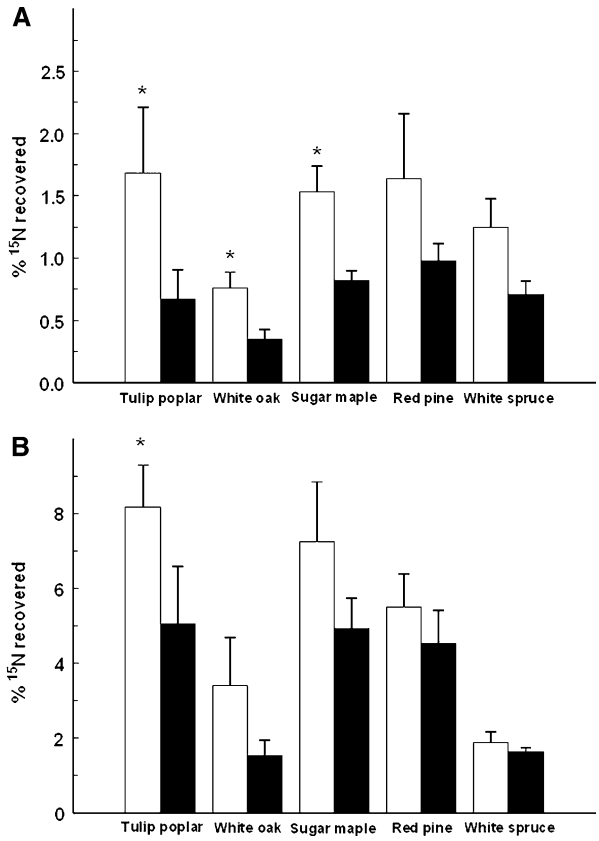

Figure 4. Recovery of ^{15}N in fine roots receiving NH_4^+ (*white bar*) and glycine (*black bar*) treatments at **A** 12 h and **B** 14 days following injection. Symbols (*) denote significant differences ($P \leq 0.05$) between treatments. Note differences in *Y*-axis scales between the two graphs. *Bars* are mean ± SE, $n = 6$–9.

Discussion

Microbial Mediation of Soil N Availability

Soil microorganisms at all sites rapidly incorporated both forms of added N, with up to 36 and 47% of our ^{15}N amendments recovered in MBN within 24 h of soil injections for ammonium- and glycine-treated cores, respectively (Table 2). Microbial immobilization of ^{15}N coincided with a rapid depletion of original labeled forms over the same

time period (Figure 3). Redistribution patterns of added ^{15}N into other soluble N pools and retention within bulk soil suggests that microbial N turnover was a function of soil C to N balance at each site. Generally, soils with narrow C:N ratios are thought to promote higher rates of N processing due to C limitation, whereas microbial function is generally considered more N-limited in soils with a wider C:N ratio (Paul and Clark 1989). Amino acids differ from DIN in their dual function as a source of both metabolic C and N. Therefore, we predicted stands with narrow soil C:N ratios would transform a greater proportion of glycine-N to DIN, compared to N-limited ecosystems where soil microorganisms would retain amino acid-N and C. Data from this experiment are consistent with that prediction. For example, soil N availability was relatively high in sugar maple and tulip poplar, where average C:N ratios in the top 7 cm (13–14) were the lowest recorded among forest types and significantly lower than those of red pine (22) or white spruce (23). Corresponding to these differences, we found that initial recovery of ^{15}N-glycine as DON was 41–56% lower than recovery of $^{15}NH_4^+$ as DIN for the two AM-dominated forest types (Figure 3A, C). Moreover, microbial-^{15}N immobilization in sugar maple was significantly higher during the first 12 h of sampling for cores receiving ^{15}N-glycine. In contrast, we observed no significant difference in recovery between ^{15}N-glycine and $^{15}NH_4^+$ as DON and DIN, respectively, at the first sampling period for any of the EM-dominated stands (Figure 3B, D, E).

Greater immobilization of ^{15}N derived from glycine than NH_4^+ does not necessarily imply microbial preference for glycine-N because dilution effects arising from differences in soil concentrations of amino acid- and NH_4^+-N at each site could mask total microbial uptake for each N form. Moreover, we did not apply correction factors in calculating ^{15}N-MBN. Site-specific differences in extraction efficiency for fumigated soils could complicate cross-ecosystem comparisons of microbial immobilization of our substrates. However, when considering, (1) the majority of the glycine label disappeared from soil DON within 45 min, and (2) mineralization of glycine-N to DIN was higher than the conversion of NH_4^+ to DON under sugar maple and tulip poplar, we believe soil microorganisms in the two AM-dominated stands were utilizing glycine primarily as a C source. Additional support for this hypothesis comes from a companion study that investigated linkages between in situ glycine turnover and the overall decomposability of soil C at these same sites (McFarland and others 2010). Data from that study indicate soils under sugar maple and tulip poplar differed sharply from the EM-dominated stands with respect to their C economy. We found the rate of ^{13}C-labeled glycine turnover to be significantly higher and labile soil C pools to be significantly lower for the two AM-dominated stands than for red pine or white spruce, indicating that rapid immobilization of glycine in tulip poplar and sugar maple may be more a response of C-limitation than N-limitation for microbial growth.

Ecosystem N Retention

With the exception of sugar maple, the majority of our NH_4^+ and glycine label remained in the non-biomass fraction of soil N after cycling through the microbial biomass. After 14 days, retention of ^{15}N in root-free bulk soil ranged from 41 ± 3% (sugar maple) to 83 ± 10% (tulip poplar) for the NH_4^+ treatment, and from 47 ± 7% (sugar maple) to 90 ± 6% (tulip poplar) for the glycine treatment. We have no explanation for relatively poor recovery of both labels in sugar maple soils at the first sampling period, other than mass flow away from the injection site due to steady precipitation shortly following the onset of our treatment applications. Nevertheless, sugar maple was the only site where we witnessed a significant decline in label over time (Figure 1).

Sugar maple soils tend to have high rates of nitrification and leaching (Lovett and Mitchell 2004). As NO_3^- is not a preferred N source for sugar maple (Templer and Dawson 2004; but see Fahey and Yavitt 2005), soils under these trees tend to have lower plant-mediated N retention relative to co-occurring species, for example, beech, yellow birch, or hemlock. Moreover, ecosystem N export is negatively correlated with C:N ratio of the forest floor in forest ecosystems of north-eastern North America (Lovett and others 2002). Low N demand by a relatively C-stressed soil microbial community could explain in part why soil ^{15}N retention was significantly lower in sugar maple than the other forest types, particularly red pine, because both northern temperate stands developed on well-drained sandy soils. Our cores extended only 12 cm below the litter layer, which is a relatively small fraction of the total soil depth in the sugar maple forest. Similarly, we did not measure nitrification rates, so we cannot quantify how much of the unrecovered ^{15}N in sugar maple was either exported from the coring area or resided in lower, unsampled soil horizons.

Short-term redistribution of ^{15}N described here follows patterns of incorporation reported in similar

studies (Näsholm and others 1998; McFarland and others 2002; Kaye and others 2003; Clemmensen and others 2007). Microbes rapidly immobilized labile ^{15}N corresponding to a concomitant decline in availability of the ^{15}N tracer within soluble N pools. Two weeks later, less than 25% of our label was recoverable as DIN, DON, or microbial N at any site, but total recovery of ^{15}N in the bulk soil remained statistically unchanged regardless of the N form initially applied. Microbial assimilation is an important N retention pathway (Zak and others 1990; Zogg and others 2000), but despite uncertainty in the efficiency of chloroform-fumigated soil extractions, microbial immobilization at its peak accounted for less than 50% of total ^{15}N recovery. This suggests that other unmeasured processes, for example, fixation within clay minerals, chemical reactions with soil humus, or production of recalcitrant microbial residues from rapid microbial turnover also contributed to the accumulation of stable soil N.

The relative importance of clay and organic matter in transforming our labile N additions to non-exchangeable forms likely varied with the edaphic characteristics of each site. For instance, the mineral fraction of white oak soils contained up to 40% clay, likely lowering plant and microbial uptake and creating strong ^{15}N retention capacity. However, low clay content at the remaining sites may have abiotically mediated N stabilization via direct chemical inclusion into recalcitrant SOM. Viewed broadly, biotic and abiotic N immobilization obviously have important implications for plant nutrition and N acquisition strategies.

Plant N Uptake

In short-term competition events for N, plants are generally considered to be poor competitors against soil microorganisms (Jackson and others 1989; Zak and others 1990). In our study, fine root recovery of ^{15}N within 24 h of treatment application was approximately an order of magnitude less than microbial immobilization. Though these results appear to conform to the paradigm of plant–microbial competition for N, it is noteworthy that most studies addressing plant–microbial competition for N, including our own, regard the soil microbiota as a black box. However, there are serious flaws inherent to this interpretation. First, the chloroform-fumigation procedure used for extracting and quantifying microbial N is indiscriminate in that it lyses cells from all living organisms in the soil. This includes functional groups such as mycorrhizae, which are part of the plant complex and may not be ''competing'' with the plant for N. Second, as demonstrated by Ruess and others (2006), a large fraction of fine root biomass in boreal forest ecosystems is extremely small (<350 μm diameter). These fine roots are nearly impossible to separate from the soil matrix and are easily fragmented during soil manipulation. Yet, they are the most active in nutrient absorption, and have the highest N content of all root size classes (Pregitzer and others 2002). In many pulse-chase isotope experiments, including this one, it is very possible that a large portion the N attributed to microbial immobilization is actually plant N assimilated in very fine root tips. The resulting analytical bias against plant uptake is proportional to the magnitude of this experimental error.

Fine root N ranged from 7.6 to 16.5 mg g^{-1} and was highest in the AM-dominated stands, tulip poplar and sugar maple, and lowest in the EM-dominated stands, white oak and white spruce. In comparison, Pregitzer and others (2002) recorded fine root N values from the same sites that ranged from 8.5 to 30 mg g^{-1} depending on tree species and root order. Fine root N decreases with increasing root order and it is possible that the lower N concentrations among our fine root samples are attributable to loss of some first order roots (200–300 μm diameter) during the initial processing in our field laboratory. Similarly, N sequestered within extramatrical hyphae in symbiotic association with these fine roots would be even more difficult to isolate from free-living heterotrophic biomass N. Assuming a large part of the nutrients acquired by mycorrhizae fungi are eventually transported to the host, plants may be better ''competitors'' for soil N than currently recognized.

The fact that plant and microbial ^{15}N sequestration initially (<12 h) increased for both N forms, implies that both groups of organisms were effectively targeting the same N resources. Additionally, plants are long-lived relative to microbes and thus we expected fine root acquisition of ^{15}N to increase over time as plants capitalized on tracer remobilized following microbial turnover (Harrison and others 2007). Sequential sampling over 14 days revealed that fine roots steadily accrued the ^{15}N label while recovery of ^{15}N in MBN declined. However, given our experimental design, we were unable to determine whether increased plant recovery of our ^{15}N label stems from microbial turnover or simply delayed transfer of ^{15}N absorbed by extramatrical hyphae of mycorrhizal fungi. Moreover, our estimates of plant uptake are likely conservative due to translocation of the ^{15}N tracer

to aboveground sinks. For example, glycine metabolism in plant roots can yield serine and other products that are preferentially transported out of the root system (Schmidt and Stewart 1999). Similarly, root assimilation of inorganic N, an energy-expensive process, which involves affixing C skeletons to these N forms, can also result in a significant amount of N transport from the root system. However, unlike NO_3^-, which can be accumulated in the root vacuolar compartment as well as reduced for assimilation, NH_4^+ becomes toxic at high concentrations and thus must be assimilated rather quickly. Therefore, in stands where nitrification potential and perhaps root NO_3^- uptake is high (for example, tulip poplar), differences in plant assimilatory pathways for inorganic N may account for comparatively high accumulations of ^{15}N, particularly in cores receiving $^{15}NH_4^+$.

At all sites we established plant capacity to absorb glycine intact, which is not extraordinary in light of a myriad of solution culture studies, which have demonstrated direct amino acid uptake for a broad spectrum of plant species (Bajwa and Read 1985; Kielland 1994; Persson and Näsholm 2001; Finzi and Berthrong 2005; Svennerstam and others 2007; Krab and others 2008). The dogma that plants rely solely on inorganic N for their nutrition has been challenged both in the laboratory and the field (Virtanen and Linkola 1946; Kielland 1994; Schmidt and Stewart 1999; Raab and others 1999; Persson and Näsholm 2001; Miller and Bowman 2003; Finzi and Berthrong 2005; Xu and others 2006), but the usefulness of these studies in establishing plant capacity to utilize alternate N forms only becomes relevant to plant nutrition when examined in view of soil N availability as mediated by microbial, including mycorrhizal, competition for these N forms.

Uptake data of tracer ^{15}N alone may be misleading in assessing the relevance of different N forms to plant nutrition due to differences in availability of endogenous N (sensu Kielland and others 2006). Therefore, we adjusted our estimates of fine root N uptake to account for differential isotope dilution between treatments based on soil concentrations of DIN and FAA-N at each site (Figure 6). To broaden the scope of our comparison, we included plant uptake data from EM-dominated balsam poplar stands growing in the same landscape as our white spruce stands (McFarland and others 2002). We found that relative uptake of amino acid-N versus DIN was significantly ($P \leq 0.05$) lower than 1:1 in tulip poplar and significantly higher than 1:1 in red pine

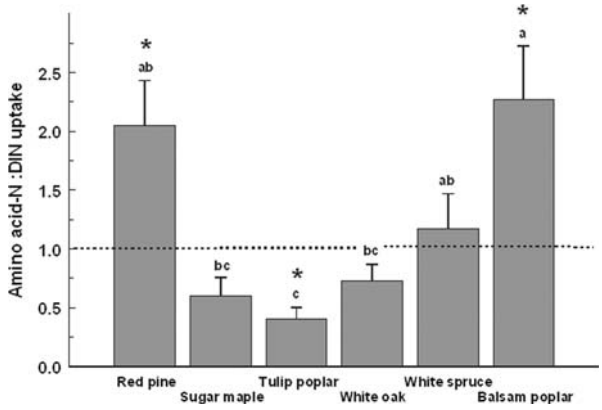

Figure 6. Relative uptake of free amino acids versus DIN for all forest types. *Horizontal stippled line* represents the 1:1 uptake ratio between amino acids and DIN. Mean ± SE, n = 6–9. Calculations for plant N uptake are based on total fine root ^{15}N accumulation at the second sampling period, 2 h following treatment application. *Letters above the bars* indicate differences ($P \leq 0.05$) among sites. Symbols (*) indicate ratios for which 95% C.I. were not found to include 1:1.

and balsam poplar; however, white oak, sugar maple, and white spruce were statistically near unity with respect to the two N forms. Additionally, both EM-dominated white oak and AM-dominated tulip poplar had higher uptake rates for DIN than amino acid-N. Though these observations appear to indicate lack of physiological preference for amino acids based on mycorrhizal association, pool adjusted uptake was significantly different among forest types ($F_{5,34} = 6.02$, $P < 0.001$), and a substantial fraction of this variation was explained by mycorrhizal association. Plant uptake of amino acid-N versus DIN was three-fold higher in EM-dominated stands (1.6 ± 0.2) than AM-dominated stands (0.5 ± 0.1). Thus, FAA appears be an important component of the N economy in all these stands. What remains unclear is whether plant uptake of N is determined more by the availability of N forms or an evolved physiological preference for a particular N form linked to mycorrhizal association (AM versus EM). This uncertainty stems largely from our ignorance of the true extent of the involvement of mycorrhizal association in determining the patterns in N uptake observed in this study. Regardless, our data suggest that the relative importance of inorganic and organic N forms to the N economy of plants could be connected in part to the distribution of these major mycorrhizal types.

Several aspects of our experimental approach limit our interpretations of plant uptake of the ^{15}N tracer. For one, we could not account for secondary

consumption of ^{15}N by plants, particularly in later sampling periods. Cores treated with $^{15}NH_4^+$ yielded detectable quantities of ^{15}N-labeled DON within 45 min of treatment application. We suspect at least some of this ^{15}N released to the DON pool represents exoenzyme production for decomposition of SOM (Sinsabaugh and Moorhead 1994), but the specific chemistry of this pool remains unknown. Once incorporated into the chemical architecture of soil microorganisms, it is impossible to deduce what N forms become plant-available as the microbial pool turns over and the ^{15}N tracer is remobilized. Consequently, over the long-term (>24 h), treatment applications become irrelevant as we cannot discern whether plant ^{15}N absorption reflects uptake of organic or inorganic compounds or from what source (microbial versus bulk soil) that N is derived.

We are equally cautious about our interpretations concerning plant access to both N treatments in the field. In theory, enhanced N availability associated with label additions could have temporarily overwhelmed microbial transporter systems, giving plants access to N outside their usual resource niche for N nutrition (McKane and others 2002). However, in this study, we do not feel our N additions were excessive. K_m values for uptake kinetics of soil amino acids, for instance, suggest that microorganisms typically have low-affinity transport systems. In general, soil concentrations of FAA or NH_4^+ required to saturate the potential for soil microbial uptake are in the millimolar range (Vinolas and others 2001), while our N additions were significantly less.

Conclusion

Quantitative relationships between production and the availability of limiting resources cannot be established until reliable and accurate estimates of organic N cycling and uptake by plants are obtained. Our research provides important insights into the cycling dynamics of labile N in forested ecosystems. To our knowledge, this study is the first to use a common experimental approach to develop quantitative patterns of microbial utilization and plant uptake of inorganic and organic N across a broad geographic and taxonomic range of forest ecosystem species. We found that although plant N uptake was low in comparison to microbial immobilization, accumulation of our ^{15}N tracer in plant tissue over several weeks indicated that they are effective long-term sinks. Plant apparent N preference is governed to a large extent by N availability as mediated by patterns of microbial utilization, and immobilization within abiotic soil pools; however, we could not discount the influence of distinctive plant–fungal symbioses in regulating plant N nutrition. Our data suggest plant capacity to directly absorb amino acids is a pervasive characteristic for a variety of forest types across a large latitudinal gradient, regardless of dominant mycorrhizal association. Moreover, the finding that the ratio of fine root amino acid uptake was 3× higher for stands dominated by EM fungi than AM fungi suggests that mycorrhizal type mediates plant uptake for different N forms. This discrimination of N form by mycorrhizal type is probably due to unique functional attributes of AM versus EM fungi in adaptation to physiochemical properties of the soil environments in which they have evolved.

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