

Cycling Dynamics of NH_4^+ and Amino Acid Nitrogen in Soils of a Deciduous Boreal Forest Ecosystem

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ABSTRACT

Conventional studies of nitrogen (N) cycling in forest ecosystems have focused on inorganic N uptake as the primary source of N for plant metabolism. More recently, however, alternative sources of N for plant nutrition, such as free amino acids, have gained attention, particularly in nutrient-limited systems. Using a multiple stable isotope (^{13}C and ^{15}N) design, that allowed us to simultaneously assess root uptake of ammonium (NH_4^+) and glycine, we compared the cycling dynamics of NH_4^+ and amino acid N within the soils of several interior Alaskan floodplain balsam poplar stands. Our design included multiple sampling periods extending from 45 min to 14 days, which permitted us to study interpool transfers of our carbon (C) and N isotopes over time. Microbial biomass N was the largest sink of both ^{15}N -ammonium and glycine. Percent recovery of ^{15}N for this pool was an order of magnitude larger than fine-root ^{15}N uptake for most sampling periods. Although recovery of ^{15}N in fine-root biomass was small, amino acid N and

NH_4^+ were assimilated at approximately the same rate irrespective of sampling period, and total recovery was still increasing 2 weeks after application. Recovery of ^{15}N in bulk soil samples did not vary significantly over time for either treatment. However, bulk soil ^{13}C declined steadily during the experiment, measuring less than 30% recovery of added label after 14 days. We suspect that the majority of ^{13}C lost from our soils was respired. Soil microorganisms strongly outcompeted plants in the short term for both NH_4^+ and amino acid N. However, amino acid N appears to cycle through soil N pools at approximately the same rate as inorganic N forms. The similarity in uptake patterns for inorganic and organic N suggests that these stands are meeting part of their N requirements directly from amino acids.

Key words: amino acid; glycine; ammonium; balsam poplar; ^{13}C and ^{15}N ; organic nitrogen uptake; microbial biomass N; floodplain; Alaska.

INTRODUCTION

Organic nitrogen (N) is the predominant form of N found in arctic and subarctic soils (Walker 1989; Kielland 1995), existing in a variety of forms from amino acids to heterocyclic N compounds such as purines and pyrimidines (Schulten and Schnitzer 1998). Although fluxes of inorganic N in northern

ecosystems have been studied in detail (Giblin and others 1991; Van Cleve and others 1983), relatively little is known about the role of organic N sources in the N economies of plants in these systems. Moreover, despite the fact that a broad range of plant species have demonstrated the capacity to use organic sources of N (Stribley and Read 1980; Finlay and others 1992; Chapin and others 1993; Kielland 1994, 1997; Schimel and Chapin 1996; Raab and others 1999; Näsholm and others 1998, 2000), most regional and global models predicting ecosystem

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response to changing climate or altered land-use patterns have focused on inorganic N cycling in calculations of net ecosystem carbon (C) flux (Running and Hunt 1993; Melillo and others 1993).

In particular, studies in arctic and boreal ecosystems have shown that annual N mineralization is insufficient to account for the quantity of N absorbed annually by vegetation (Shaver and others 1991; Ruess and others 1996). This would suggest that our understanding of the relationship between ecosystem production and soil N dynamics is still incomplete. Plants in arctic and boreal ecosystems must be sequestering organic N to supplement their N requirements; thus, sequestration represents a major consideration for elucidating the function of N in controlling ecosystem production.

Over the past decade, researchers have begun to assess the relative importance of organic N to the nutritional requirements of plants in cold environments with low-quality soil organic matter (Finlay and others 1992; Chapin and others 1993; Kielland 1994, 1997; Michelsen and others 1996, 1998; Schimel and Chapin 1996). Low soil temperatures reduce the rate of organic matter decomposition, thereby reducing rates of net N mineralization (Yin 1992). Still, the overall availability of N may actually be enhanced relative to the products of mineralization due to elevated amino acid production associated with increased proteolytic activity in soils with a higher proportion of organic matter (Chapin and others 1988; Raab and others 1999). Concentrations and turnover of free amino acids in tundra and taiga environments can be up to an order of magnitude greater than for ammonium (Kielland 1995; Jones and Kielland 2002). Moreover, the energetics associated with the assimilation of amino acid versus inorganic N forms suggest that the former should be a preferable N source for plants. This argument could also be extended to microorganisms, both free-living and symbiotic, since amino acids should provide a high-quality source of metabolic C as well as N. Outside of laboratory manipulations (Jones 1999), however, there is little direct empirical evidence to support this contention.

Competition for N between plants and microorganisms exists to varying degrees in all terrestrial ecosystems, so to discuss plant N uptake without considering the role of microorganisms essentially excludes one of the major factors controlling N availability to plants. For instance, several pulse-chase experiments that have quantified recovery rates of added N label in vegetation and soil pools have found that microbes are a stronger sink for this N source than plants (Jackson and others 1989; Zak

and others 1990; Schimel and Chapin 1996; but see Lipson and Monson 1998). Yet other observations concerning the long-term fate of inorganic ^{15}N applied to various grassland and forest ecosystems are less conclusive (Hart and others 1993). In some instances, plants and microbes demonstrated a similar capacity for sequestering the added label; whereas in others, plants appeared to dominate over the microbial pool in accumulating ^{15}N . Understanding the factors controlling N availability to plants therefore requires a more comprehensive approach to elucidate the pathways for both inorganic and organic N cycling. In intact forest ecosystems, where multiple sinks and processes interact to transform and cycle N, it is difficult to establish patterns for these processes by studying only one form of the resource, that is, NH_4^+ or NO_3^- within an isolated pool. There is a need for more integrated studies that focus on interpool transfers of other N forms, particularly in ecosystems where plants may have the capacity to utilize soluble forms of organic N.

Here we examine, concurrently, the cycling dynamics of NH_4^+ and amino acid N in the soils of a deciduous boreal forest ecosystem dominated by ectomycorrhizal trees and shrubs. We predicted that in nutrient-limited systems, such as taiga forests, where low soil temperatures can slow mineralization rates, organic N sources would assume a greater role than inorganic N sources (for example, NH_4^+) in supplying the annual N requirements of vascular plants. Our study had two objectives. The first was to provide in situ experimental evidence that vegetation in balsam poplar communities along floodplains in interior Alaska can directly absorb amino acids. Secondly, we evaluated the partitioning of inorganic and organic N between plants and soil microorganisms. We addressed these objectives by using a multiple stable isotope design that allowed us to simultaneously assess root uptake of NH_4^+ and glycine.

MATERIALS AND METHODS

Study Site

Our study area was the Bonanza Creek LTER site, 20 km southwest of Fairbanks, Alaska ($64^\circ 51'\text{N}$, $147^\circ 43'\text{W}$; elevation, approximately 120 m). Glacially fed, the Tanana River winds along multiple channels through this landscape, creating a floodplain of alkaline soils that support a mosaic of plant communities representing all stages of the primary-successional sequence. The successional development of these communities begins with sand bar

Table 1. Stand Characteristics for Each of the Three Floodplain Balsam Poplar Study Sites in the Bonanza Creek Experimental Forest

Parameter	Site		
	Stand 1	Stand 2	Stand 3
Soil temperature ($^{\circ}\text{C}$) ^a	9.65 ± 0.18	9.69 ± 0.20	8.40 ± 0.14
Percent total soil carbon			
Organic horizon	32.3 ± 1.5	30.3 ± 4.1	26.3 ± 6.5
Mineral horizon	1.2 ± 0.2	1.3 ± 0.4	2.0 ± 0.6
Percent total soil nitrogen			
Organic horizon	1.8 ± 0.0	1.6 ± 0.1	1.4 ± 0.4
Mineral horizon	0.08 ± 0.01	0.08 ± 0.02	0.13 ± 0.03
Stem density ^b	567	867	922
Basal area ^c	28.6	36.9	44.3
Total tree biomass ^d	1.27 × 10 ⁵	1.68 × 10 ⁵	1.99 × 10 ⁵
Total litterfall ^e	2460.0	2461.0	1828.9
Mycorrhizal root tips ^f	41.3 ± 5.6	61.8 ± 6.6	30.8 ± 3.1
Percent mycorrhizal infection ^g	92	94	97

^aAverage soil temperature measured at 7-cm depth during August 1998.

^bStem density = stems · ha⁻¹

^cBasal area = m² ha⁻¹

^dTotal tree biomass = kg · ha⁻¹ as measured in June 1998

^eLitterfall = kg · ha⁻¹ y⁻¹ collected from September 1997 to September 1998

^fMycorrhizal root tips are live ectomycorrhizal (ECM) root tips per meter balsam poplar fine root

^gPercent mycorrhizal infection is # number of healthy ECM tips per total tips counted. Dead tips or tips that were older or not easily distinguishable as ECM were not counted (Lansing unpublished).

colonization by horsetails (*Equisetum* spp.) and willow (*Salix* spp.) and culminates in a mixture of slow-growing black spruce forest (*Picea mariana*) and muskeg on older terraces (Viereck and others 1993).

In floodplain forests, balsam poplar (*Populus balsamifera*) is the dominant deciduous community type, transitional between thin-leaf alder thickets (*Alnus tenuifolia*) and white spruce (*Picea glauca*) stages of the chronosequence. During the alder stage, rapid N₂ fixation takes place concurrently with mineralization and nitrification. Most of the soil N for the entire chronosequence accumulates during this stage of development (Van Cleve and others 1993b). However, as the alder community succeeds to a closed canopy balsam poplar stand, both N₂ fixation and net nitrification decline (Van Cleve and others 1993a; Uliassi and Ruess 2002).

A combination of field and lab evidence suggests that there are two mechanisms driving N limitation in balsam poplar stands. First, secondary chemicals (tannins) leached from balsam poplar litter may suppress the N₂- fixation rate and the gross N mineralization rate (Schimel and others 1996). Second, microbial N immobilization may be enhanced by the release of labile C compounds. Low-molecular-weight phenolics from balsam poplar litter increased soil respiration in incubation studies, indi-

cating that they are used as microbial substrates (Sugai and Schimel 1993; Schimel and others 1996). The overall effect of these two processes is a reduction in N availability, as a balsam poplar canopy becomes dominant.

Our experiment was conducted in three mature balsam poplar stands distributed along a 5-km stretch of the Tanana River (Table 1). Vegetation structure for these sites includes a closed canopy of balsam poplar with a dense thin-leaf alder understory. Basal area for stems larger than 5 cm averaged 36.7 m² ha⁻¹ and 1.8 m² ha⁻¹ for balsam poplar and alder, respectively, with densities for poplar ranging from 567 to 922 stems ha⁻¹ among the three stands. Total litterfall averaged 279 ± 13 g · m⁻² y⁻¹ across the three stands during the 1998–99 growing season. Rose (*Rosa acicularis*) and high-bush cranberry (*Viburnum edule*) are prominent within the shrub layer, filling understory canopy gaps previously occupied by decadent alder shrubs.

Average daily soil temperatures measured at a depth of 7 cm during the 1998 growing season ranged from a minimum of 3.8°C in May to a maximum of 11.4°C in late July. The soil, classified as typic cryofluvent, consists of an alluvium of fine to medium sand grains overlain by a well-developed organic profile extending to more than –8 cm

in some places. Soil C:N ratios average 19.0 for the organic layer and 16.0 for the mineral soil (J. W. McFarland unpublished). Soil pH was not measured in these stands; however, chemical analysis of mineral horizons in similar stands indicates that they are calcareous and therefore alkaline (Marion and others 1993). Values for the forest floor are actually mildly acidic, ranging between 5.6 and 6.4 depending on depth (Van Cleve and others 1983).

Field

Our tracer experiment was conducted during August 1998 in previously established 30×30 m plots, one plot per stand. Within each plot, there were three subplots; each subplot contained three injection grids, one for each of three treatments. The entire design was replicated across three stands. Injection grids measured 81×15 cm and consisted of six identical templates with 37 holes each (222 total/grid). Each grid was injected with one of three treatment solutions in the upper 10 cm of soil. The treatment solutions were as follows: (a) $^{15}\text{NH}_4^+$ plus U- $^{13}\text{C}_2$ -glycine (ammonium treatment), (b) NH_4^+ plus U- $^{13}\text{C}_2$ [^{15}N]-glycine (glycine treatment), or (c) distilled water (control).

Using doubly labeled glycine in the second treatment allowed us to evaluate the root uptake of intact amino acids by comparing the ratio of ^{15}N and ^{13}C found within fine-root tissue to the 2:1 ratio of ^{13}C and ^{15}N found in the tracer (Nasholm and others 1998). In theory, a ratio of less than 2:1 indicates that at least a portion of the ^{15}N sequestered by fine roots receiving this treatment was mineralized from glycine prior to assimilation. Unlabeled ammonium was added to the second treatment to mirror any fertilization effect created by the simultaneous addition of labeled ammonium and glycine in the first treatment. The injection volume was 2 ml, which applied approximately $0.39 \text{ g } ^{13}\text{C m}^{-2}$ and $0.22 \text{ g } ^{15}\text{N m}^{-2}$ for both labeled solutions. Total N additions for each treatment averaged $10.51 \mu\text{gN g}^{-1}$ dry soil ($5.255 \mu\text{g}$ as NH_4^+ -N and $5.255 \mu\text{g}$ as glycine-N). Each 2-ml aliquot was delivered by inserting the needle to a 10-cm depth and emptying the repeating pipette as the needle was withdrawn.

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 (Figure 1). The center of each coring hole exactly matched the center of its respective injection template. Theoretically, this permitted us to remove a soil core 12 cm in depth with a known amount of added label ($600 \mu\text{g } ^{15}\text{N}$ and $1040 \mu\text{g } ^{13}\text{C}$ per core; see Calculations section). Both the injection grids and the coring grids were constructed of 0.32-cm Lexan sheets, which were

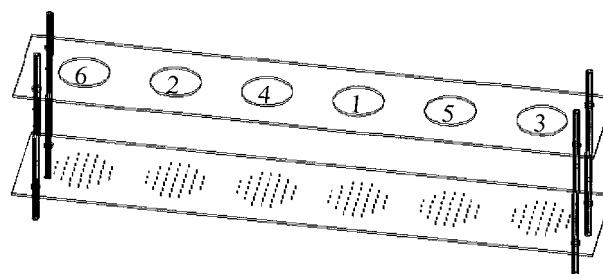


Figure 1. Coring scheme used for each sampling period. Treatments are as follows: TRT A (labeled ammonium treatment) = $^{15}\text{NH}_4^+\text{SO}_4$ + U- $^{13}\text{C}_2$ -glycine; TRT B (doubly labeled glycine treatment) = $[\text{NH}_4^+]\text{SO}_4$ + U- $^{13}\text{C}_2$ [^{15}N]-glycine; TRT C (control) = deionized H_2O . Cores within each grid were injected to 10 cm and harvested to 12 cm at 45 min; 2, 12, and 24 h; and 7 (168 h) and 14 (336 h) days. Sampling periods were randomized within grids. Sampling periods: 1 = $T_{45\text{min}}$, 2 = $T_{2\text{h}}$, 3 = $T_{12\text{h}}$, 4 = $T_{24\text{h}}$, 5 = $T_{168\text{h}}$, 6 = $T_{336\text{h}}$.

flexible enough to mold to the surface of the forest floor. Grids were held in position by four steel pins buried to a depth of 20 cm, which made it easy to properly align the coring grids over the respective injection grids.

Within each stand, subplots were separated by a minimum of 5 m. Injecting a complete subplot took approximately 30 min, after which we began harvesting the first in a series of cores for each treatment. Cores within each grid were harvested to 12 cm at 45 min; 2, 12, and 24 h; and 7 and 14 days. Initial processing was conducted on site in a tent laboratory. Immediately following sampling, 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 (a) total soil C and N, (b) extractable dissolved inorganic N (DIN) and dissolved organic N (DON), and (c) microbial N. No rocks were found in any of the cores collected; therefore, soils were hand-sorted and mixed to minimize any disturbance associated with sieving. After initial processing, all root and soil samples were frozen with liquid N_2 in the field and stored on ice for transport to our laboratory facilities.

Laboratory

Root samples were thawed and floated briefly in distilled water to remove all remaining organic matter. We separated roots by size. Roots measuring over 1 mm in diameter were classified as coarse; roots measuring 1 mm or less were classified as fine. Fine roots were freeze-dried and ground using a Wiley mill (40 mesh) and subsequently powdered

using a ball mill to ensure complete homogenization within each sample. Soil moisture content was determined by drying subsamples at 70°C for 36 h. These subsamples were ground using a ball mill. Both roots and soils were then analyzed for C, N, ^{13}C , and ^{15}N using a Europa Scientific continuous flow mass spectrometer (PDZ Europa, Inc.).

Samples for determination of DIN were extracted in the field with 0.5M K_2SO_4 containing 5 ppm phenyl mercuric acetate for 1 h, vacuum-filtered through Whatman no. 1 filter paper, and stored frozen until analysis. After thawing, these samples were analyzed for NH_4^+ and NO_3^- by flow injection colorimetry using a Lachat autoanalyzer (Keeney and Nelson 1982) and prepared for ^{15}N analysis by a diffusion procedure. The procedure entails pipetting 20 ml of the sample into a 140-ml plastic specimen container. A 4-mm disk cut from Whatman GF/D glass fiber filters was suspended on a stainless steel wire above the solution in the plastic container. The disk was acidified with 10–15 μl of 1.0M H_2SO_4 . Five acid-washed glass beads were added to the container 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, each container was spiked with 50 μl of a 100-ppm ($^{14}\text{NH}_4$) $_2\text{SO}_4$ solution (0.366% ^{15}N). Approximately 0.2 g of MgO was added to each container just prior to sealing. The container was then placed on an orbital shaker table at 85 rpm for 5 days. Standards with a known atom % ^{15}N were analyzed along with the samples to evaluate diffusion efficiency.

Microbial biomass N (MBN) was determined using a fumigation–extraction technique (Brookes and others 1985). Soils were fumigated in the field with ethanol-free chloroform for 24 h in a modified pressure cooker, transported to our laboratory under vacuum, and extracted in 250-ml glass beakers with 0.5M K_2SO_4 . Fumigated and DIN extracts were digested using a modified micro-Kjeldahl procedure (Bremner and Mulvaney 1982) and analyzed colorimetrically with a Technicon continuous flow autoanalyzer (Whitledge and others 1981). DON was calculated as the difference between digested and undigested extracts. MBN was calculated as the difference between fumigated N and DON. No correction factor (K_{ec}) was used in the calculations.

Fumigated and dissolved organic N extracts were diffused in a Mason jar unit described by Khan and others (1997). Approximately 10 ml of the Kjeldahl digest was pipetted into the Mason jar unit. Two quartz filter disks (Whatman QM-A) were placed on stainless steel holders attached to the lid of each

Mason jar and acidified with 10 μl of 0.5M H_2SO_4 . Again, five acid-washed glass beads were added to each jar along with 10 ml of 10M NaOH. The jar was sealed and heated to 45°C overnight.

Calculations

All isotope values for DIN, DON, MBN, fine-root N and C, and soil N and C are reported as percent (%) recovery of added label, with the exception of the fine-root C pool, which is also reported in delta notation. We used delta notation in this instance so we could observe, with greater resolution, the subtle enrichment of this pool against background. $\delta^{13}\text{C}$ was calculated using the following formula:

$$\delta^{13}\text{C} = 1000 \cdot$$

$$(\text{sample } \%^{13}\text{C} - \text{PDB } \%^{13}\text{C}) / \text{PDB } \%^{13}\text{C}$$

The percent of added label recovered in a particular pool was determined by multiplying the ^{13}C or ^{15}N atom percent enrichment (APE) of the pool by the pool size (μg N or C per gram dry soil) and dividing this value by the amount of label added to the core. 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 a site prior to use in estimating enrichment. For each core, the area injected with label was over twice as large as the area extracted for analysis. Therefore, we used the surface area ratio of the injection template to the coring template (Figure 1) to estimate the fraction of label (600 μg ^{15}N and 1040 μg ^{13}C) that was injected into each treatment core.

Statistical Analysis

All C and N pools were analyzed using analysis of variance (ANOVA) with subplots nested within stands. Response variables were either percent of added label (^{15}N or ^{13}C) recovered or pool size (μg N or C). Factor effects tested in these analyses included stand, subplot (within stand), treatment, and sampling period. Multiple comparisons for relevant factor effects were conducted using Tukey HSD tests. We assumed plot, treatment, and sampling period to be fixed and subplot to be random. All inferences regarding pool dynamics are made at the stand level.

Due to missing values from two of our stands, we were faced with an unbalanced design in many of our analyses. Therefore, we opted to use a mixed-model analysis (PROC MIXED, SAS Systems version 6.12, 1996; SAS Institute, Cary, NC, USA) to evaluate variance within all our extractable N pool

Table 2. Mean Percent Recovery (± 1 SE) of Added ^{15}N within Various Plant and Soil Pools

N form	Pool	Recovery of added ^{15}N (%)					
		45 min	2 h	12 h	24 h	168 h	336 h
NH_4^+	DIN	20.90 \pm 3.55	20.96 \pm 3.84	10.95 \pm 2.93	1.96 \pm 0.15	8.41 \pm 1.48	4.71 \pm 1.07
	DON	16.09 \pm 5.52	13.64 \pm 4.08	14.01 \pm 7.58	21.54 \pm 4.89	7.15 \pm 4.19	6.15 \pm 1.35
	Microbial N	45.89 \pm 9.16	49.90 \pm 16.0	26.80 \pm 7.74	64.20 \pm 11.7	21.17 \pm 3.96	12.56 \pm 3.44
	Fine-root N	0.07 \pm 0.01	0.17 \pm 0.03	0.52 \pm 0.10	0.63 \pm 0.17	1.39 \pm 0.28	1.64 \pm 0.22
	Bulk soil N	58.93 \pm 5.89	71.14 \pm 6.98	68.44 \pm 6.06	79.30 \pm 11.9	67.04 \pm 6.28	73.37 \pm 6.94
Glycine	DIN	9.16 \pm 3.69	7.73 \pm 2.74	7.03 \pm 0.93	1.55 \pm 0.39	4.80 \pm 0.48	3.10 \pm 0.38
	DON	24.56 \pm 5.26	16.06 \pm 4.94	10.48 \pm 1.82	18.61 \pm 2.75	6.26 \pm 2.95	7.06 \pm 1.25
	Microbial N	30.70 \pm 16.0	53.05 \pm 7.39	21.70 \pm 4.94	37.95 \pm 6.07	19.19 \pm 2.70	10.34 \pm 2.61
	Fine-root N	0.08 \pm 0.02	0.24 \pm 0.04	0.31 \pm 0.08	0.52 \pm 0.07	0.94 \pm 0.18	1.39 \pm 0.28
	Bulk soil N	58.41 \pm 6.29	60.56 \pm 7.29	57.85 \pm 3.62	69.19 \pm 9.89	50.04 \pm 6.56	65.00 \pm 10.7

N, nitrogen; NH_4^+ , ammonium; DIN, dissolved inorganic nitrogen; DON, dissolved organic nitrogen

Since stand was not a significant factor effect in any of the analyses (see Table 3), nine replicate cores, three from each stand, were pooled together for each nitrogen substrate at each time period.

Note that not all pools contain complete replication.

data sets. We included a repeated-measures component in our design to model variation within subplots across all sampling periods and chose autoregressive order one as our covariance structure within subjects.

For root data from the $^{13}\text{C}^{15}\text{N}$ -glycine treatment, we regressed excess ^{13}C against excess ^{15}N for each time period (Nasholm and others 1998) and compared the slopes for each regression line to a slope of 2 (injection ratio of C:N for glycine) using a two-sided *t*-test for comparing two slopes.

RESULTS

Pool Size and Background ^{13}C and ^{15}N

N pool size varied dramatically between the various soil components. MBN (mean \pm standard error [SE]) averaged $155.7 \pm 7.9 \mu\text{g N g}^{-1}$ dry soil across all treatments and time periods. This represented approximately 3.0% of total soil N and was substantially higher than soluble organic and inorganic N pools. Although treated cores typically had a larger pool size ($F_{2,55} = 3.74$, $P = 0.03$), there was no significant shift in microbial N over time within any treatment. No significant treatment or time effects were observed in analyses of the DIN or DON pools, which averaged 9.3 ± 1.3 and $65.6 \pm 3.7 \mu\text{g N g}^{-1}$ dry soil respectively, across all treatments and sampling periods. Thus, on average, the N additions associated with each treatment enhanced the DIN pool by 56% and the DON pool by 8%. Total root biomass averaged 27.4 mg g^{-1} dry soil for all sites.

Fine roots made up less than 15% of this mass and, at 1–2% N, accounted for an average of $47 \mu\text{g N g}^{-1}$ dry soil.

Altogether, ^{15}N values for our control samples fell within reasonable ranges for ambient $\delta^{15}\text{N}$ values for all pools measured. Previous values reported from similar floodplain stands averaged -2.2% and -4.1% , respectively, for bulk soils and roots (K. Kielland unpublished). Soil $\delta^{15}\text{N}$ values for our control cores (0–12 cm) averaged $1.3 \pm 0.4\%$. Fine-root values were slightly depleted at $-0.7 \pm 1.1\%$ relative to the bulk soil pool. ^{13}C abundance in the fine-root pool averaged -28.14% (± 0.07), which is also consistent with previous measurements taken for floodplain vegetation (K. Kielland unpublished). Variation in ^{13}C and ^{15}N abundance of each pool was small for control cores across time, while $\delta^{15}\text{N}$ values for treated cores ranged one to two orders of magnitude higher than natural abundance for most pools and time periods. This result suggests that our ability to detect treatment effects in each pool over time was strong despite any fractionation associated with interpool transfer of N.

Recovery of ^{15}N and ^{13}C in Soil

Recoveries of ^{15}N from labeled ammonium and glycine for each pool are summarized in Table 2. Total recovery of ^{15}N was similar across sites ($F_{2,88} = 2.13$, $P = 0.12$) for bulk soil samples (soil from which only roots are removed). Mean recovery for bulk soil was not significantly different over time for either treatment. However, the total amount of

Table 3. The Effect of Treatment, Sampling Period, and Stand on Percent Recovery of Added Tracer within Soil Extractable Nitrogen (N) and Fine Root and Bulk Soil Carbon (C) and Nitrogen Pools

Pool	Source	df	F	P
DIN	Stand	2, 58	0.43	0.66
	Treatment	1, 58	22.21	^b
	Sampling period	5, 58	12.12	^b
	Treatment × Sampling period	5, 58	3.24	^a
DON	Stand	2, 58	2.05	0.14
	Treatment	1, 58	0.10	0.75
	Sampling period	5, 58	3.51	^a
	Treatment × Sampling period	5, 58	0.56	0.73
MBN	Stand	2, 34	0.97	0.39
	Treatment	1, 34	3.59	0.07
	Sampling period	5, 34	7.40	^b
	Treatment × Sampling period	5, 34	1.00	0.43
Fine-root C	Stand	2, 88	1.33	0.27
	Treatment	1, 88	0.09	0.76
	Sampling period	5, 88	7.82	^b
	Treatment × Sampling period	5, 88	0.71	0.61
Fine-root N	Stand	2, 88	1.33	0.27
	Treatment	1, 88	3.11	0.08
	Sampling period	5, 88	26.76	^b
	Treatment × Sampling period	5, 88	0.72	0.61
Bulk soil C	Stand	2, 88	1.82	0.17
	Treatment	1, 88	2.10	0.15
	Sampling period	5, 88	18.17	^b
	Treatment × Sampling period	5, 88	1.22	0.31
Bulk soil N	Stand	2, 88	2.13	0.12
	Treatment	1, 88	4.86	0.03
	Sampling period	5, 88	1.33	0.26
	Treatment × Sampling period	5, 88	0.26	0.93

MBN, microbial biomass nitrogen; DIN, dissolved inorganic nitrogen; DON, dissolved organic nitrogen
Data were analyzed using a mixed model ANOVA ($\alpha = 0.05$)

^a $P \leq 0.01$

^b $P \leq 0.001$

^{15}N recovered from the NH_4^+ -amended ($69.7 \pm 3.0\%$) cores was on average 9% higher across all time periods than soil from cores that received glycine ($60.2 \pm 3.1\%$; $F_{1,88} = 4.86$, $P = 0.03$). More importantly, however, the high rate of recovery for ^{15}N in the bulk soil pool after 14 days indicates that most of the labeled N applied to our soils was retained over time. In contrast to ^{15}N , recovery of ^{13}C in bulk soil samples declined steadily over time (Figure 2), measuring less than 30% after 14 days. Initial recovery values for samples harvested at 45 min ranged between 45% and 50% of added label, depending on the treatment combination. The rapid loss of ^{13}C in the initial hours of the experiment suggests that glycine represents a good energy source for soil microorganisms (although we can't differentiate root respiration from microbial respiration). Given the high N retention and rapid C loss,

it would appear that microbes are assimilating glycine to utilize the carbon skeleton for metabolism rather than for biosynthesis.

Of the various components of bulk soil N, MBN represented the largest sink for ^{15}N , regardless of treatment. It is clear that glycine represents a relatively labile N source for microbial assimilation since the recovery rates for glycine ^{15}N were of the same magnitude as those for $^{15}\text{NH}_4^+$. Enrichment within this pool was very rapid for both treatments, averaging 46% recovery for ammonium and 31% recovery for glycine at 45 min after injection. Percent recovery of ^{15}N for this pool ranged from 10% to 64%, depending on the treatment and sampling period. MBN in both ^{15}N treatments varied more or less in concert over time, since there was no significant time × treatment interaction ($F_{5,34} = 1.00$, $P = 0.43$); however, recovery rates appeared to be

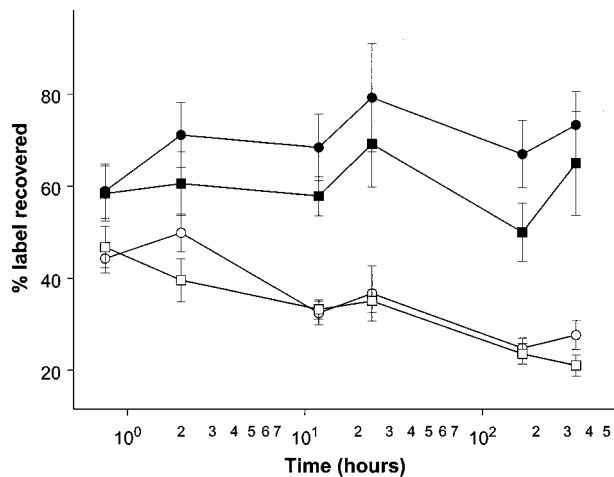


Figure 2. Percent recovery of added ^{13}C and ^{15}N in bulk soil carbon and nitrogen pools over time. ● = labeled ammonium treatment ($^{15}\text{NH}_4^+ + ^{13}\text{C}$ -glycine); ■ = doubly labeled glycine treatment ($^{14}\text{NH}_4^+ + ^{13}\text{C}^{15}\text{N}$ -glycine). Open symbols represent soil carbon; solid symbols represent soil nitrogen. Values are averaged across cores ($n = 9$). Data are means ± 1 standard error of the mean.

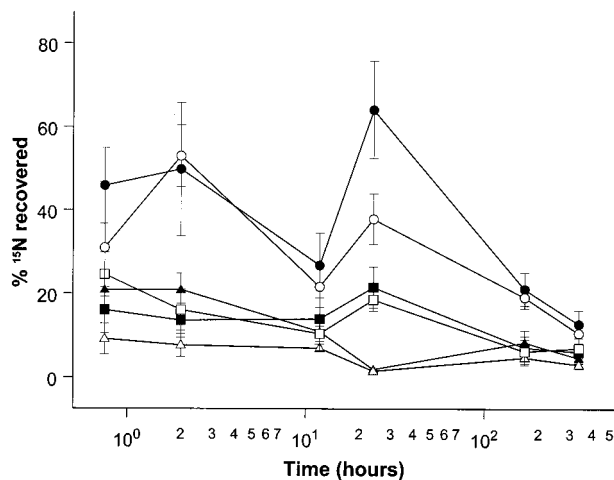


Figure 3. Percent recovery of added ^{15}N as DIN, DON, or microbial N pools against time. ● = microbial N pool; ▲ = DIN pool; ■ = DON pool. Open symbols represent ammonium treatment ($^{15}\text{NH}_4^+ + ^{13}\text{C}$ -glycine); solid symbols represent doubly labeled glycine ($^{14}\text{NH}_4^+ + ^{13}\text{C}^{15}\text{N}$ -glycine) treatment. Data are means ± 1 standard error of the mean.

higher in the ammonium treatment than for glycine at some time periods (Figure 3). After 24 h, microbial immobilization of added ^{15}N was almost 70% higher in the ammonium treatment than it was for glycine. We observed no differences between the labeled treatments in the microbial pool after 24 h.

The amount of label recovered as DIN was low.

Initial recoveries of DIN- ^{15}N varied from 9% for glycine-amended cores to 21% for those receiving ammonium. Microbial immobilization was rapid for the ammonium treatment, since most of the tracer disappeared from the DIN pool within 24 h after injection. ^{15}N enrichment within the DIN pool peaked again at 7 days for ammonium cores before falling below 5% recovery at 14 days. Across all sampling periods, less than 10% of the label was recovered as DIN in soils receiving glycine, indicating that this N form was either retained within the microbial pool or remained in the soil as organic N (DON). Still, recovery of ^{15}N -DIN administered as glycine was highest in the first sampling period, suggesting that at least part of this substrate was rapidly mineralized and released as inorganic N. Within 24 h, the DIN pool accounted for less than 2% of the glycine label. Recovery peaked again at 7 days, but not to the degree that we observed in the ammonium treatment. No significant treatment effect was detected in the DIN pool on the final sampling date.

Although the distribution of label over time in the DON pool did not vary significantly with treatment application ($F_{5,58} = 0.56$, $P = 0.73$), we did observe distinct patterns of DON cycling when compared with the DIN and microbial pools. Label recovered as DON averaged 16% for the NH_4^+ treatment and 25% for the glycine treatment at the first sampling period. These values declined slowly for both treatments, until the fourth sampling period (24 h), after which % recovery of ^{15}N increased for both N sources. This reenrichment of the DON pool after 24 h corresponds to a concomitant increase in microbial biomass ^{15}N (Figure 3) and a decrease in extractable inorganic ^{15}N .

Recovery of both ^{15}N tracers in the microbial pool peaked at 2 h and again at 24 h even though percent recovery of ^{15}N in bulk soil showed no significant change over time ($F_{5,88} = 1.33$, $P = 0.26$) for either ammonium- or glycine-amended soils. If labeled N were following a path of immobilization, mineralization, and excretion as excess NH_4^+ or NO_3^- we would expect to see a steady increase in microbial ^{15}N corresponding to a steady decrease in DIN ^{15}N and vice versa as N was released from the microbial pool. Percent recovery of ^{15}N in the microbial pool did not increase steadily over time, but it oscillated significantly during the first four sampling periods; whereas recovery of ^{15}N as DON increased for both treatments between 12 and 24 h. However, after 2 weeks, the amount of label recovered as DON had fallen to about 6%–7%, similar to the values observed for DIN. These observations suggest (a) that some fraction of the micro-

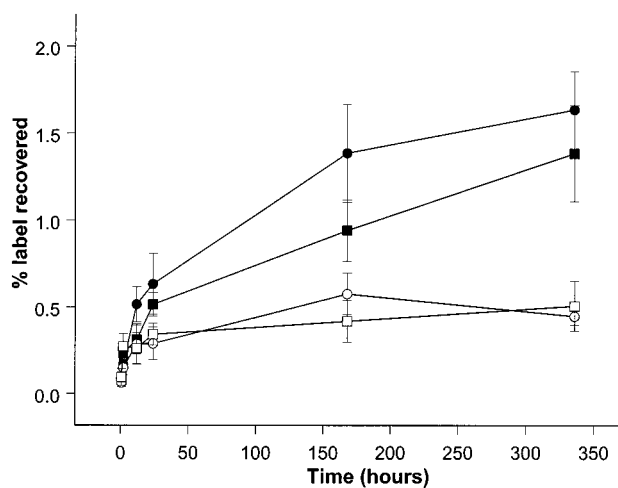


Figure 4. Percent recovery of added ^{13}C and ^{15}N in fine-root carbon and nitrogen pools over time. ● = labeled ammonium treatment ($^{15}\text{NH}_4^+$ + ^{13}C -glycine); ■ = doubly labeled glycine treatment ($^{14}\text{NH}_4^+$ + $^{13}\text{C}^{15}\text{N}$ -glycine). Open symbols represent root carbon; solid symbols represent root nitrogen. Data are means \pm 1 standard error of the mean.

bial population might be releasing extracellular enzymes (into the DON pool) for degradation of more complex organic substrates (see Discussion), and (b) that most of the ^{15}N remaining in the soil after 2 weeks must be locked up in a recalcitrant (non-extractable) organic N form.

Root Uptake

Amino acid N and NH_4^+ were taken up by fine-root biomass at approximately the same rate (Figure 4); overall, there was no treatment effect on percent recovery of ^{15}N in fine roots ($F_{1,88} = 3.11$, $P = 0.08$). Although total recovery of ^{15}N in fine-root biomass was small, averaging 1.39% and 1.64% for glycine and NH_4^+ , respectively, at 14 days, total recovery was still increasing after 2 weeks in both treatments. In contrast, fine-root C showed a 2% enrichment of ^{13}C over the first 24 h, but no significant change over the next 13 days (Figure 5). Enrichment of the fine-root C pool stopped somewhere between 12 and 24 h, suggesting that any glycine ^{15}N sequestered by fine roots after 12 h was not assimilated as an intact amino acid.

To evaluate this idea, we regressed molar excess ^{13}C against molar excess ^{15}N in fine roots for each sampling period (Nasholm and others 1998). This calculation allowed us to determine how fine-root ^{13}C and ^{15}N from each sampling period compared to the 2:1 C:N injection ratio that was administered to soils receiving the doubly labeled glycine treatment

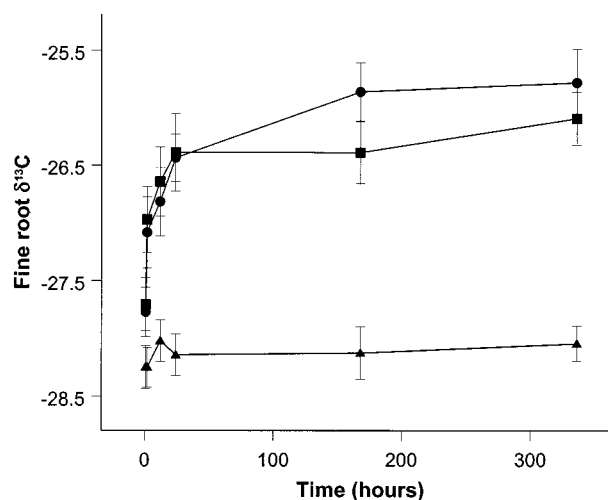


Figure 5. Change in $\delta^{13}\text{C}$ values of the fine-root carbon pool over time. This figure is provided to show treatment effects at a finer resolution. ● = labeled ammonium treatment ($^{15}\text{NH}_4^+$ + ^{13}C -glycine); ■ = doubly labeled glycine treatment ($^{14}\text{NH}_4^+$ + $^{13}\text{C}^{15}\text{N}$ -glycine); ▲ = deionized water. Data are means \pm 1 standard error of the mean.

(Figure 6). Our data demonstrate an enrichment of ^{13}C in excess of the 2:1 ratio within fine-root biomass for the first 2 hours of the experiment, after which fine-root ^{13}C enrichment began to decline, indicating an excess of ^{15}N relative to ^{13}C in roots from subsequent time periods. Slopes for all time periods except 12 h are significantly different from a 2:1 ratio.

DISCUSSION

To date, much of the research in these balsam poplar stands concerning soil N has focused on the conventional pathways of nutrient acquisition by plants—namely, mineralization and nitrification (Klingensmith and Van Cleve 1993; Van Cleve and others 1993b). Yet we know that these traditional pathways cannot account for all of the N absorbed by plants in floodplain balsam poplar stands. Using the sum of mineralization, fixation, and precipitation inputs of N to estimate apparent N uptake in balsam poplar, Ruess and others (1996) determined that apparent plant uptake values would have to be increased threefold to account for fine-root production in these stands. Herein we discuss the hypothesis that part of the unexplained N in balsam poplar biomass can be explained by direct uptake of organic N.

In our experiment, microbial biomass accounted for the largest biologically active fraction of labeled

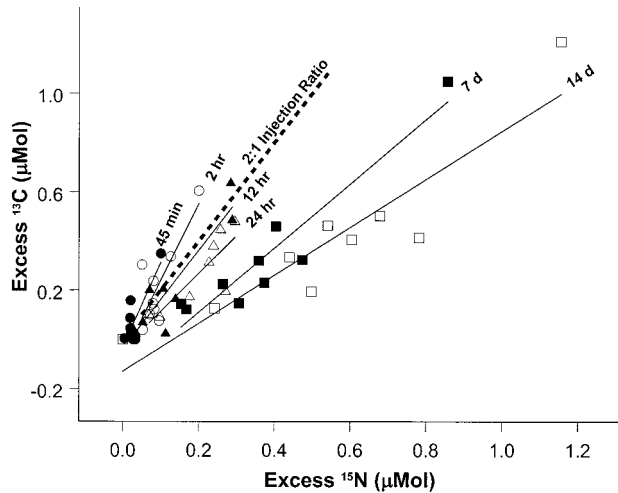


Figure 6. The relationship between excess ^{13}C (μMol) and excess ^{15}N (μMol) in fine roots at all time periods. Data are from the doubly labeled glycine treatment only. Units are per gram dry weight. Sampling periods are plotted above their respective regression lines. Slopes and adjusted R^2 values for each regression are as follows: ●, 45 min ($s = 3.44$, $R^2_{\text{Adj}} = 62.4$); ○, 2 h ($s = 3.13$, $R^2_{\text{Adj}} = 61.1$); ▲, 12 h ($s = 2.00$, $R^2_{\text{Adj}} = 82.6$); △, 24 h ($s = 1.54$, $R^2_{\text{Adj}} = 62.6$); ■, 168 h ($s = 1.30$, $R^2_{\text{Adj}} = 88.3$); □, 336 h ($s = 0.98$, $R^2_{\text{Adj}} = 82.5$). The dotted line represents the 2:1 injection ratio of C:N administered with the doubly labeled glycine treatment.

N. In the first 24 h of sampling, we recovered up to 65% of the ^{15}N we injected as ammonium within the microbial N pool. Perhaps of equal interest, however, over 50% of the ^{15}N administered as glycine was also recovered within this pool over the same time period. This is not surprising in light of recent work examining amino acid N turnover rates in soils from taiga ecosystems (Jones and Kielland 2002). In a study of 10 contrasting soil types, Jones (1999) used a cocktail of 15 different ^{14}C -labeled amino acids in an incubation experiment designed to assess the effects of soil type, depth, and temperature on the decomposition rates of these N sources by soil microbial populations. Their results suggest that amino acid uptake and assimilation in soils is a very rapid process, with half-lives ranging from 1 to 12 h depending on the soil type and temperature. Other ^{15}N tracer studies have reported rates and magnitudes of microbial N immobilization similar to our own (Jackson and others 1989; Schimel and Chapin 1996; Zogg and others 2000). Zak and others (1990) found that microbial immobilization of N in a northern hardwood forest in early spring was an order of magnitude higher than plant uptake. They concluded that microbial N retention could actually reduce the potential for N losses from this

system at a time when N export is at a maximum prior to overstory development.

The fluctuations of microbial N over the initial 24 h of the experiment could reflect microbial metabolism of absorbed amino acids. In the case of glycine, for example, microbes may be assimilating the amino acid, stripping N from the carbon skeleton and excreting excess N (Barraclough 1997). Some of the C could be used for microbial biosynthesis. However, during a companion experiment conducted in floodplain white spruce stands, we measured a rapid pulse of $^{13}\text{CO}_2$ prior to extracting cores treated with labeled glycine (J. W. McFarland unpublished). Most likely, a significant amount of the assimilated glycine in our balsam poplar soils is used as an energy source. The fact that we observed a decrease in the recovery of ^{13}C within the bulk soil pool supports this hypothesis. Across all stands, % ^{13}C recovered in the bulk soil pool dropped from almost 50% to just over 20% 2 weeks after injection. Although our evidence is largely circumstantial, we suspect that a large portion of the missing ^{13}C was respired.

The idea that microbes are mining DON for carbon could explain the ^{15}N results that we see in the glycine treatment. However, since microbial ^{15}N values vary more or less conjointly over time for both the ammonium and glycine treatments, it suggests that microbes are also utilizing N and could be both N and C limited. If microbial growth were limited only by N, this would help explain the rapid immobilization of N observed in both treatments within the first two sampling periods. However, if organisms were also energy limited, the addition of N could stimulate microbial activity to a point where labile soil C is temporarily exhausted. Some of the immobilized ^{15}N might be used in enzyme synthesis and then released as extracellular enzymes to decompose more recalcitrant organic substrates for C acquisition. This would account for the temporary decline in microbial biomass ^{15}N recovery for both treatments after 12 h, since consistent recovery of ^{15}N in the bulk soil pool at this sampling period confirms that the label is not leaving the soil matrix. The idea that our N additions stimulated microbial growth to the point of C limitation also suggests that our tracer additions were of sufficient quantity to induce a fertilization effect. We did witness significantly higher values for MBN in labeled cores versus control cores; however, when calculated on a mass basis, our N additions for each treatment represented no more than 8% of the MBN pool. If our N additions resulted in a significant fertilization effect on soil microorganisms, we would anticipate some fluctuation of MBN during

the initial stages of the experiment as new generations of microbes adjusted to the altered C:N balance in the soil. Since we observed no significant change in MBN over time for any treatment, we believe that our ^{15}N results reflect more of a natural cycling of N into and out of the pool than a fluctuation of pool size brought about by fertilization.

Regardless, we can say with certainty that the flux of glycine- and ammonium-derived N into and out of this pool is rapid. Yet the ultimate fate of the label once it is released from the microbial pool is still unclear. Two observations indicate that most of the label lost from the microbial and soluble N pools is eventually incorporated into a more recalcitrant pool of soil N (Perakis and Hedin 2001). First, all three of our extractable N pools showed a decline in ^{15}N at the end of our sampling regime. Second, analysis of the ^{15}N content of the bulk soil revealed no significant change over time for either treatment. Finally, other researchers working in boreal forest ecosystems have found a similar relationship between the ^{15}N content of soil biota and the amount of label retained within the soil's organic profile (for example, see Nasholm and others 1998).

Clearly, not all of the label was retained within the soil organic complex. A portion was taken up by roots, both directly and following release from the microbial pool. Overall plant ^{15}N uptake during the course of the experiment was low (less than 2% recovery) in comparison to uptake by microorganisms (12%–64% recovery), but it was the only pool that was increasing in enrichment after 14 days. Moreover short-term uptake patterns show that to a limited extent plants can compete directly for amino acid N. Our results suggest that 75% of the ^{13}C acquired by fine roots occurred within the first 24 h. In our regression analysis relating excess ^{13}C to excess ^{15}N , slopes from the first and second sampling periods are greater than 2, indicating an enrichment of ^{13}C to ^{15}N that exceeds the injection ratio. Slopes from subsequent sampling periods reflect a decrease in excess ^{13}C concomitant with a rise in excess ^{15}N . It seems that in the initial hours of the experiment, fine roots competed directly for amino acid N, taking up the doubly labeled amino acid intact. After 12 h, however, given the rapidity with which ^{15}N was immobilized within the MBN pool, it is possible that our additions of glycine were exhausted. Since fine-root ^{15}N continued to increase throughout subsequent sampling periods, plants must have begun assimilating N released from microbial or mycorrhizal mineralization of glycine.

Our values for ^{13}C and ^{15}N content of roots are based on analysis of the solid fraction of fine-root

biomass (less than 1 mm diameter) only. We did not measure the $\delta^{13}\text{C}$ or ^{15}N of materials that were transported out of fine roots to the rest of the plant. Consequently, the plants may have absorbed more of the applied N tracers than is directly evident from our data. It is reasonable to assume that plants are translocating part of the N taken up by fine roots to aboveground tissues. If ^{15}N were translocated and ^{13}C remained in the root as part of a structural or metabolic C pool (that is, ectomycorrhizae within the root), this would explain why we observed an excess enrichment of ^{13}C in the fine-root C pool for the first two sampling periods. It would also indicate that our original analyses underestimated plant N uptake. To test this idea, we estimated what cumulative uptake could have been had plant N uptake been relatively constant for the duration of the experiment, regardless of N form, and translocation of N to other parts of the plant were taking place. Multiplying an average uptake rate calculated from the first two sampling periods by 336 h yields total plant uptake for the entire experiment. For both treatments, plant recovery of ^{15}N increases from just under 2% to just over 29%. These estimations are purely speculative; however, they do suggest an upper limit for the quantity of ^{15}N that could have been transported aboveground.

Several processes could contribute to the low recovery of ^{15}N in fine-root tissue during the course of our experiment. Virtually all of the balsam poplar fine roots in each of our stands are colonized by some type of ectomycorrhizal fungus (Table 1) (Lansing unpublished). These fungi have demonstrated some capacity to hydrolyze proteins to sequester N (Abuzinadah and others 1986; Abuzinadah and Read 1986); however, proteolytic degradation is strongly influenced by soil pH. The optimum pH range for proteolytic activity for many of these fungi is between 3.0 and 4.5 (Read 1991; but see Dahne and others 1995). In an experiment where soil alkalinity was augmented through liming, researchers found that uptake of N by mycorrhizal plants was reduced with increasing soil pH regardless of whether the N source was lyophilized fungal tissue or ammonium (Anderson and others 1997). Due to the alkalinity of the soils along the Tanana floodplain (Van Cleve and others 1993a), it is possible that the mycorrhizal fungi in these forests are confronted with a suboptimal environment for proteolytic degradation and subsequent immobilization of organic N. In these N-limited soils, saprotrophic fungi and other soil microorganisms may be the superior competitors for nutrients.

Given the ubiquitous nature of ectomycorrhizal hyphae in these stands, a more likely explanation

for the low recovery of ^{15}N in the fine-root pool is N retention within the hyphal network (Aber and others 1998). Although it is widely accepted that the mutualistic association between host and mycobiont acts to facilitate the host's ability to acquire nutrients, many of the details concerning the nutritional requirements of mycorrhizal fungi in natural ecosystems remain unknown. For example, it has been demonstrated in Scots pine (*Pinus sylvestris*) seedlings that up to 32% of the N assimilated by the fungal symbiont was retained within the external mycelium despite the fact that this tissue represented less than 16% of the total fungal biomass (Colpaert and others 1996). Labeled N that is diverted to extramatrical mycelial growth instead of being assimilated by the host plant would explain both the low accumulation of ^{15}N in fine roots and the high retention of ^{15}N in bulk soil samples. Since we made no effort to retain extramatrical fungal biomass in our processing of roots, the only fungal tissue likely to remain prior to isotopic analysis of the fine-root biomass would be a portion of the mantle surrounding each root tip and the tissue within the root. Thus, external hyphal biomass could represent an ecologically important yet unquantified sink for N in floodplain soils.

CONCLUSION

In our study, we used a relatively simple tracer technique to follow the fate of ammonium and glycine labeled with ^{15}N through soil and root pools in a floodplain balsam poplar stand. In the short term (under 12 h), plants competed directly for amino acid N, as evidenced by the rapid enrichment of fine-root ^{13}C . It is impossible, however, to assess long-term patterns for fine-root uptake of amino acids using our experimental design, given the high turnover rates of amino acids within these floodplain soils (Jones and Kielland 2002). The label we introduced to both ammonium- and glycine-amended soils appeared to be rapidly immobilized and transformed by microbes. Plants accounted for only a small fraction of the total ^{15}N recovered; the vast majority of applied N remained in the soil matrix at the end of the experiment. This implies that soil microorganisms play an important role in N cycling processes, both as mediators of N availability to plants and as regulators for ecosystem N retention.

Although plants were poor competitors with microbes in the short-term competition for soil N, plants are long-lived compared to soil microorganisms and could capitalize on the continuous turnover of these substrates by sequestering some of the

products of short-term N fluxes. The steady increase of ^{15}N in our fine-root biomass supports this idea. Moreover, because we were not able to detect any difference in fine-root ^{15}N values between the ammonium and glycine treatment at the outset of the experiment, we believe that intact amino acids could prove to be a significant fraction of fine-root N uptake in these stands. Over the course of a growing season, such a strategy could result in a significant portion of soil organic N being fixed in above- and belowground plant tissue.

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