2005

The Uptake of Amino Acids by Microbes and Trees in Three Cold-Temperate Forests

Adrien C. Finzi

Sean T. Berthrong
Butler University, sberthro@butler.edu

Ecology, 86(12), 2005, pp. 3345–3353

THE UPTAKE OF AMINO ACIDS BY MICROBES AND TREES IN THREE COLD-TEMPERATE FORESTS

ADRIEN C. FINZI[1] AND SEAN T. BERTHRONG[2]

Department of Biology, Boston University, Boston, Massachusetts 02215 USA

Abstract. Amino acids are emerging as a critical component of the terrestrial N cycle, yet there is little understanding of amino acid cycling in temperate forests. This research studied the uptake and turnover of amino acid N by soil microbes and the capacity of forest trees to take up the amino acid glycine in comparison to NH_4^+ and NO_3^-. This research was conducted in three temperate forests located in northwest Connecticut, USA. The three forests differed in soil parent material and canopy tree species composition. At all three sites, amino acids were released from soil organic matter through the activity of proteolytic enzymes resulting in a pool of free amino acids in soil. Free amino acids were rapidly immobilized by soil microbes. A ^{15}N-enriched-glycine-addition experiment also showed that a significant fraction of the amino acid N taken up by soil microbes was mineralized to NH_4^+ with substantial nitrification at one site. Tree species from all three sites had the physiological capacity to absorb the amino acid glycine but took up amino acid N, NH_4^+, and NO_3^- in proportion to their availability in the soil. At the site with the highest gross fluxes of N, nearly all the N in amino acids was mineralized, and fine roots assimilated inorganic N much more rapidly than amino acid N. At the two sites with slower rates of gross amino acid production, the pool of free amino acids was larger, and fine roots assimilated amino acid N almost as fast as inorganic N. This study demonstrates that amino acids are an important component of the N cycle in temperate forests.

Key words: *amino acid; inorganic nitrogen; microorganism; nitrogen cycling; temperate forest.*

INTRODUCTION

Research on the cycle of soil N in temperate forests has largely focused on inorganic forms (NH_4^+, NO_3^-). This focus has partly reflected a long-standing belief that trees take up only inorganic N. It has also reflected the belief that trees are inferior competitors to soil microbes and that only the by-products of microbial-N metabolism (i.e., NH_4^+ and NO_3^-) are available for uptake by trees (e.g., historical perspectives in Melillo [1981], Kaye and Hart [1997], Schimel and Bennett [2004]). Observations that net primary production (NPP), N uptake, N fluxes in litterfall, and N use efficiency are highly correlated with the rate of net N mineralization (e.g., Pastor et al. 1984, Nadelhoffer et al. 1985, Zak et al. 1989, Reich et al. 1997) have also reinforced the belief that the supply of inorganic N regulates ecosystem processes.

Recent studies show that plants from numerous biomes on earth have the physiological capacity to assimilate organic N in the form of amino acids (e.g., Kielland 1997, Schmidt and Stewart 1999, Nasholm et al. 2000, Lipson and Nasholm 2001, Bennett and Prescott 2004). In all ecosystems in which amino acids have been measured, freely extractable amino acids exist in soils (e.g., Kielland 1995, Raab et al. 1999, Jones et al. 2004), and they are assimilated by plants in direct competition with soil microbes (e.g., Nasholm et al. 1998, McFarland et al. 2002, Bardgett et al. 2003). Amino acids can turn over extremely rapidly in boreal forests (Jones and Kielland 2002) and temperate agronomic ecosystems (Jones 1999). In temperate agronomic soils, amino acids also contribute to N mineralization (Barraclough 1997, O'Dowd et al. 1999, Jones et al. 2004). Thus, it is apparent that amino acids are a critical component of the terrestrial N cycle (Chapin et al. 2002, Schimel and Bennett 2004).

With the past emphasis on inorganic N cycling, we do not know the extent to which amino acids contribute to the cycle of soil N in temperate forests. To address the gap in our understanding of the soil N cycle, this research addressed the following questions. What is the rate of amino acid production in temperate forest soils? Are amino acids readily consumed by soil microbes and are they are substrate for mineralization? Do temperate forest trees have the physiological capacity to take up intact amino acids? At what rate are amino acids taken up by tree roots relative to NH_4^+ and NO_3^-? Are different forms of N taken up in proportion to their availability in the field?

MATERIALS AND METHODS

Study sites

This research was conducted on two sites at the Great Mountain Forest (GMF), Norfolk, Connecticut (the Es-

Manuscript received 27 September 2004; revised 9 May 2005; accepted 31 May 2005. Corresponding Editor: J. P. Schimel.

[1] E-mail: afinzi@bu.edu

[2] Present address: Department of Biology, Duke University, Durham, North Carolina 27708 USA.

ker and Granite sites) and one site in the Housatonic State Forest, North Canaan, Connecticut, USA (the Dolomite site, all sites located at 42° N, 73°15′ W). From 1930 to present, the mean annual temperature in this area is 7°C, and the mean annual precipitation is 1330 mm (R. Russ, *personal communication*).

The three study sites are named according to the origin of the soil parent material. The soils at the Esker site (GMF) are derived from a glacial stream, and forests grow on a thick accumulation of sand and abundant cobbles >20 m above granite bedrock. The soils are Entisols classified as Typic Udorthents (Hill et al. 1980). The soils are coarse textured and acidic. Of the three sites, Esker soils have the lowest content of total C and N (Appendix A). The site is dominated by *Pinus strobis* (78% of total tree basal area [BA]), *Tsuga canadensis* (7% of BA), *Acer rubrum* (7% of BA), and *Betula* sp. (7% of BA).

The soils at the Granite site are derived from glacial till overlying granite bedrock. Soil development is more extensive than at the Esker site. The soils are Inceptisols classified as Typic Dystrochrepts (Hill et al. 1980). The depth to bedrock is <1 m throughout the area. Soils at the Granite site are strongly acidic, low in available base cations, but high in total C and intermediate in N with a large C-to-N ratio (Appendix A). The Granite site is codominated by *Prunus serotina* (18% of BA), *Fagus grandifolia* (17% of BA), *Acer rubrum* (17% of BA), *Quercus rubra* (16% of BA), *Tsuga canadensis* (12% of BA), and *Pinus strobis* (9% of BA).

The soils on the Dolomite site are derived from glacial till overlying dolomite ($CaMg(CO_3)_2$). The soils are mesic Inceptisols and classified as Aquic Eutrochrepts (Hill et al. 1980). The depth to bedrock is <1.5 m. By comparison to the Granite and Esker sites, the soils at the Dolomite site are finer textured with a higher pH (Appendix A). These soils are intermediate in C content, high in N content, and have the smallest C-to-N ratio of the three sites (Appendix A). The Dolomite site is dominated by *Fraxinus americana* (42% of basal area), *Acer saccharum* (27% of BA), *Carya ovata* (9% of BA), and *Populus grandidentata* (9%).

Plant and soil analyses

Four replicate 20 × 20 m plots were identified at each site. The replicate plots were located 50–200 m from one another. With the exception of gross proteolysis, all analyses were based on soil cores collected 15 September 2003. Gross proteolysis was measured in soils collected 15 June 2001 and 1 October and 1 November 2002 (Berthrong and Finzi, *in press*). Two soils cores were collected per plot per sample date at a minimum distance of 10 m. Soil cores were taken from the top 15 cm of mineral soil using a 5 cm diameter soil bulk density sampler. The mineral soil cores were stored on ice for transport back to the laboratory.

All measurements were made within 72 h of soil core collections. Initially all the samples were sieved through an 8-mm brass mesh to remove rocks and coarse roots. The sieved samples were homogenized by hand and stored in polyethylene bags at 4°C. To answer the questions outlined in the *Introduction*, the following analyses were done: (1) the pools of amino acid and inorganic N were extracted less than 24 h after sample collection; (2) the rates of gross proteolysis, gross NH_4^+ cycling, and gross NO_3^- cycling began within 24 h of sample collection; (3) the rate of N uptake by excised fine roots was measured within 48 h of sample collection; (4) the addition of ^{15}N-labeled glycine to soils was started within 72 h of sample collection; and (5) after 72 h subsamples of all soils were dried at 110°C for 5 d to determine initial soil moisture content. The two cores per plot were analyzed separately for each measurement.

Soil N pools and fluxes

The pools of amino acid-N, NH_4^+, and NO_3^- were determined by extracting 30-g samples of soil in 100 mL of 2 mol/L KCl (Sheperd et al. 2001). Ammonium concentrations were measured using the phenolate method on an autoanalyzer (Lachat Quickchem 8000, Zellweger Analytics, Milwaukee, Wisconsin, USA). Nitrate N was determined by the hydrazine sulfate method (Lachat Quickchem 8000). The concentration of N in amino acids was determined by color reaction with ninhydrin (Rosen 1957). Concentrations of amino acid N were determined by comparing the optical absorbance (570 nm) of the samples relative to a standard curve using leucine and correcting for contamination by NH_4^+ (Berthrong and Finzi, *in press*).

The rates of gross proteolysis were measured using a modified version of the methods outlined in Watanabe and Hayano (1995) and Lipson et al. (1999). This technique measures the accumulation of amino acids in soil slurries in the absence of an exogenous source of protein and microbial uptake. In brief, 3-g samples of soil were placed in 100-mL bottles to which were added 10 mL of 0.05 mol/L sodium citrate buffer. The slurries were incubated at 22°C for 5 h on a shaker table at 120 oscillations/min. A 5-h incubation was chosen because the rate of gross proteolysis is linear over this period as determined by the kinetics of proteolysis (Berthrong and Finzi, *in press*). Each sample received an 0.4-mL aliquot of toluene to inhibit microbial uptake of amino acids during the incubation. Immediately following incubation, 3 mL of trichloroacetic acid solution (TCA) was added to each sample thereby halting the activity of the proteolytic enzymes. The samples were then centrifuged and filtered through Whatman #1 filter paper (Whatman, Clifton, New Jersey, USA) and stored frozen at −4°C until analysis. The concentration of amino acids was quantified using the ninhydrin procedure of Rosen (1957). The rate of gross proteolysis was cal-

culated as the difference between the concentration of amino acids in soils before and after the 5-h incubation.

Gross N transformations were measured by isotope dilution (Hart et al. 1994). Two 20-g subsamples were taken from each sieved soil core and placed in 250-mL plastic bottles. Both samples were initially labeled with 1 mL of a 0.6 mg/L, 99-atom%-enriched $(^{15}NH_4)_2SO_4$ solution prepared in nanopure water. Using a pipette, the 1-mL sample of ^{15}N was applied drop-by-drop across the surface of the soil. One of the samples was extracted in 2 mol/L KCl after 15 min while the remaining sample incubated for 24 h at 22°C after which time it was also extracted in 2 mol/L KCl. The same procedure was repeated in a second set of 20-g samples to which we added 1 mL of 0.9 mg/L, 99-atom%-enriched $K^{15}NO_3$.

Gross rates of NH_4^+ and NO_3^- mineralization and immobilization were calculated using the equations of Hart et al. (1994). The initial and final concentrations of NH_4^+ and NO_3^- in the extracts were determined by analysis on an autoanalyzer (Lachat QuickChem FIA+ 8000 Series, Zellweger Analytics). The atom%-^{15}N excess of the NH_4^+ and NO_3^- in the extracts of the initial and incubated samples was determined by diffusing the N in each sample onto an acidified cellulose disc followed by analysis on a mass spectrometer (Europa Integra, Cheshire, UK) at the University of California, Davis, California, USA. The diffusion procedure is described in the next section.

^{15}N-labelled glycine uptake by soil microbes

A ^{15}N-enriched-glycine-addition experiment was used to study the uptake of amino acids by soil microbes and the redistribution of amino acid N to dissolved organic N (DON), microbial biomass N, NH_4^+, NO_3^-, and non-extractable pools in soil. This experiment was conducted in the Dolomite and Granite site soils only. Two 30-g subsamples of soil from each sieved soil core were placed into separate 250-mL plastic bottles. A 1-mL aliquot of 7.4 mg N/L, 98-atom%-enriched glycine was added to each sample, drop-by-drop across the surface of the soil sample. One sample was extracted in 2 mol/L KCl after 15 min and the second sample was extracted after exactly 24 h.

A sequential extraction procedure was used to recover ^{15}N as DON, NH_4^+, NO_3^-, and in microbial biomass and soils (Holmes et al. 2003). The sequential extraction procedure begins by separating the pool of DON and inorganic N from microbial biomass and soil N pools. Immediately after extracting with 0.5 mol/L K_2SO_4, the samples were filtered through a plastic syringe fitted with a 0.45-μm filter. Forcing the supernatant through the filter separates DON and inorganic N from most of the N contained in floating microbial cells and organic matter. The filtrate was then run on an autoanalyzer for inorganic N concentrations (Lachat QuickChem FIA+ 8000 Series) and the content of ^{15}N in the NH_4^+ and NO_3^- pools analyzed by mass spectrometry following the acid diffusion procedure. The pool of extractable DON was also measured in this extraction. A 5-mL subsample of the filtrate was persulfate digested and analyzed for DON as NO_3^- on an autoanalyzer (Cabrera and Beare 1993). The ^{15}N content of the DON pool was also measured by mass spectrometry following the acid diffusion procedure.

The microbial-biomass N pool was estimated using a modification of the fumigation-extraction technique (Brooks et al. 1985). Briefly, the 0.45-μm filters were placed back into the plastic bottles containing the residual soil, and both were fumigated with CH_3Cl for 5 d in a vacuum desiccator. Following the 5-d fumigation all samples were extracted in 0.5 mol/L K_2SO_4. The extracts were persulfate digested, the concentration of N in microbial biomass determined as NO_3^- on an autoanalyzer, and the ^{15}N content of the microbial biomass pool determined by mass spectrometry following the acid diffusion procedure. The soil remaining in each bottle was scraped into a tin dish, dried to constant mass at 60°C for 3 d, ground, and a 5-mg sample sent for analysis of ^{15}N content on a mass spectrometer to estimate the non-extractable fraction in soil.

The atom%-^{15}N excess of all fractions except soils was determined by diffusing the N in each sample onto an acidified cellulose disc followed by analysis on a mass spectrometer at the University of California, Davis (Brooks et al. 1989). In brief, 1 mL of the ^{15}N-labeled sample was added to a specimen cup containing 40 mL of 2 mol/L KCl and 6.43 μmol ^{14}N (as $[^{14}NH_4]_2SO_4$). The addition of ^{14}N brought the concentration and isotope ratio of N into the optimal detection range for mass spectrometry. A cellulose disc (Whatman #3) was acidified with 10 μL of 2.5 mol/L $KHSO_4$ and suspended on a metal wire at the top of each specimen cup. To each specimen cup, we added 270 mg MgO to volatilize the NH_4^+ to NH_3, which was then trapped on the acidified cellulose disc. The diffusion lasted for 7 d at 20°C after which time the acidified filter discs were air dried for 3 d in a desiccator with a sulfuric acid trap, rolled into tin capsules, and then sent for isotope analysis. The same procedure was used to diffuse NO_3^--based samples (i.e., DON, microbial biomass N, and NO_3^- pools) with the exception that 160 mg of Devarda's Alloy was added to each specimen cup. Devarda's Alloy reduces NO_3^- to NH_4^+.

Excised root nutrient uptake studies

The uptake of glycine, NH_4^+, and NO_3^- was quantified with the use of excised roots (e.g., Treseder and Vitousek 2001). A 1-g subsample of the fine roots (<2 mm in diameter) was hand-picked from each replicate core collected within each 20 × 20 m plot, rinsed free of soil in distilled water, and combined into a single sample. Three soil-free, 40-mg wet-mass samples of fine roots from each plot at each site were placed into replicate 5 × 5 cm cheesecloth bags. Root lengths varied from 2 to 4.5 cm. Each fine root bag was rinsed in

0.5 mmol/L $CaCl_2$ for 20 min. The bags were then incubated for 30 min in 300 μmol N/L solutions of 98-atom%-enriched ^{15}N-glycine, $^{15}NH_4Cl$, or $K^{15}NO_3$ containing 0.5 mmol/L $CaCl_2$ (for membrane integrity), and 0.01 mol/L sucrose (as an energy source; Jackson et al. 1990). After incubation, the fine root bags were rinsed for 10 min in four different solutions of 1 mmol/L KCl to remove any ^{15}N adsorbed to the root (Jackson and Reynolds 1996). Fine roots were then dried at 60°C for 4 d, ground, and analyzed for ^{15}N content on a mass spectrometer at the University of California, Davis. The roots not used in uptake studies were weighed and then dried for 4 d at 110°C to determine moisture content and background $\delta^{15}N$.

Data analysis

The four 20 × 20 m plots at each site were considered replicates and statistical analysis was based on $n = 12$ (3 sites × 4 replicates). Thus all within-plot samples were averaged prior to statistical analysis. The three sample dates on which proteolysis was measured were averaged prior to statistical analysis. Differences among sites in the pools and gross fluxes of amino acid N, NH_4^+, and NO_3^- were analyzed by one-way ANOVA with site as the main effect. The ^{15}N-enriched glycine tracer experiment was analyzed by repeated measures ANOVA with the percentage recovery of ^{15}N as the dependent variable and site and form of recovery (i.e., DON, microbial biomass N, NH_4^+, NO_3^-, soil) as the main effects with two levels for time (15 min, 24 h). The excised root nutrient uptake kinetic study was analyzed by two-way ANOVA with site and form (i.e., glycine, NH_4^+, NO_3^-) as the main effects. The Ryan-Einot-Gabriel-Welsch Multiple Range Test was used to analyze post-hoc differences among means in all statistical analyses and all analyses were carried out using SAS (SAS 1987).

The motivation for the ^{15}N-enriched-glycine addition experiment was to compare the immobilization and transformation of N in glycine between sites and among different pools over 24 h. Total isotope recovery ranged from 74% to 112% (see *Results*). To account for differences in total isotope recovery between sites and time periods in this study, the percentage recovery of ^{15}N in each pool within a site and sample time was rescaled to 100%. This was achieved by dividing the percentage recovery of ^{15}N within a particular form by the total isotope recovery at that site and time period. For example, a mean of 73% of the ^{15}N was recovered in the NH_4^+ pool at the granite site after 15 min, when total isotope recovery was 112%. This value was rescaled to 65% (i.e., 73%/112%), and so on for all other values in the data set. The underlying assumption in this rescaling is that the percentage recovery of ^{15}N within a site and sample period is representative of the movement of N from the amino acid glycine independent of whether the isotope was under-recovered or over-recovered.

TABLE 1. Among-site and between-form comparisons of the concentration of N (μg N/g soil) in amino acid and inorganic N pools in the top 15 cm of mineral soil and the percentage of the inorganic N pool composed of NO_3^- in soils collected 15 September 2003.

Site	Amino acid N (μg N/g)	Inorganic N ($NH_4^+ + NO_3^-$) (μg N/g)	NO_3^- (%)
Esker	$2.65^{a,B}$ (0.18)	$3.77^{b,A}$ (0.13)	21^{b} (3)
Granite	$3.53^{a,A}$ (0.20)	$4.02^{b,A}$ (0.08)	22^{b} (3)
Dolomite	$2.30^{a,B}$ (0.15)	$7.50^{a,A}$ (0.15)	56^{a} (3)

Notes: Different superscript, lowercase letters indicate a significant difference among sites ($P < 0.05$). Different superscript, uppercase letters indicate a significant difference between the concentration of amino acid N and inorganic N within a site ($P < 0.05$). The research was conducted on two sites at the Great Mountain Forest, Norfolk, Connecticut (the Esker and Granite sites), and one site in the Housatonic State Forest, North Canaan, Connecticut, USA (the Dolomite site).

RESULTS

Pools and fluxes of amino acids and inorganic N

The concentration of extractable amino acid N was highest at the Granite site and lowest at the Dolomite site but among-site differences were not statistically significant (Table 1). The concentration of inorganic N was significantly higher at the Dolomite site than the Esker and Granite sites, and significantly more of the inorganic N was in the form of NO_3^- (Table 1). At the Esker and Dolomite sites, the concentration of inorganic N in soil was significantly higher than the concentration of amino acid N. At the Granite site, the concentration of inorganic N in soils was not significantly different than amino acid N (Table 1).

The rates of gross proteolysis, gross NH_4^+ mineralization, and gross nitrification were significantly higher at the Dolomite site than the Esker and Granite sites (Table 2). Similarly, the rates of gross NH_4^+ and NO_3^- immobilization were significantly higher at the Dolomite than the Esker and Granite sites (Table 2). Net mineralization, calculated as the difference between the gross production and consumption of NH_4^+ and NO_3^-, was significantly higher at the Dolomite site than the Esker and Granite sites.

Recovery of ^{15}N-labeled glycine

At the Dolomite site, the percentage recovery of ^{15}N summed across all forms (herein ''total isotope recovery'') was 112 ± 9% and 82 ± 9% after 15 min and 24 h, respectively. At the Granite site, total isotope recovery was 74 ± 10% and 105 ± 10% after 15 min and 24 h, respectively.

The percentage recovery of ^{15}N-enriched glycine varied among forms, between sites, and through time (Appendix B). Recovery of ^{15}N after 15 min was significantly greater in microbial biomass at the Granite site than at the Dolomite site where recovery as NH_4^+ was significantly greater (Fig. 1A, B). The recovery of N in DON, as NO_3^- and in non-extractable fractions, ac-

TABLE 2. Gross rates of proteolysis and NH_4^+ and NO_3^- production ($\mu g\ N \cdot g^{-1} \cdot 24\ h^{-1}$), gross rates of NH_4^+ and NO_3^- immobilization ($\mu g \cdot g^{-1} \cdot 24\ h^{-1}$), and net mineralization ($\mu g \cdot g^{-1} \cdot 24\ h^{-1}$) (means, with SE in parentheses).

Research site	Gross proteolysis	Gross NH_4^+ mineralization	Gross nitrification	Gross NH_4^+ immobilization	Gross NO_3^- immobilization	Net mineralization
Esker	2.09[b] (−0.17)	4.21[b] (−0.15)	0.92[b] (−0.24)	3.06[b] (−0.35)	0.41 (−0.14)	1.66[b] (−0.38)
Granite	2.65[b] (−0.16)	4.49[b] (−0.86)	1.00[b] (−0.07)	2.82[b] (−0.41)	0.67 (−0.22)	2.00[b] (−0.66)
Dolomite	4.04[a] (−0.62)	7.21[a] (−0.57)	4.91[a] (−0.68)	4.85[a] (−0.38)	1.21 (−0.85)	6.06[a] (−0.93)

Note: Different superscript letters within a column indicate a significant ($P < 0.05$) difference in N transformation rate among sites.

counted for <10% of isotope recovery at both sites. After 24 h, the single largest recovery of ^{15}N was as NO_3^- at the Dolomite site (Fig. 1C). By contrast, most of the ^{15}N remained in microbial biomass and NH_4^+ pools at the Granite site with little nitrification (Fig. 1D). The recovery of ^{15}N in microbial biomass was not significantly different between the two sites, but recovery as NH_4^+ was significantly greater at the Granite site (Fig. 1C, D). The recovery of ^{15}N within DON and non-extractable pools was still <10% of that initially applied at both sites (Fig. 1C, D).

N uptake by fine roots

The physiological capacity of plant roots to take up N varied significantly among forms and sites (form × site interaction, $P < 0.0001$). Fine roots from all sites took up NH_4^+ significantly ($P < 0.001$) more rapidly than glycine, which was taken up significantly more rapidly than NO_3^- (Fig. 2A). Fine roots collected from the Dolomite site took up NH_4^+ and NO_3^- significantly more rapidly than fine roots from the Granite and Esker sites (Fig. 2A). The rate of glycine uptake was significantly faster at the Dolomite and Esker sites than at the Granite site. Fine roots from the Dolomite site had a physiological capacity to take up inorganic N (i.e., the sum of NH_4^+ and NO_3^- uptake rates in Fig. 2A) fivefold greater than that of glycine. The ratio of inorganic-N uptake to glycine uptake was ~2 at the Esker and Granite sites, significantly lower than that observed at the Dolomite site (Fig. 2B).

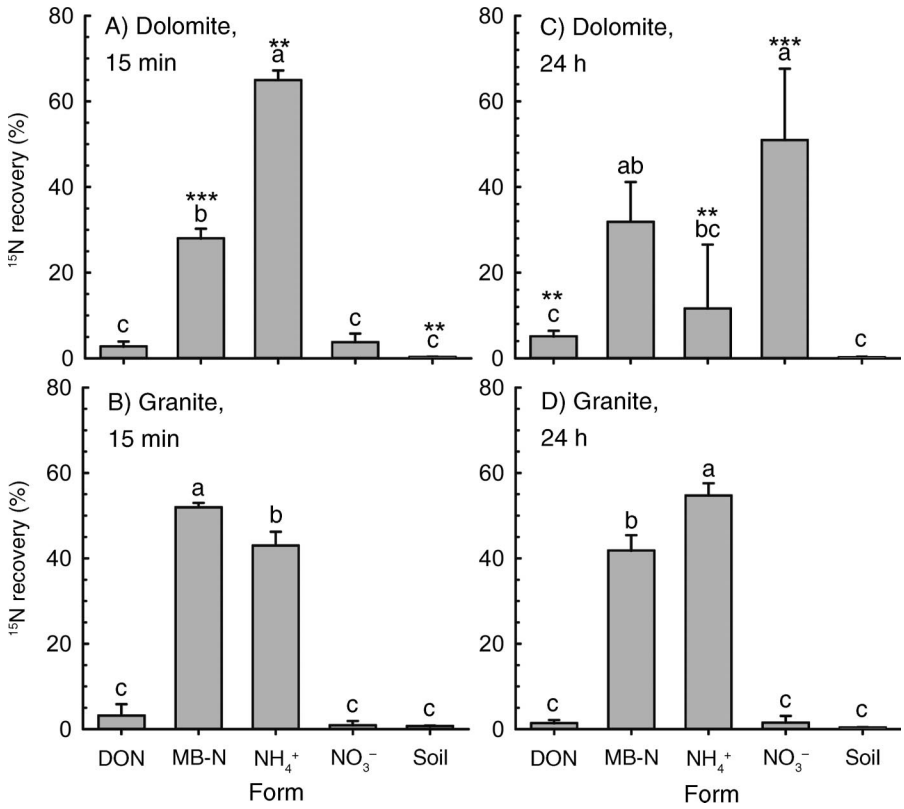

FIG. 1. Percentage recovery of ^{15}N-enriched glycine in dissolved organic nitrogen (DON), microbial-biomass N (MB-N), NH_4^+, NO_3^-, or non-extractable soil pools ("soil") after 15 min and 24 h at each site (means + 1 SE). Different letters above bars indicate a significant ($P < 0.001$) difference in the percentage recovery of ^{15}N within a site and time period. Asterisks above the bars indicate a significant between-site difference in the percentage recovery of ^{15}N within a given pool: $^{**}P < 0.01$; $^{***}P < 0.001$.

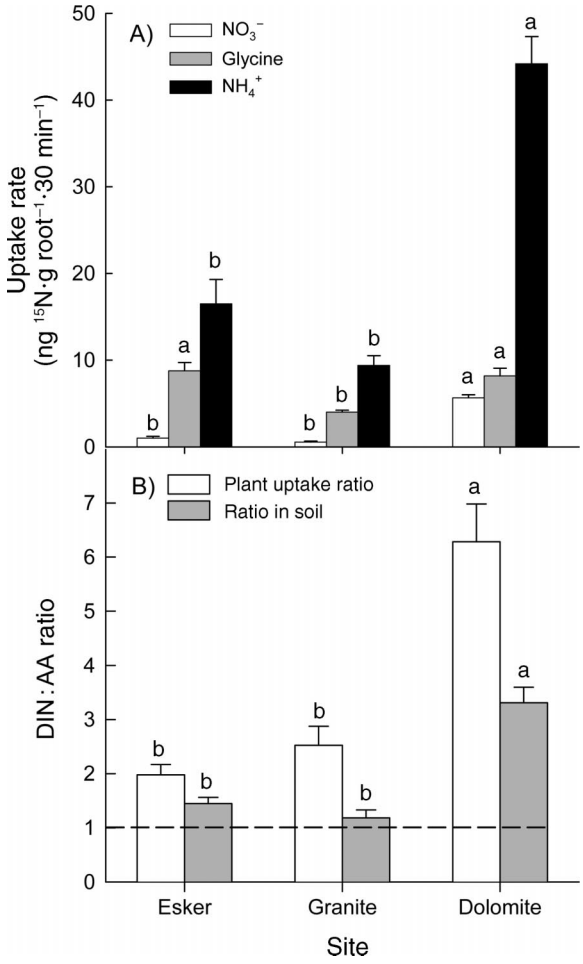

FIG. 2. (A) The uptake rate of 300 μmol/L solutions of NH_4^+ (black bars), glycine (gray bars), and NO_3^- (white bars) in roots taken from the three different sites (means + 1 SE). Superscript letters indicate a significant ($P < 0.001$) among-site difference in the uptake of a particular form of N. (B) The ratio of inorganic N uptake (sum of NH_4^+ uptake and NO_3^- uptake in panel (A), DIN) to glycine uptake in excised roots (white bars) and the ratio of inorganic N to amino acid (AA) N concentrations in the field (gray bars; data from Table 1). Superscript letters indicate a significant difference ($P < 0.01$) in ratios among sites. The dashed line in (B) corresponds to a ratio of 1.

DISCUSSION

There is an emerging consensus that amino acids are a critical component of the terrestrial N cycle (Neff et al. 2003, Schimel and Bennett 2004). While amino acid cycling has been widely studied in arctic (e.g., Jones and Kielland 2002), alpine (e.g., Lipson et al. 1999, Raab et al. 1999), boreal (e.g., Nasholm et al. 1998, McFarland et al. 2002), and temperate agronomic ecosystems (e.g., Barraclough 1997, Jones et al. 2004, 2005), there are virtually no comparable data on amino acid cycling in temperate forests. This study clearly demonstrated that amino acids are a critical component of the N cycle in temperate forests. Amino acids were rapidly released from soil organic matter (SOM) through the activity of proteolytic enzymes (Table 2), a pool of freely extractable amino acids was present in soils (Table 1), amino acids were rapidly immobilized and mineralized by soil microbes (Fig. 1), and temperate forest trees had the capacity to take up the amino acid glycine and appeared to assimilate amino acid N relative to inorganic N in proportion to their abundance in the soil (Fig. 2).

All the soil-N cycling measurements made in this study were conducted in the laboratory, some measurements were made in different years, and both sources of variation could affect the interpretation of data. For example, proteolysis was measured in soil slurries, a condition that is not present in these upland soils. Similarly, gross fluxes of NH_4^+ and NO_3^- were measured in sieved soils, and sieving soils can increase the availability of C and N for microbial metabolism, thereby affecting N cycling. Finally, proteolysis was measured in 2001 and 2002, whereas gross N cycling was measured in 2003. Despite these limitations, this research provided important insights into amino acid cycling in these temperate forests (listed above) that are discussed in detail in the following section.

Similarly, the data on N uptake from excised fine roots must be interpreted with care (e.g., Nasholm et al. 2000). First, this study examined the physiological capacity of fine roots to take up different forms of N at a single concentration (i.e., 300 μmol N/L). It is not known whether the uptake of these different forms of N would follow the same relative ranking across a concentration gradient. Second, nutrient uptake studies using excised roots typically exclude mycorrhizal fungi that can be very important to plant N capture (Abuzinadah and Read 1989, Hodge et al. 2001). Third, the species of the roots were unknown and could have varied from sample to sample within a site. Fourth, the cut ends of the roots were placed directly into the ^{15}N solutions leaving open the possibility that some portion of the recovered ^{15}N diffused into the roots through the cut ends. Finally, glycine, NH_4^+, and NO_3^- have different mobility in soil that could affect root physiology and the appearance of affinity for different forms. NH_4^+ is positively charged and adsorbs readily onto the cation-exchange capacity of temperate forest soils rendering NH_4^+ largely immobile. By contrast, glycine and NO_3^- are neutral and negatively charged, respectively; they do not adsorb as readily onto exchange sites in soil; and both forms are mobile in soil water, often moving to existing root surfaces through transpirational water (Raven et al. 1992, Chapin et al. 2002). Therefore, plants may not produce as many carriers for the uptake of glycine and NO_3^- as they might for NH_4^+, giving rise to the pattern of N uptake observed in this study. Despite these uncertainties however, there were very clear among-site variations in the form of N taken up that reflected variations in the pool sizes and fluxes of amino acids and inorganic N among the three forest

types. The excised root N uptake data therefore appear to be illustrative of broad, among-site patterns in N uptake by dominant tree species.

Soil N cycling

Schimel and Bennett (2004) proposed that the depolymerization of N bound in SOM controls the rate at which N can be converted to available forms. Amino acids are one type of N that is depolymerized from SOM via the activity of proteolytic enzymes (Watanabe and Hayano 1995), and previous studies have shown that amino acids can be a substrate for N mineralization (Barraclough 1997, Jones et al. 2004). The data collected in this study are consistent with the hypothesis that the rate of depolymerization controls the transformation of N to available forms. The rate of proteolysis was positively correlated with the rate of gross NH_4^+ mineralization ($\sigma = 0.75$, $P < 0.01$). Similarly, the rate of gross NH_4^+ mineralization was positively correlated with the rate of gross nitrification ($\sigma = 0.87$, $P < 0.001$). Although there are many other forms of organic N that can be depolymerized from SOM (e.g., nucleic acids and amino sugars; Schimel and Bennett 2004), in these temperate forests the flow of N to available forms is highly correlated with, and therefore possibly regulated by, the release of amino acids from SOM.

Amino acids can turn over extremely rapidly in soil. Jones (1999) found that the mean half-life of amino acids in surface soils (0–10 cm depth) collected from 10 agroecological zones in the United Kingdom was 1.6 ± 0.6 h. Similarly, Jones and Kielland (2002) estimated that the pool of amino acid N turned over ~20 times per day in the organic horizons of black spruce forests in Alaska, USA. The ^{15}N-enriched glycine study presented here also demonstrated the potential for rapid amino acid N turnover (Fig. 2). Within 15 min of application ~54% of the ^{15}N in the labeled glycine was recovered as NH_4^+ (Fig. 1). After 24 h, there was substantial nitrification, accounting for ~26% of the N originally applied as glycine (Fig. 1).

Soil C-to-N ratios have long been recognized as a control over net mineralization and nitrification (e.g., Pastor et al. 1984, Ollinger et al. 2002) by dictating the degree to which microbial function is C and/or N limited (Paul and Clark 1996). Typically, narrow C-to-N ratio soils have high rates of net mineralization because microbes are more strongly C limited than N limited (e.g., Finzi et al. 1998). By contrast, wide C-to-N ratio soils have low rates of net mineralization because microbial function remains strongly N limited. Hypothetically, the same principle could explain the rapid turnover of amino acids in soil (e.g., Jones and Kielland 2002, Jones et al. 2004). Amino acids are a labile source of both C and N for microbial metabolism. Amino acids taken up by microbes in narrow C-to-N ratio soils are likely to be assimilated for their C content, thereby releasing NH_4^+ to the soil solution (Jones et al. 2004, Schimel and Bennett 2004). By contrast, in ecosystems with wide C-to-N ratios microbial function is likely to be more strongly N limited than C limited and much of the amino acid N taken up by microbes would be retained within microbial biomass (Schimel and Chapin 1996).

The content of N in soil at the Dolomite site was significantly greater than at the Granite site (491 ± 23 and 392 ± 9 g N/m^2, respectively). Similarly, the C-to-N ratio of soil at the Dolomite site was significantly lower than at the Granite site (12.7 ± 0.3 and 19.9 ± 0.6, respectively, Appendix A). Consistent with the hypothesis that soil C-to-N ratios control the turnover of N within amino acids, microbial-^{15}N immobilization of labeled glycine was significantly lower and mineralization to $^{15}NH_4^+$ significantly higher at the Dolomite site after 15 min than at the Granite site (Fig. 1A, B). After 24 h, significantly more of the $^{15}NH_4^+$ at the Dolomite site was nitrified whereas there was much greater retention of ^{15}N within microbial biomass and NH_4^+ pools at the Granite site (Fig. 1C, D). Thus it appears that amino acids are principally, but not exclusively, used as a labile source of C at the Dolomite site whereas at the lower-N-status granite site, microbial function appears to be more strongly N limited with efficient recycling of amino acid N within microbial biomass and NH_4^+ pools (Fig. 1).

Plant uptake

Fine roots of the trees at each site had the physiological capacity to take up the amino acid glycine (Fig. 2). There were, however, important among-site differences in the rate at which glycine was taken up relative to inorganic N (Fig. 2B), and these differences appeared to reflect among-site variation in available forms (e.g., Raab et al. 1999, Weigelt et al. 2005). Fine roots of the trees from the Dolomite site took up inorganic N significantly more rapidly than glycine (Fig. 2B), and the availability of inorganic N at this site was significantly greater than amino acid N (Tables 1 and 2). By contrast, the rate of inorganic-N uptake relative to glycine uptake was significantly lower at the Esker and Granite sites (Fig. 2B), where the availability of amino acid N in soil was higher (Tables 1 and 2). Additional support for this interpretation of the data comes from Berthrong and Finzi (*in press*), who measured the concentration of extractable amino acid N and inorganic N at the Esker, Granite, and Dolomite sites at three points during the growing season in 2001 and 2002. They found that the concentration of inorganic N was significantly higher than amino acid N on four of six sample dates at the Dolomite site. By contrast, the concentration of amino acid N was significantly higher than inorganic N on four of six sample dates at the Granite site. The Esker site was intermediate in that inorganic N concentrations were significantly higher than amino acid N on one sample date but on two other sample dates amino acid N was significantly higher than inorganic N (Berthrong and Finzi, *in press*).

Excised root nutrient uptake studies show that in cold ecosystems plant species take up glycine more rapidly than NH_4^+ (Chapin et al. 2002). The ratio of glycine to NH_4^+ uptake in boreal trees is ~1.3 (Chapin et al. 1986, Nasholm et al. 1998) and increases to 2.1 in arctic vascular plants (Chapin et al. 1993, Kielland 1994) and 5 in nonvascular arctic plants (Kielland 1997). The ratio of glycine to NH_4^+ uptake in this study ranged from 0.53 at the Esker site to 0.42 and 0.19 at the Granite and Dolomite sites, respectively. Variations in glycine-to-NH_4^+ uptake ratios from artic tundra to boreal and temperate forests coincide with a latitudinal gradient in net N mineralization (lowest in arctic and highest in temperate forests; Nadelhoffer et al. 1985, Giblin et al. 1991, Shaver et al. 1991, Ruess et al. 1996, Reich et al. 1997). This analysis raises the hypothesis that amino acids contribute less to plant growth and forest productivity in the temperate zone than at higher latitudes (Schimel and Bennett 2004).

Conclusions

To our knowledge this is the first study of amino acid cycling in temperate forests that linked the production and turnover of amino acids to patterns of root-N uptake. These data clearly showed variation in the production and turnover of amino acids among three cold-temperate forests (Table 2, Fig. 1) and significant differences in the rate of glycine vs. NH_4^+ vs. NO_3^- uptake by dominant tree species at each site (Fig. 2). The data collected in this study support the hypothesis that the N status of an ecosystem can have a significant effect on amino acid vs. inorganic-N availability for plant growth (Schimel and Bennett 2004). In particular, the high-N-status Dolomite site cycled N significantly more rapidly than the lower-N-status Esker and Granite sites (Table 2, Fig. 1). At the Dolomite site, N uptake by roots was faster than at the Granite site, with much greater uptake rates for inorganic N than amino acid N (Fig. 2). The opposite was true at the Esker and Granite sites where rates of amino acid cycling and N mineralization were slower (Table 2), the pool sizes of amino acid N and inorganic N were similar in size (Table 1), and where tree roots took up the amino acid glycine at a rate much more comparable to inorganic N. As more studies begin to compare rates of amino acid and inorganic N cycling with patterns of nutrient capture in fine roots, we will increasingly understand the importance of amino acids to the cycle of soil N and as a form of N that supports terrestrial productivity.

Acknowledgments

The Childs Family and Jody Bronson provided invaluable logistical support for the research at the Great Mountain Forest. Thanks to the State of Connecticut, Department of Environmental Protection, Natural Area Preserves Program for granting us access to the forests on the Canaan Mountain. Thanks to Eddie Brzostek, Bridgid Curry, and Jennifer Talbot for their help in the laboratory. Thanks to Eddie Brzostek, Jenny Talbot, Vikki Rodgers, Anne Gallet-Budynek, Joshua Schimel, and two anonymous reviewers for their comments on an earlier draft of this manuscript. This research was supported by a grant from the United States Department of Agriculture (2000-00782).

Literature Cited

Abuzinadah, R., and D. J. Read. 1989. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants V. Nitrogen transfer in birch (*Betula pendula* L.) infected with different mycorrhizal fungi. New Phytologist **112**:55–60.

Bardgett, R. D., T. C. Streeter, and R. Bol. 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology **84**:1277–1287.

Barraclough, D. 1997. The direct or MIT route for nitrogen immobilization: a N-15 mirror image study with leucine and glycine. Soil Biology and Biochemistry **29**:101–108.

Bennett, J. N., and C. F. Prescott. 2004. Organic and inorganic nitrogen nutrition of western red cedar, western hemlock and salal in mineral N-limited cedar–hemlock forests. Oecologia **141**:468–476.

Berthrong, S. T., and A. C. Finzi. *In press*. Amino acid and inorganic nitrogen cycling in three temperate forests of southern New England. Biogeochemistry.

Brooks, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid extraction method to measure microbial biomass nitrogen. Soil Biology and Biochemistry **17**:837–842.

Brooks, P. C., J. M. Stark, B. B. McInteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated 15-N analysis. Soil Science Society of America Journal **53**:1707–1711.

Cabrera, M. L., and M. H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal **57**: 1007–1012.

Chapin, F. S., P. Mattson, and H. Mooney. 2002. Principles of terrestrial ecosystem ecology. Springer, New York, New York, USA.

Chapin, F. S., L. Moilanen, and K. Kielland. 1993. Preferential use of organic nitrogen for growth by a nonmycorrhizal arctic sedge. Nature **361**:150–153.

Chapin, F. S., K. Van Cleve, and P. R. Tyron. 1986. Relationship of ion absorption to growth rate in taiga trees. Oecologia **69**:238–242.

Finzi, A. C., N. Van Breemen, and C. D. Canham. 1998. Canopy tree soil interactions within temperate forests: species effects on soil carbon and nitrogen. Ecological Applications **8**:440–446.

Giblin, A. E., K. J. Nadelhoffer, G. R. Shaver, J. A. Laundre, and A. J. McKerrow. 1991. Biogeochemical diversity along a riverside toposequence in arctic Alaska. Ecological Monographs **61**:415–435.

Hart, S. C., J. M. Stark, E. A. Davidson, and M. K. Firestone. 1994. Nitrogen mineralization, immobilization and nitrification. Pages 985–1018 *in* A. L. Page, editor. Methods of soil analysis. Part 21. Microbiological and biochemical properties. Soil Science Society of America, Madison, Wisconsin, USA.

Hill, D. E., E. H. Sautter, and W. N. Gunick. 1980. Soils of Connecticut. Bulletin Number 787. Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA.

Hodge, A., C. D. Campbell, and A. H. Fitter. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature **413**:297–299.

Holmes, W. E., D. R. Zak, K. S. Pregitzer, and J. S. King. 2003. Soil nitrogen transformations under *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* following 3 years exposure to elevated CO2 and O-3. Global Change Biology **9**:1743–1750.

Jackson, R. B., J. H. Manwaring, and M. M. Caldwell. 1990. Rapid physiological adjustment of roots to localized soil enrichment. Nature **344**:58–60.
Jackson, R. B., and H. L. Reynolds. 1996. Nitrate and ammonium uptake for single- and mixed-species communities grown at elevated CO_2. Oecologia **105**:74–80.
Jones, D. L. 1999. Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biology and Biochemistry **31**:613–622.
Jones, D. L., and K. Kielland. 2002. Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. Soil Biology and Biochemistry **34**:209–219.
Jones, D. L., D. Shannon, T. J. Fortune, and J. F. Farrar. 2005. Plant capture of amino acids is maximized under high soil amino acid concentrations. Soil Biology and Biochemistry **37**:179–181.
Jones, D. L., D. Shannon, D. V. Murphy, and J. Farrar. 2004. Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. Soil Biology and Biochemistry **36**:749–756.
Kaye, J. P., and S. C. Hart. 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology and Evolution **12**:139–143.
Kielland, K. 1994. Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. Ecology **75**:2373–2383.
Kielland, K. 1995. Landscape patterns of free amino acids in arctic tundra soils. Biogeochemistry **31**:85–98.
Kielland, K. 1997. Role of free amino acids in the nitrogen economy of arctic cryptogams. Ecoscience **4**:75–79.
Lipson, D., and T. Nasholm. 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia **128**:305–316.
Lipson, D. A., S. K. Schmidt, and R. K. Monson. 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology **80**:1623–1631.
McFarland, J. W., R. W. Ruess, K. Kielland, and A. P. Doyle. 2002. Cycling dynamics of NH4+ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. Ecosystems **5**:775–788.
Melillo, J. M. 1981. Nitrogen cycling in deciduous forests. Ecological Bulletin **33**:427–442.
Nadelhoffer, K. J., J. D. Aber, and J. M. Melillo. 1985. Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. Ecology **66**:1377–1390.
Nasholm, T., A. Ekblad, A. Nordin, R. Giesler, M. Hogberg, and P. Hogberg. 1998. Boreal forest plants take up organic nitrogen. Nature **392**:914–916.
Nasholm, T., K. Huss-Danell, and P. Hogberg. 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology **81**:1155–1161.
Neff, J. C., F. S. Chapin, and P. M. Vitousek. 2003. Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. Frontiers in Ecology and the Environment **1**:205–211.
O'Dowd, R. W., D. Barraclough, and D. W. Hopkins. 1999. Nitrogen and carbon mineralization in soil amended with D- and L-leucine. Soil Biology and Biochemistry **31**:1573–1578.
Ollinger, S. V., M. L. Smith, M. E. Martin, R. A. Hallett, C. L. Goodale, and J. D. Aber. 2002. Regional variation in foliar chemistry and N cycling among forests of diverse history and composition. Ecology **83**:339–355.
Pastor, J., J. D. Aber, C. A. McClaugherty, and J. M. Melillo. 1984. Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecology **65**:256–268.
Paul, E. A., and F. E. Clark. 1996. Soil microbiology and biochemistry. Second edition. Academic Press, San Diego, California, USA.
Raab, T. K., D. A. Lipson, and R. K. Monson. 1999. Soil amino acid utilization among species of the Cyperaceae: plant and soil processes. Ecology **80**:2408–2419.
Raven, J. A., B. Wollenweber, and L. L. Handley. 1992. A comparison of ammonium and nitrate as nitrogen sources for photolithotrophs. New Phytologist **121**:19–32.
Reich, P. B., D. F. Grigal, J. D. Aber, and S. T. Gower. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. Ecology **78**:335–347.
Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Archives of Biochemistry and Biophysics **67**:10–15.
Ruess, R. W., K. Van Cleve, J. Yarle, and L. A. Vierck. 1996. Contributions of fine root production and turnover to carbon and nitrogen cycling in taiga forests of the Alaskan interior. Canadian Journal of Forest Research **26**:1326–1336.
SAS. 1987. SAS/STAT guide for personal computers. Version 6. SAS Institute, Cary, North Carolina, USA.
Schimel, J., and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology **85**:591–602.
Schimel, J. P., and F. S. Chapin. 1996. Tundra plant uptake of amino acid and NH4+ nitrogen in situ: plants compete well for amino acid N. Ecology **77**:2142–2147.
Schmidt, S., and G. R. Stewart. 1999. Glycine metabolism by plant roots and its occurrence in Australian plant communities. Australian Journal of Plant Physiology **26**:253–264.
Shaver, G. R., K. J. Nadelhoffer, and A. E. Giblin. 1991. Biogeochemical diversity and element transport in a heterogeneous landscape, the north slope of Alaska. Pages 105–126 *in* M. G. Turner and R. H. Gardner, editors. Quantitative methods in landscape ecology. Springer, New York, New York, USA.
Shepherd, M., A. Bhogal, G. Barrett, and C. Dyer. 2001. Dissolved organic nitrogen in agricultural soils: effects of sample preparation on measured values. Communications in Soil Science and Plant Analysis **32**:1523–1542.
Treseder, K. K., and P. M. Vitousek. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology **82**:946–954.
Watanabe, K., and K. Hayano. 1995. Seasonal-variation of soil protease activities and their relation to proteolytic bacteria and *Bacillus* spp in paddy field soil. Soil Biology and Biochemistry **27**:197–203.
Weigelt, A., R. Bol, and R. D. Bardgett. 2005. Preferential uptake of soil nitrogen forms by grassland plant species. Oecologia **142**:627–635.
Zak, D. R., G. E. Host, and K. S. Pregitzer. 1989. Regional variability in nitrogen mineralization, nitrification, and overstory biomass in northern Lower Michigan. Canadian Journal of Forest Research **19**:1521–1526.

APPENDIX A

A table presenting selected properties of each study site is available in ESA's Electronic Data Archive: *Ecological Archives* E086-184-A1.

APPENDIX B

A table presenting results of the repeated-measures ANOVA for the percentage of recovery of ^{15}N-labeled glycine between sites is available in ESA's Electronic Data Archive: *Ecological Archives* E086-184-A2.