LATITUDINAL PATTERNS OF AMINO ACID CYCLING AND PLANT N UPTAKE AMONG NORTH AMERICAN FOREST ECOSYSTEMS

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Jack W. McFarland, B.A.

Fairbanks, Alaska

December 2008

ABSTRACT

Interest in the role of organic nitrogen (N) to the N economy of forest ecosystems is gaining momentum as ecologists revise the traditional paradigm in N cycling to emphasize the importance of depolymerization of soil organic matter (SOM) in controlling the bioavailability of N in forest soils. Still, there has yet to be a coordinated effort aimed at developing general patterns for soil organic N cycling across ecosystems that vary in climate, SOM quality, plant taxa, or dominant mycorrhizal association: ectomycorrhizae (EM) vs. arbuscular mycorrhizae (AM). In this study, experimental additions of $^{13}C^{15}N$-glycine and $^{15}NH_4^+$ were traced *in situ* through fine root and soil N pools for six North American forest ecosystems in an effort to define patterns of plant and microbial N utilization among divergent forest types.

Recovery of ^{15}N in extractable soil pools varied by N form, forest type, and sampling period. At all sites, immobilization by the soil microbial biomass represented the largest short-term (<24 h) biotic sink for NH_4^+ and amino acid-N, but differences in microbial turnover of the two N forms were linked to cross-ecosystem differences in SOM quality, particularly the availability of labile carbon (C). At the conclusion of the experiment, microbial N turnover had transferred the majority of immobilized ^{15}N to non-extractable soil N pools. By comparison, fine root uptake of NH_4^+ and glycine-N was low (<10% total tracer recovery), but ^{15}N enrichment of this pool was still increasing at the final sampling period. Since there was no significant loss of ^{15}N tracer within the bulk soil after 14 days for any forest type except sugar maple, it suggests plants have the capacity

to capitalize on multiple N turnover events and thus represent an important long-term sink for ecosystem N.

Plants in all stands had some capacity to absorb glycine intact, but plant N preference again varied by forest type. Relative uptake of amino acid-N versus inorganic N was lowest in tulip poplar and highest in red pine and balsam poplar, while white oak, sugar maple, and white spruce stands were statistically near unity with respect to the two N forms. However, N uptake ratios were threefold higher in EM-dominated stands than in AM-dominated stands indicating mycorrhizal association in part mediated plant N preference. Thus, amino acids represent an important component of the N economies of a broad spectrum of forest ecosystems, but their relevance to plant nutrition likely varies as a function of microbial demand for C as well as N.

Dedicated to

Claire and Marie McGrattan

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

CHAPTER 1

CHAPTER 2

LIST OF TABLES

Page

PREFACE

This dissertation contains three chapters, each of which were written and formatted for publication in separate journals. Chapter 1 was published in 2002 in *Ecosystems*. Chapters 2 and 3, respectively, were submitted to *Soil Biology and Biochemistry* and *Ecosystems*. Though each chapter has several co-authors, as lead author, I am responsible for the writing, data analysis, and the majority of the laboratory and field work presented here. Therefore, I take sole responsibility for any errors in this study. The use of 'we' throughout each manuscript represents a collaborative effort in the design of the project as well as editing of the manuscripts.

Roger Ruess and Knut Kielland were instrumental in helping me develop the research questions presented here, and devise sound strategies for testing the hypotheses arising from those questions. Much of the inspiration for the development of this study derives from Knut's own dissertation work in the tundra communities encompassing the Toolik Lake Arctic Long Term Ecological Research site. However, I am particularly grateful for Roger's time and effort in training me as an ecologist and challenging me to see the bigger picture as I wandered through various microcosms of thought.

As is common with most research projects, there are a great number of people behind the scenes who graciously contribute their time and knowledge to ensure the success of the project. The cross-ecosystem comparisons presented here would have been impossible without extraordinary logistical support from Ron Hendrick and Lee Ogden at the University of Georgia and Kurt Pregitzer and Andy Burton at Michigan Technological University. In addition, Ron and Andy's knowledge of the temperate

stands used in this study was a great resource that I tapped frequently throughout the writing process. Terry Chapin took time out of his very busy schedule to spend time with me sampling in the field as well as review each manuscript prior to submission. Lola Oliver and Tim Quintal of the Forest Soils Laboratory provided invaluable technical assistance during much of the laboratory analyses. Lola deserves special thanks for the thousands of samples she has run on the mass spectrometer for our lab group over the years. Amy Zacheis, Dana Thomas, and Julie McIntyre were all helpful in determining the appropriate statistical analyses for each data set.

Field work in ecology often involves long hours under inclement conditions, particularly for experimental designs which require 'round the clock' sampling effort. Jeffrey Farmer, Greg Maurer, Jason Schneider, Joe Hicks, Jennifer Mitchell, and Kevin Petrone contributed many hours of hard work helping me collect data; without their assistance this study could not have been accomplished. Above all, I am indebted to Greg who cheerfully spent most of an uncomfortably hot summer crisscrossing Georgia and Michigan in a 1973 Dodge cargo van, without air conditioning, to collect samples from the temperate sites.

Finally, I thank my parents, Jeanne and Wes, for their unwavering support and encouragement during the many years it has taken me to complete this project. They have stood by me since day one, and I am truly grateful for their persistence.

GENERAL INTRODUCTION

It is generally agreed that nitrogen (N) is the nutrient most often limiting forest ecosystem production (Vitousek et al. 1991), and considerable effort over the last several decades has been directed towards elucidating factors regulating N availability to plants. A central process in the terrestrial N cycle, N mineralization, particularly the net accumulation of mineral N, has long been considered the key component of soil N cycling defining soil fertility with respect to plant growth (Vitousek and Howarth 1979). However, our improved understanding of the complexity of soil N cycling dynamics has generated a greater awareness of the limitations of this metric.

Net N mineralization is a useful indicator of N supply to plants in ecosystems where the cycling of soil N is rapid enough to minimize competition for N between plants and soil microorganisms, but in marginal ecosystems, measures of net N mineralization can greatly underestimate the amount of N supplied to plants. For example, traditional approaches to summarizing ecosystem N budgets have failed to account for a large percentage of annual plant N increments in alpine (Labroue and Carles 1977), arctic (Shaver et al. 1991, Giblin et al. 1991), and taiga ecosystems (Ruess et al. 1996). The discrepancy between N supply and plant N demand is due in part to the fact that net accumulation of soluble NO_3^- or NH_4^+, or a lack thereof, is a poor predictor of overall N cycling since gross N mineralization rates can be masked by microbial immobilization (Stark and Hart 1997). Similarly, whole-soil processes are often misrepresented by microcosm studies of soil N cycling which treat the soil as a homogenous medium despite strong evidence of spatial and temporal variation in soil nutrient availability.

More importantly though, plant plasticity in N acquisition, principally the ability to utilize organic N forms, has forced a general reconsideration of factors controlling plant N nutrition in terrestrial ecosystems.

Evidence for direct plant uptake of organic N dates back a century (Hutchison and Miller 1911), but the ecological significance of this N assimilatory pathway over a range of plant species and geographical locations has only recently started to materialize. Organic N is the predominant form of soil N for most terrestrial ecosystems. By some estimates, 75% of soil organic N consists of proteinaceous material and heterocyclic N compounds (Schulten and Schnitzer, 1998). Studies covering a broad range of plant taxa from arctic, (Kielland 1994) boreal (Persson and Näsholm 2001), temperate (Finzi and Berthrong 2005), tropical (Endres and Mercier, 2003), and agricultural (Jämtgård et al. 2008) ecosystems have demonstrated plant capacity to "short-circuit" the N mineralization loop by directly absorbing soil organic N, particularly free amino acids (FAA). In natural environments, plants likely utilize on other forms of organic N, e.g. nucleic acids (Clinton et al. 1995), but most studies of plant organic N uptake have focused on proteinaceous compounds given the relatively high abundance of amino acid N in soil (Senwo and Tabatabai 1998). Together these observations suggest that the N economy of plants can no longer be modeled solely on the availability of mineral N. This recognition has lead to a revision of the traditional view of terrestrial N cycling whereby the key process regulating bioavailable N has shifted from N mineralization to exoenzyme-mediated depolymerization of polymeric soil organic N (Chapin et al. 2002).

While ecologists are now beginning to appreciate the role of organic N in the N economies of plants in forested ecosystems, there are several caveats to the approaches used by many researchers studying the contribution of organic N to plant nutrition. First, much of the aforementioned work examining plant absorption of FAA has been conducted on either excised roots or plants grown in hydroponics. Even investigations of plant FAA uptake in soil are often highly controlled pot experiments where ecologically realistic plant-microbial interactions for N are unlikely. Second, simple organic N compounds, e.g. amino acids, are a source of labile carbon (C) as well as N. Therefore, studies of plant uptake of organic N should also consider the C status of the soil microbiota since differences in competition for various organic N sources may be driven as much by C limitation as by microbial demand for N. Third, plant and microbial use of N can be mutualistic as well as competitive, particularly in ecosystems where a significant portion of the microbial biomass (foremost mycorrhizal fungi) is actually part of the plant complex and thus not in direct competition with plants for N. Additionally, despite strong evidence that mycorrhizal fungi vary widely in their capacity to mobilize and assimilate nutrients for their host, linkages between plant N uptake and mycorrhizal type have never been explicitly tested across a broad spectrum of forest ecosystems. Such oversights can detract from our understanding of plant nutrition as well as the underlying processes associated with soil N turnover.

The overall goal of my research was to address the relative importance of inorganic vs. organic N cycling across a broad latitudinal gradient of forest ecosystems that differ with respect to climate, overstory taxonomy (gymnosperm- vs. angiosperm-

dominated forests), soil organic matter quality, and dominant mycorrhizal association: ectomycorrhizae (EM) vs. arbuscular mycorrhizae (AM). I initiated this study in 1998 to address four broad objectives. My first objective was to ascertain, *in situ*, whether plants within each forest type could directly absorb soil FAA. Secondly, I sought to estimate, at the stand level, the relative contribution of inorganic and organic N sources to the N economy of plants within each community. Thirdly, I assessed the compartmentalization of inorganic and organic N within soil pools and evaluated the partitioning of these N sources between plants and microorganisms. My final objective was to determine how differences in soil organic matter quality, particularly the availability of labile substrate influences the turnover dynamics of FAA along a latitudinal gradient of forest soils.

These research objectives are aimed at testing hypotheses that not only develop a predictive framework for plant N usage, but that directly contribute to broader patterns for N cycling in terrestrial forest ecosystems. In accordance with the new paradigm in terrestrial N cycling, I hypothesized that direct plant uptake of amino acid N across this range of forest ecosystems is linked directly to soil organic matter turnover and inversely correlated with rates of inorganic N mineralization. In N-limited boreal forests where low soil temperatures retard rates of N mineralization, I predicted that amino acids would assume a greater role in supplying the annual N requirements of vascular plants than in temperate stands where N mineralization rates are typically higher. Additionally, since EM-dominated soil communities generally exhibit more extensive hyphal networks and a greater capacity to degrade complex organic substrates than AM communities (Smith and Read 1997), I also hypothesized that mycorrhizal association mediates, to some degree,

the turnover of inorganic N vs. organic N and consequently the ratio of inorganic to organic N uptake by plants. Across plant taxa, I expected gymnosperms to exhibit a greater reliance on organic N than angiosperms due to the higher rates of fine root turnover, higher substrate quality, and thus higher rates of N mineralization typically associated with angiosperms. However, within a taxon I predicted that the relative importance of organic vs. inorganic N to plant nutrition should follow mycorrhizal differences (higher uptake of organic N in EM vs. AM). In response to differences in C availability, I anticipated turnover rates for FAA, would be lowest among temperate deciduous forests and highest among temperate and boreal evergreen forests where plant litter chemistry and reduced soil temperatures are believed to act as a constraint to C cycling.

LITERATURE CITED

Chapin FS, Matson P, Mooney H. 2002. Principles of terrestrial ecosystem ecology. Springer-Verlag, New York, New York, USA.

Clinton PW, Newman RH, Allens RB. 1995. Immobilization of ^{15}N in forest litter studied by ^{15}N CPMAS NMR spectroscopy. Eurasian Journal of Soil Science 46: 551-556.

Endres L, Mercier H. 2003. Amino acid uptake and profile in bromeliads with different habits cultivated *in vitro*. Plant Physiology and Biochemistry 41:181-187.

Finzi AC, Berthrong ST. 2005. The uptake of amino acids by microbes and trees in three cold-temperate forests. Ecology 86:3345-3353.

Giblin, AE, Nadelhoffer KJ, Shaver GR, Laundre JA, McKerrow AJ. 1991.

Biogeochemical diversity along a riverside toposequence in arctic Alaska. Ecological Monographs 61:415-435.

Hutchison HB, Miller NHJ. 1911 The direct assimilation of organic and inorganic forms of nitrogen by higher plants. Journal of Agricultural Science 4:282-302.

Jämtgård S, Näsholm T, Huss-Danell K. 2008. Characteristics of amino acid uptake in barley. Plant and Soil 302:221-231.

Kielland K. 1994. Amino acid absorption by Arctic plants: implications for plant nutrition and nitrogen cycling. Ecology 75:2373-2383.

Labroue L, Carles J. 1977. Le cycle de l'azote dans les solsapins du Pic du Midi de Bigorre (Hautes-Pyrennees). Oecologia Plantarum. 12:55-77.

Persson J, Näsholm T. 2001. Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4:434–438

Ruess RW, Van Cleve K, Yarie J, Viereck LA. 1996. Contributions of fine root production and turnover to the carbon and nitrogen cycling in the taiga forests of the Alaskan interior. Canadian Journal of Forest Research 26:1326-1336.

Schulten HR, Schnitzer M. 1998. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26:1-15.

Senwo ZN, Tabatabai MA. 1998. Amino acid composition of soil organic matter. Biology and Fertility of Soils 26:235–242

Shaver GR, Nadelhoffer KJ, Giblin AE. 1991. Biogeochemical diversity and element transport in a heterogeneous landscape, the North Slope of Alaska. pp. 105-126. Quantitative Methods in Landscape Ecology. Springer-Verlag, New York.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. Academic Press, London.

Stark JM, Hart SC. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385:61-64.

Vitousek P M, Gosz JM, Grier CC, Melillo JM, Reiners WA, Todd RL. 1979. Nitrate losses from disturbed ecosystems. Science 204:469–474.

Vitousek, P.M. and Howarth, R.W. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87-115.

.

CYCLING DYNAMICS OF NH_4^+ AND AMINO ACID NITROGEN IN SOILS OF A DECIDUOUS BOREAL FOREST ECOSYSTEM[1]

ABSTRACT

Conventional studies of nitrogen 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 assess simultaneously root uptake of ammonium (NH_4^+) and glycine, we evaluated the contribution of organic N to the nitrogen economy of an interior Alaskan floodplain balsam poplar stand. Our design included multiple sampling periods extending from 45 minutes to 14 days, which permitted us to study the cycling of our C and N isotopes within root and soil pools 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 two weeks after

---

[1] McFarland JW, Ruess RW, Kielland K, Doyle AP. 2002. Cycling dynamics of NH_4^+ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. Ecosystems 5:775–788.

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, accounting for 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 out competed 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

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 understood 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 response to changing climate or altered land-use patterns focus on inorganic N cycling in

calculations of net ecosystem carbon flux (Running and others 1993; Melillo and others 1993).

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

Research over the past decade has begun to assess the relative importance of organic N to the nutritional requirements of plants in cold environments with low soil organic matter quality (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 that 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 carbon as well as nitrogen. Outside of laboratory manipulations (Jones 1999), however, there is little direct empirical evidence to support this statement.

Competition for N between plants and microorganisms exists to varying degrees in all terrestrial ecosystems and to discuss plant N uptake in isolation of microorganisms essentially excludes a major factor controlling N availability to plants. For instance, several pulse-chase experiments which 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, while in others plants appeared to dominate over the microbial pool in accumulating ^{15}N. Understanding the factors controlling nitrogen availability to plants therefore requires a more comprehensive approach in elucidating 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, i.e. NH_4^+ or NO_3^- within an isolated pool. There is a need for more integrated studies that focus on inter-pool transfers of other N forms, particularly in ecosystems where plants may have the capacity to utilize soluble forms of organic nitrogen.

Here we examine, concurrently, the cycling dynamics of NH_4^+ and amino acid N in soils of a deciduous boreal forest ecosystem dominated by ectomycorrhizal trees and shrubs. We predict that in nutrient limited systems, such as taiga forests, where low soil temperatures can slow mineralization rates, organic N sources will assume a greater role than inorganic N sources (e.g., 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 using a multiple stable isotope design that allowed us to assess simultaneously root uptake of NH_4^+ and glycine.

MATERIALS AND METHODS

Study sites

Our study area was the Bonanza Creek Long Term Ecological Research (LTER) site, 20 km SW of Fairbanks, Alaska (64°51'N, 147°43'W, elevation ~120 m). Glacially fed, the Tanana River winds along multiple channels through this landscape, creating a floodplain of alkaline soils supporting a mosaic of plant communities representing all stages of the primary-successional sequence. The successional development of these communities begins with sand bar 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 N_2 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 1993a). However as the alder community progresses to a closed canopy balsam poplar stand, both N_2 fixation and net nitrification decline (Van Cleve and others 1993b; Uliassi and Ruess 2002).

A combination of field and lab evidence suggests there are two mechanisms driving N limitation in balsam poplar stands. First, secondary chemicals (tannins) leached from balsam poplar litter may suppress N_2-fixation and gross N mineralization rates (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, indicating 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 1.1). Vegetation structure for these sites includes a closed canopy of balsam poplar with a dense thin-leaf alder understory. Basal area for stems > 5 cm averaged 36.7 $m^2 \cdot ha^{-1}$ and 1.8 $m^2 \cdot ha^{-1}$ 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 $g \cdot m^{-2} \cdot yr^{-1}$ 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 7 cm depth 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 depths > 8 cm in some places. Soil C:N ratios average 19.0 for the organic layer and 16.0 for the mineral soil (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 sampling

Our tracer experiment was conducted during August 1998 in previously established 30 x 30 m plots, one plot per stand. Within each plot, there were three subplots, each of which contained three injection grids, one for each of three treatments. The entire design was replicated across three stands. Injection grids measured 81 x 15 cm and consisted of 6 identical templates with 37 holes each (222 total/grid). Each grid was injected with one of 3 treatment solutions in the upper 10 cm of soil. Treatment solutions

were (1) $^{15}NH_4^+$ plus U-[$^{13}C_2$]-glycine (ammonium treatment), (2) NH_4^+ plus U-[$^{13}C_2$] [^{15}N]-glycine (glycine treatment), or (3) distilled water (control).

Using doubly-labeled glycine in the second treatment allowed us to evaluate 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 (Näsholm 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 brought about by the simultaneous addition of labeled ammonium and glycine in the first treatment. Injection volume was 2 ml, which applied approximately 0.39 g $^{13}C \cdot m^{-2}$and 0.22 g $^{15}N \cdot m^{-2}$ for both labeled solutions. Total N additions for each treatment averaged 10.51 μg N •g^{-1}dry soil (5.255 μg as NH_4^+-N and 5.255 μg as glycine-N). Each 2 ml aliquot was delivered by inserting the needle to 10cm depth and emptying the repeating pipette as the needle was withdrawn.

Coring grids consisted of 6 holes large enough to allow a soil corer with an inside diameter of 5.5 cm to pass through unobstructed (Figure 1.1). The center of each coring hole matched exactly 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 μg ^{15}N and 1040 μg ^{13}C per core—see calculations below). Both injection grids and coring grids were constructed of 0.32 cm lexan sheets which were flexible enough to mould 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 five meters. Injecting a complete subplot took approximately thirty minutes, 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 d; initial processing was conducted on site in a tent laboratory. Immediately following sampling, each core was split vertically into 2 equal halves. One half was used for sorting and freezing roots for ^{13}C and ^{15}N analysis. The other half was used for ^{13}C and ^{15}N analysis of (1) total soil C and N, (2) extractable dissolved inorganic N (DIN) and dissolved organic N (DON), and (3) microbial N. No rocks were found in any of the cores collected, and thus soils were hand-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 analyses

Root samples were thawed and floated briefly in distilled water to remove all remaining organic matter. We separated roots by size. Roots > 1 mm diameter were classified as coarse, while those ≤ 1 mm 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 #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 a 50 μl of a 100 ppm $(^{14}NH_4)_2SO_4$ solution (0.366% ^{15}N). Approximately 0.2 g of MgO was added to each container just prior to sealing, which was then placed on an orbital shaker table at 85 rev min^{-1} for 5 d. 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 these 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 off 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 to observe, with greater resolution, the subtle enrichment of this pool against background. $\delta^{13}C$ was calculated using the following formula:

$$\delta^{13}C = 1000 * (\text{sample \% } ^{13}C - \text{PDB \% } ^{13}C)/\text{PDB \% } ^{13}C$$

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 1.1), to estimate the fraction of label (600 μg ^{15}N and 1040 μg ^{13}C) that was injected into each treatment core.

Statistical analyses

All C and N pools were analyzed using 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's HSD tests. We assumed subplot, 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) to evaluate variance within all our extractable N pool 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 $^{13}C^{15}N$-glycine treatment we regressed excess ^{13}C against excess ^{15}N for each time period (Näsholm 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

Nitrogen pool size varied dramatically between the various soil components. MBN (mean ± S.E.) averaged 155.7 ± 7.9 μg N •g^{-1} dry soil across all treatments and time periods. This represented ≈ 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 ± 3.7 μg N •g^{-1} dry soil respectively, across all treatments and sampling periods. Thus, the N additions associated with each treatment enhanced the DIN pool by 56% and the DON pool by 8% on average. 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% nitrogen, accounted for an average of 47 μg N •g^{-1} dry soil.

Altogether, ^{15}N values for our control samples fell within reasonable ranges for ambient δ ^{15}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 (Kielland *unpublished data*). Soil δ ^{15}N values for our control cores (0-12 cm) averaged 1.3 ± 0.4‰. Fine root values were slightly depleted at –0.7 ± 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 (Kielland *unpublished data*). Variation in ^{13}C and ^{15}N abundance of each pool was small for control cores across time, while $\delta\ ^{15}N$ values for treated cores ranged 1-2 orders of magnitude higher than natural abundance for most pools and time periods. This suggests that our ability to detect treatment effects in each pool over time was strong despite any fractionation associated with inter-pool transfer of N.

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

Recoveries of ^{15}N from labeled ammonium and glycine are summarized for each pool in Table 1.2. 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 ^{15}N recovered from the NH_4^+-amended (69.7 ± 3.0 %) cores was on average 9% higher across all time periods than soil from cores which received glycine (60.2 ± 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 d 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 1.2), measuring less than 30% after 14 d. 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 carbon loss, it would appear that microbes are assimilating glycine to utilize the carbon skeleton for metabolism rather than for biosynthesis (See Discussion).

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 as recovery rates for glycine ^{15}N were of the same magnitude as those for $^{15}NH_4^+$. Enrichment within this pool was very rapid for both treatments, averaging 46% recovery for ammonium and 31% recovery for glycine, 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 as there was no significant time x treatment interaction ($F_{5,34} = 1.00$, $P = 0.43$); however, recovery rates appeared to be higher in the ammonium treatment than for glycine at some time periods (Figure 1.3). 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, as 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 d for ammonium cores before falling below 5% recovery at 14 d. 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 nitrogen. Within 24 h the DIN pool accounted for less than 2% of the glycine label. Recovery peaked again at seven 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.

Though 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 re-enrichment of the DON pool after 24 h corresponds to a concomitant increase in microbial biomass ^{15}N (Figure 1.3) 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 oscillated significantly during the first four sampling periods, while recovery of ^{15}N as DON increased for both treatments between 12 and 24 h. However, after two weeks, the amount of label recovered as DON had fallen to 6-7%, similar to the values observed for DIN. These observations suggest: 1) that some fraction of the microbial population might be releasing extracellular enzymes (into the DON pool) for degradation of more complex organic substrates (see Discussion), and 2) that most of the ^{15}N remaining in the soil after two weeks must be locked up in a recalcitrant (non-extractable) organic N form.

Root nitrogen uptake

Amino acid N and NH_4^+ were taken up by fine root biomass at approximately the same rate (Figure 1.4); 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 d, total recovery was still increasing after two weeks in both treatments. In contrast, fine root carbon showed a 2 ‰ enrichment of ^{13}C over the first 24 h, but no significant change over the next 13 days (Figure 1.5). Enrichment of the fine root carbon 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 (Näsholm and others 1998). This 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 1.6). Our data demonstrate an enrichment of ^{13}C in excess of the 2:1 ratio within fine root biomass for the first two 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 the nitrogen 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 three-fold to account for fine root production in these stands. Herein we discuss the hypothesis that part of the unexplained nitrogen 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 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 though, was that 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 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 1-12 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 nitrogen from the carbon skeleton and excreting excess nitrogen (Barraclough 1997). Some of the carbon could be used for microbial biosynthesis. However, during a companion experiment conducted in floodplain white spruce stands we measured a rapid pulse of $^{13}CO_2$ prior to extracting cores treated with labeled glycine (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% two weeks after injection. Though our evidence is largely circumstantial, we suspect 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 nitrogen- and carbon-limited. If microbial growth were limited only by nitrogen 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 carbon 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 our ^{15}N results reflect more a natural cycling of nitrogen 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 show a decline in ^{15}N at the end of our sampling regime. Second, analysis of the ^{15}N content of the bulk soil reveals no significant change over time for either treatment. Finally, others 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 (e.g., Näsholm 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 (< 2% recovery) in comparison to microorganisms (12-64% recovery), but it was the only pool that was increasing in enrichment after 14 d. Moreover short-term uptake patterns show that plants can compete to a limited extent for amino acid N directly. 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 two, marking an enrichment of ^{13}C to ^{15}N which 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 though, 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 (< 1 mm diameter) only. We did not measure the δ ^{13}C or ^{15}N of materials that were transported out of fine roots to the rest of the plant. Consequently, 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 nitrogen 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 (i.e., ectomycorrhizae within the root), this would explain why we observed an excess enrichment of ^{13}C in the fine root carbon 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 to 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 roots fine roots in each of our stands are colonized by some type of ectoycorrhizal fungus (Table 1.1; Lansing *unpublished data*). 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 (Andersson and others 1997). Due to the alkalinity of the soils along the Tanana floodplain (Van Cleve and others 1993a), it is possible that mycorrhizal fungi in these forests could be 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 might be the superior competitors for nutrients.

Given the ubiquitous nature of ectomycorrhizal hyphae in these stands, perhaps 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). Though 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 nitrogen assimilated by the fungal symbiont is retained within the external mycelium despite the fact that this tissue represented less than 16% of total fungal biomass (Colpaert and others 1996). Labeled nitrogen 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 that 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, plants (<12 h) directly competed 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, and 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.

Though 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 turnover. 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 treatments 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.

ACKNOWLEDGEMENTS

The authors wish to thank Lola Oliver and Tim Quintal of the Forest Soils Laboratory at the University of Alaska, Fairbanks for their advice and the use of their facilities. We also

thank Jeffrey Farmer for his extraordinary effort in the field. Terry Chapin, Kurt Pregitzer, Ron Hendrick, Josh Schimel, and two anonymous reviewers greatly improved the quality of this manuscript with their comments. This research was supported through grants from the University of Alaska's Center for Global Change, the National Science Foundation's Ecosystem program, grant DEB 966537, the Bonanza Creek LTER program, and USDA NRICGP 99-35101-7859.

REFERENCES

Aber J, McDowell W, Nadelhoffer, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. Bioscience 48:921-933.

Abuzinadah RA, Read DJ. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants . I. Utilization of peptides and proteins by ectomycorrhizal fungi. New Phytologist 103:481-493.

Abuzinadah RA, Finlay RD, Read DJ. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. II. Utilization of protein by mycorrhizal plants of *Pinus contorta*. New Phytologist 103:495-506.

Andersson S, Ek H, Soderstrom B. 1997. Effects of liming on the uptake of organic and inorganic nitrogen by mycorrhizal (*Paxillus involutus*) and non-mycorrhizal *Pinus sylvestris* plants. New Phytologist 135:763-771.

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

Bremner JM, Mulvaney CS. 1982. Nitrogen—total. Page AL, editor. Methods of soil analysis. Part 2. Chemical and microbiological properties. Madison (WI):American Society of Agronomy. p595-622.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17:837-842.

Chapin, FS III, Fetcher N, Kielland K, Everett KR, Linkins AE. 1988. Productivity and nutrient cycling of Alaskan tundra: enhancement by flowing soil water. Ecology 69:693-702.

Chapin FS III, Moilanen L, Kielland K. 1993. Preferential use of organic nitrogen for growth by a nonmycorrhizal arctic sedge. Nature 631:150-153.

Colpaert JV, Van Laere A, van Assche JA. 1996. Carbon and nitrogen allocation in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* L. seedlings. Tree Physiology 16:787-793.

Dahne J, Klingelhofer M, Ott M, Rothe GM. 1995. Liming induced stimulation of the amino acid metabolism in mycorrhizal roots of Norway spruce (*Picea abies* [L.] Karst.). Plant and Soil 173:67-77.

Finlay RD, Frostegard A, Sonnerfelt AM, 1992. Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl ex Loud. New Phytologist 120:105-115.

Giblin AE, Nadelhoffer KJ, Shaver GR, Laundre JA, McKerrow AJ. 1991. Biogeochemical diversity along a riverside toposequence in arctic Alaska. Ecological Monographs 61:415-435.

Hart SC, Firestone MK, Paul EA, Smith JL. 1993. Flow and fate of soil nitrogen in an annual grassland and a young mixed-conifer forest. Soil Biology and Biochemistry. 25:431-442.

Jackson LE, Schimel JP, Firestone MK. 1989. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Biology and Biochemistry 21:409-415.

Jones DL 1999. Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biology and Biochemistry 31:613-622.

Jones DL, Kielland K. 2002 Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. Soil Biology and Biochemistry 34:209-219.

Keeney DR, Nelson DW. 1982. Nitrogen-inorganic forms. Page AL, Miller RH, Keeney DR, editors. Methods of soil analysis. Part 2. Chemical and microbiological properties, 2nd ed. Madison (WI):American Society of Agronomy. p643-698.

Khan SA, Mulvaney RL, Mulvaney CS. 1997. Accelerated diffusion methods for inorganic-nitrogen analysis of soil extracts and water. Soil Science Society of America Journal 61:936-942.

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 cryptograms. Ecoscience 4:75-79.

Klingensmith KM, Van Cleve K. 1993. Patterns of nitrogen mineralization and nitrification in floodplain successional soils along the Tanana River, interior Alaska. Canadian Journal of Forest Research. 23:964-969.

Lipson DA, Monson RK. 1998. Plant-microbe competition for soil amino acids in the alpine tundra: effects of free-thaw and dry-rewet events. Oecologia 113: 406-414.

Marion GM, Van Cleve K, Dryness CT, Black CH. 1993. The soil chemical environment along a forest primary successional sequence on the Tanana River floodplain, interior Alaska. Canadian Journal of Forest Research. 23:914-922.

Melillo JM., McGuire AD, Kicklighter DW, Moore B III, Vorosmarty CJ, Schloss AL. 1993. Global climate change and terrestrial net primary production. Nature 363:234-240.

Michelsen A, Schmidt I.K, Jonasson S, Quarmby C, Sleep D. 1996. Leaf ^{15}N abundance of subarctic plants provide field evidence that ericoid, ectomycorrhizal and nonmycorrhizal and arbuscular mycorrhizal species access different sources of nitrogen. Oecologia 105:53-63.

Michelsen A, Quarmby C, Sleep D, Jonasson S. 1998. Vascular plant 15N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. Oecologia 115:406-418.

Näsholm T, Huss-Damell K, Högberg P. 2000. Uptake of organic nitrogen in the filed by four agriculturally important plant species. Ecology 81:1155-1161.

Näsholm T, Ekblad A, Högberg P. 1998. Boreal forest plants take up organic nitrogen. Nature 392:914-917.

Perakis SS, Hedin LO. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. Ecology 82: 2245-2260.

Raab TK, Lipson DA, Monson RK. 1999. Soil amino acid utilization among species of the Cyperaceae: plant and soil processes. Ecology 80:2408-2419.

Read DJ. 1991. Mycorrhizas in ecosystems. Experientia 47:376-391.

Ruess RW, Van Cleve K, Yarie J, Vierck LA. 1996. Contributions of fine root production and turnover to the carbon and nitrogen cycling in the taiga forests of the Alaskan interior. Canadian Journal of Forest Research 26:1326-1336.

Running SW, Hunt RE. 1993. Generalization of forest ecosystem process model for other biomes, BIOME-BGC, and an application for global-scale models. Ehleringer JR, Field CB, editors. Scaling physiological processes: leaf to globe. San Diego: Academic Press. p141-158.

Schimel, JP, Chapin FS III. 1996. Tundra plant uptake of amino acid and NH_4^+ nitrogen *in situ*: plants compete well for amino acid N. Ecology 77:2142-2147.

Schimel JP, Van Cleve K, Cates RG, Clausen TP, Reichardt PB. 1996. Effects of balsam balsam poplar (*Populus balsamifera*) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. Canadian Journal of Botany 74:84-90.

Schulten HR, Schnitzer M. 1998. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26:1-15.

Shaver GR, Nadelhoffer KJ, Giblin AE. 1991. Biogeochemical diversity and element transport in a heterogeneous landscape, the North Slope of Alaska. Quantitative methods in landscape ecology. New York: Springer-Verlag New York. p105-126.

Stribley DP, Read DJ. 1980. The biology of mycorrhizae in the Ericaceae VII. The relationship between mycorrhizal infection and the capacity to utilize simple and complex organic nitrogen sources. New Phytologist 86:365-371.

Sugai SF, Schimel JP. 1993. Decomposition and biomass incorporation of ^{14}C-labeled glucose and phenolics in taiga forest floor: effect of substrate quality, successional state, and season. Soil Biology and Biochemistry. 25:1379-1389.

Uliassi, DD, Ruess RW. 2002. Limitations to symbiotic N fixation in primary succession on the Tanana river floodplain, Alaska. Ecology 83:88-103.

Van Cleve K, Oliver L, Schlentner R, Viereck LA, Dyrness CT. 1983. Productivity and nutrient cycling in taiga forest ecosystems. Canadian Journal of Forest Research 13:747-766.

Van Cleve K, Yarie J, Erikson R, Dyrness CT. 1993a. Nitrogen mineralization and nitrification in successional ecosystems on the Tanana River floodplain, interior Alaska. Canadian Journal of Forest Research 23:970-978.

Van Cleve, K., C. T. Dyness, G. M. Marion, and R. Erickson. 1993b. Control of soil development on the Tanana River floodplain, interior Alaska. Canadian Journal of Forest Research 23:941-955.

Viereck LA, Dyrness CT, Foote MJ. 1993. An overview of the vegetation and soils of the floodplain ecosystems of the Tanana River, interior Alaska. Canadian Journal of Forest Research 23:889-898.

Walker LR. 1989. Soil nitrogen changes during primary succession on a floodplain in Alaska, USA. Arctic and Alpine Research 21:341-349.

Whitledge T, Malloy S, Patton C, Wirick C. 1981. Automated nutrient analysis in seawater. Brookhaven National Laboratory Tech. Rep. BNL-51398.

Yin X. 1992. Empirical relationships between temperature and nitrogen availability across North American forests. Canadian Journal of Forest Research 22:707-712.

Zak DR, Groffman PM, Pregitzer KS, Christensen S, Tiedje JM. 1990. The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. Ecology 71:651-656.

Zogg GP, Zak DR, Pregitzer KS, Burton AJ. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81:1858-1866.

Table 1.1 Stand characteristics for each of the three floodplain balsam poplar study sites in the Bonanza Creek Experimental Forest. [a]Average soil temperature measured at 7cm depth during August 1998. [b]Stem density = stems ·ha^{-1}, [c]Basal area = m^2 ·ha^{-1}, and [d]Total tree biomass = kg ·ha^{-1} as measured in June 1998. [e]Litterfall = kg ·ha^{-1} ·yr^{-1} was collected from September 1997 to September 1998. [f]Mycorrhizal root tips are live ectomycorrhizal (ECM) root tips per meter balsam poplar fine root and [g]percent mycorrhizal infection is # 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 data*).

	Site		
Parameter	*Stand 1*	*Stand 2*	*Stand 3*
Soil temperature (˚ 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 x 10^5	1.68 x 10^5	1.99 x 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

Table 1.2 Mean percent recovery (± 1 SE) of added ^{15}N within various plant and soil pools. 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.

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

Table 1.3 The effect of treatment, sampling period, and stand on percent recovery of added tracer within soil extractable N and fine root and bulk soil C and N pools. MBN = Microbial biomass nitrogen. Data were analyzed using a mixed model ANOVA (α = 0.05). * $P \leq 0.01$; ** $P \leq 0.001$

Pool	Source	Df	F	P
DIN	Stand	2, 58	0.43	0.66
	Treatment	1, 58	22.21	**
	Sampling period	5, 58	12.12	**
	Treatment x Sampling period	5, 58	3.24	*
DON	Stand	2, 58	2.05	0.14
	Treatment	1, 58	0.10	0.75
	Sampling period	5, 58	3.51	*
	Treatment x 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	**
	Treatment x Sampling period	5, 34	1.00	0.43
Fine Root C	Stand	2, 88	2.83	0.06
	Treatment	1, 88	0.09	0.76
	Sampling period	5, 88	7.82	**
	Treatment x 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	**
Table 1.3 (cont.)				
	Treatment x 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	**
	Treatment x 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 x Sampling period	5, 88	0.26	0.93

FIGURE LEGENDS

Figure 1.1 Coring grid design for treatment application and sample extraction. Treatments are as follows: TRT A (labeled ammonium treatment) = $[^{15}NH_4]_2SO_4$ + U-$[^{13}C_2]$-glycine; TRT B (doubly- labeled glycine treatment) = $[NH_4]_2SO_4^+$ + U-$[^{13}C_2][^{15}N]$-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) d. Sampling periods were randomized within grids. Sampling Periods: 1=T_{45m}, 2=T_{2h}, 3=T_{12h}, 4=T_{24h}, 5=T_{168h}, 6=T_{336h}.

Figure 1.2 Percent recovery of added ^{13}C and ^{15}N in bulk soil carbon and nitrogen pools over time. Symbols are as follows: ● labeled ammonium treatment ($^{15}NH_4^+$ + ^{13}C-glycine); and ■ doubly-labeled glycine treatment ($^{14}NH_4^+$ + $^{13}C^{15}N$-glycine). Open and solid symbols represent soil carbon and soil nitrogen, respectively. Values are averaged across cores (n = 9) and data are means ± SE.

Figure 1.3 Percent recovery of added ^{15}N as DIN, DON, or microbial N pools against time. Symbols are as follows: ● microbial N pool; □ DIN pool; and ■ DON pool. Open and solid symbols represent ammonium ($^{15}NH_4^+$ + ^{13}C-glycine); and doubly-labeled glycine ($^{14}NH_4^+$ + $^{13}C^{15}N$-glycine) treatments respectively. Data are means ± SE.

Figure 1.4 Percent recovery of added ^{13}C and ^{15}N in fine root carbon and nitrogen pools over time. Symbols are as follows: ● labeled ammonium treatment ($^{15}NH_4^+$ + ^{13}C-glycine); and ■ doubly-labeled glycine treatment ($^{14}NH_4^+$ + $^{13}C^{15}N$-glycine). Open and solid symbols represent root carbon and root nitrogen respectively. Data are means ± SE.

Figure 1.5 Change in δ ^{13}C values of the fine root carbon pool over time. This figure is provided in order to observe treatment effects at a finer resolution. Symbols are as follows: ● labeled ammonium treatment ($^{15}NH_4^+$ + ^{13}C-glycine); ■ doubly-labeled glycine treatment ($^{14}NH_4^+$ + $^{13}C^{15}N$-glycine); and □ deionized water. Data are means ± SE.

Figure 1.6 The relationship between excess ^{13}C (μMol) and excess ^{15}N (μMol) in fine roots at all sampling periods. Data are from the doubly-labeled glycine treatment only and 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 m (s = 3.44, R^2_{Adj} = 62.4); ○ 2 h (s = 3.13, R^2_{Adj} =61.1); □ 12 h (s = 2.00, R^2_{Adj} = 82.6); △ 24 h (s = 1.54, R^2_{Adj} = 62.6); ■ 168 h (s = 1.30, R^2_{Adj} = 88.3); and □ 336 h (s = 0.98, R^2_{Adj} = 82.5). The dotted line represents the 2:1 injection ratio of C:N administered with the doubly-labeled glycine treatment.

Figure 1.1

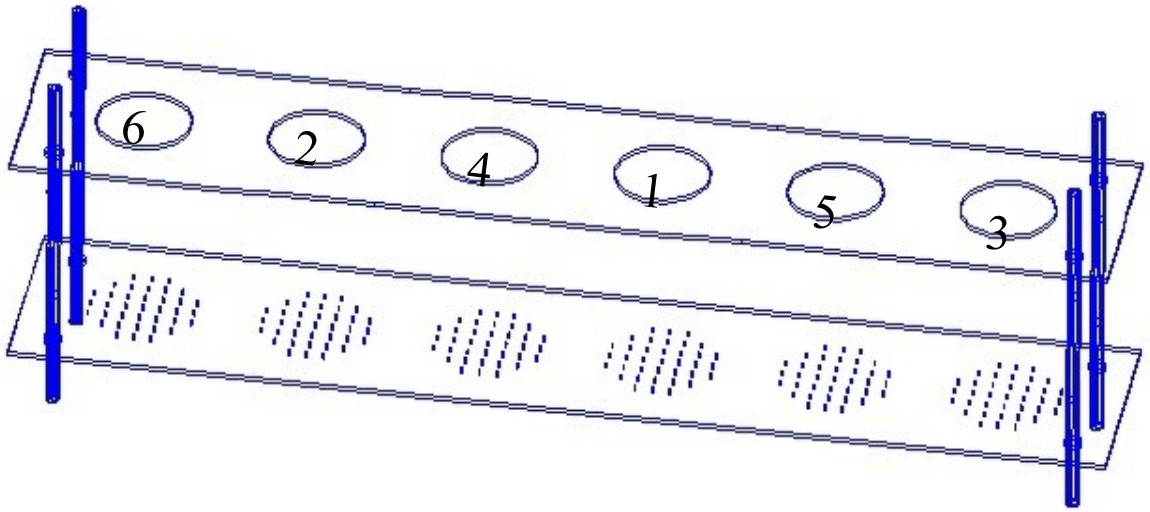

6
2
4
1
5
3

Figure 1.2

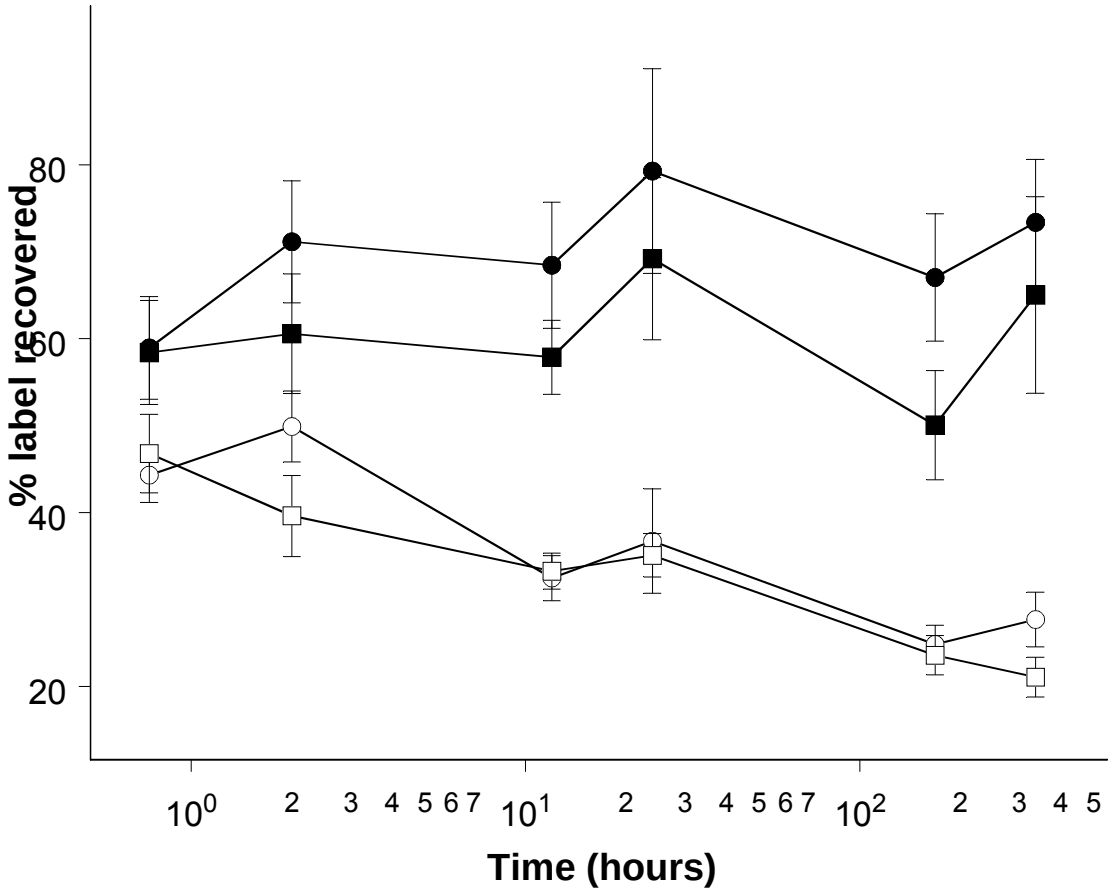

% label recovered
80
60
40
20
10^0 2 3 4 5 6 7 10^1 2 3 4 5 6 7 10^2 2 3 4 5
Time (hours)

Figure 1.3

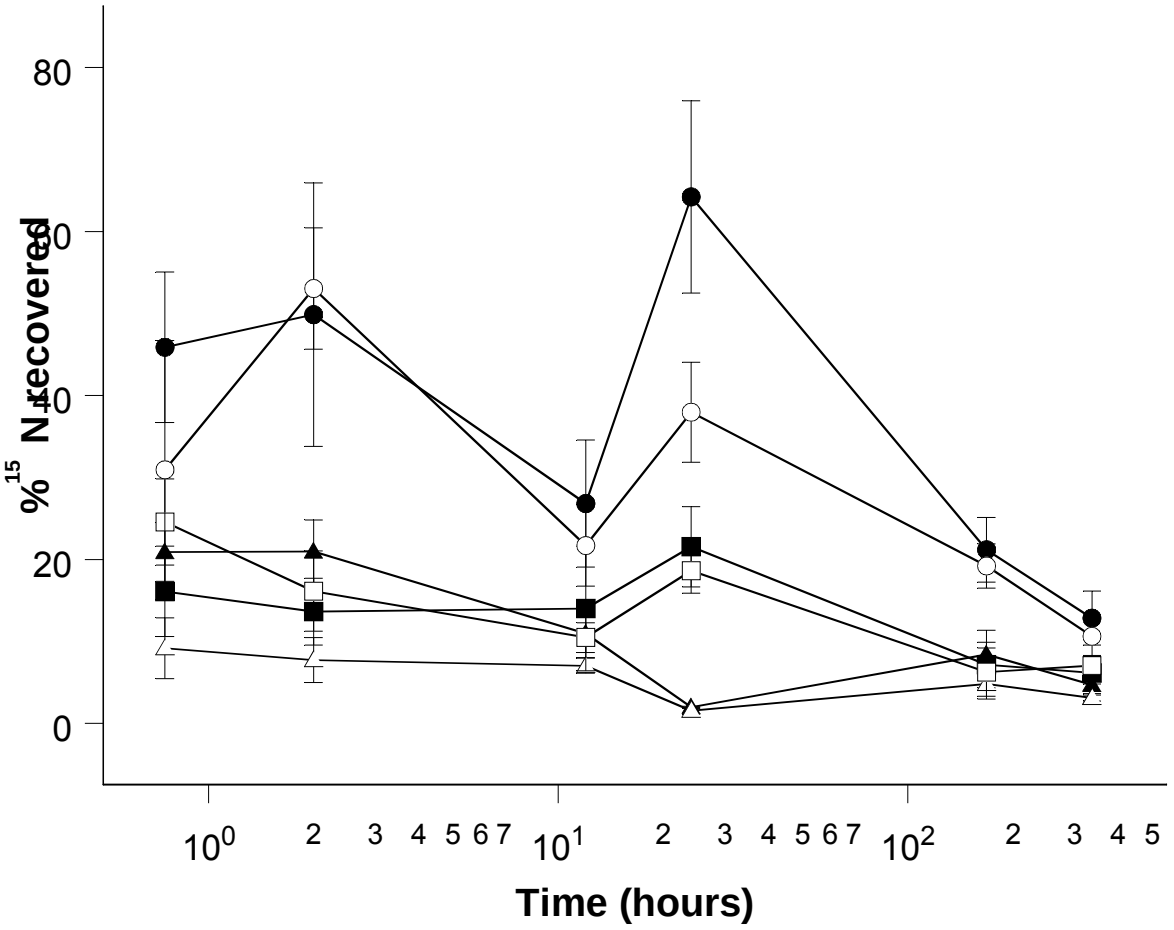

80
60
40
20
0
% 15N recovered
10^0
2
3
4
5
6
7
10^1
2
3
4
5
6
7
10^2
2
3
4
5
Time (hours)

Figure 1.4

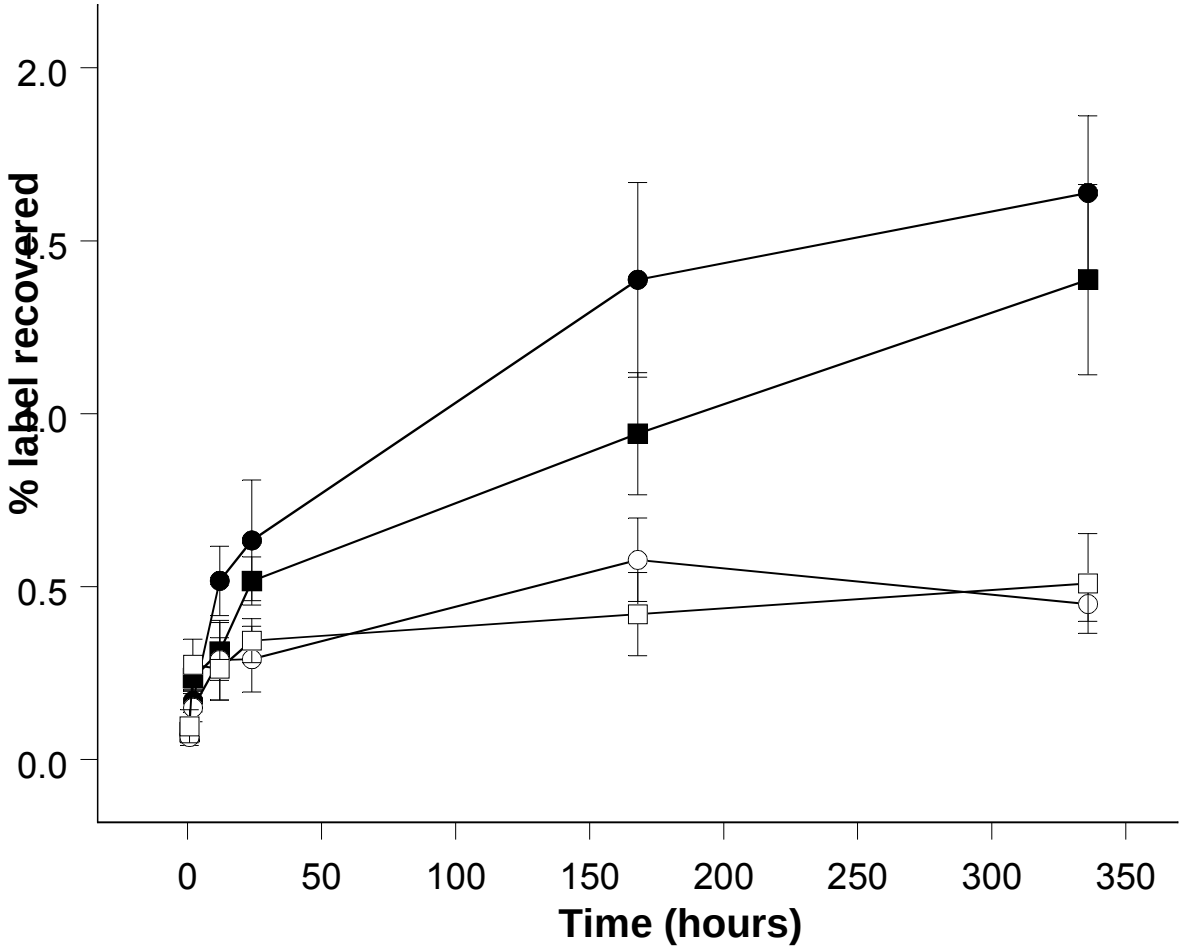

2.0
1.5
1.0
0.5
0.0
% label recovered
0
50
100
150
200
250
300
350
Time (hours)

Figure 1.5

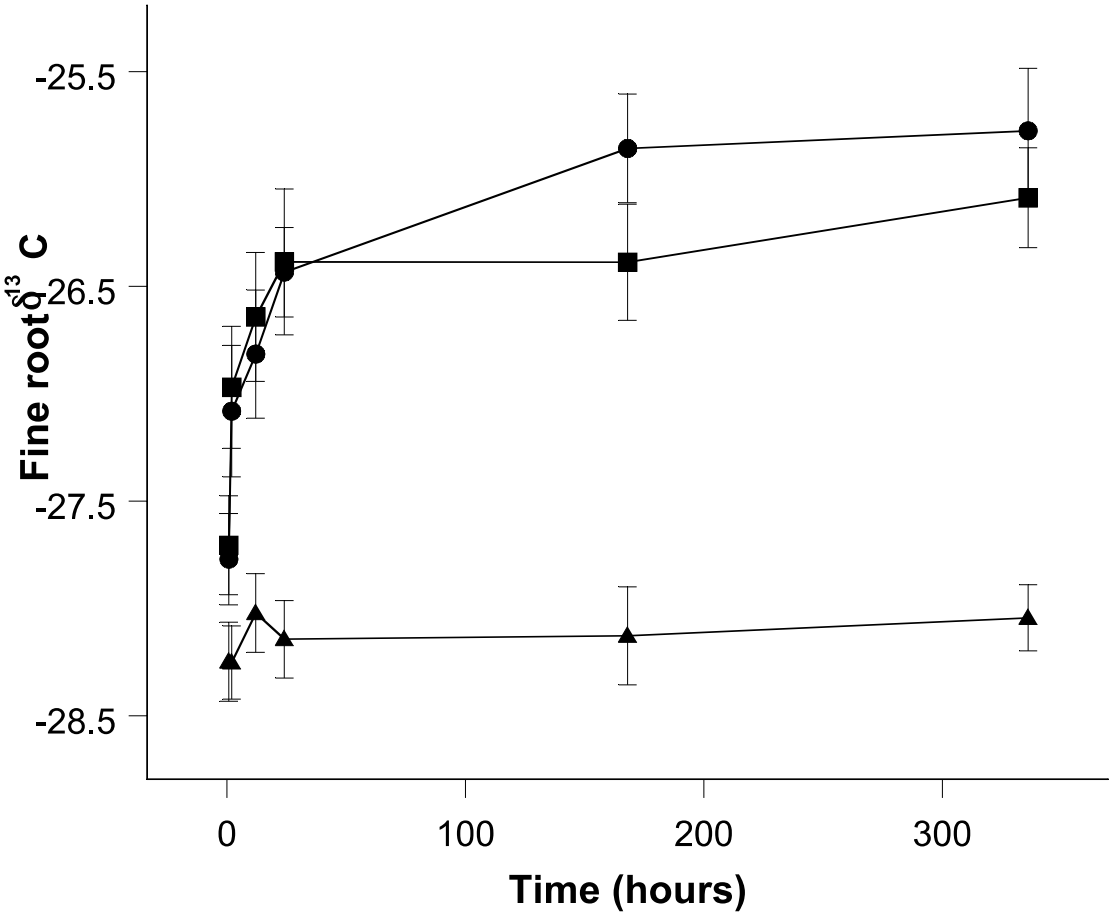
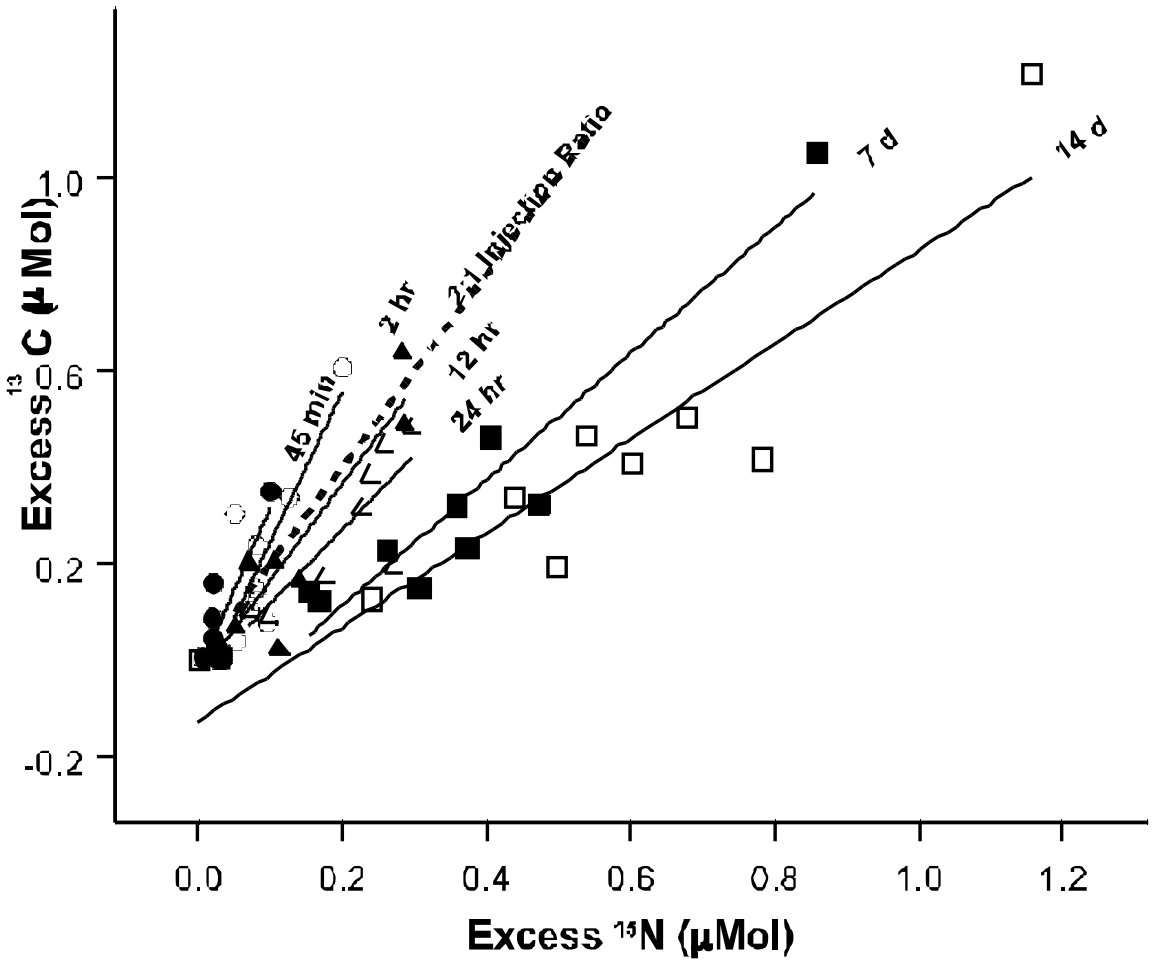


Figure 1.6


Excess ^{13}C (µMol)
1.0
0.6
0.2
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Excess ^{15}N (µMol)
45 min
2 hr
2:1 Injection Ratio
12 hr
24 hr
7 d
14 d

GLYCINE MINERALIZATION *IN SITU* CLOSELY CORRELATES WITH SOIL CARBON AVAILABILITY ACROSS SIX NORTH AMERICAN FOREST ECOSYSTEMS[2]

ABSTRACT

Free amino acids (FAA) constitute a significant fraction of dissolved organic nitrogen (N) in forest soils and play an important role in the N cycle of these ecosystems. However, relatively little attention has been given to their role as labile carbon (C) substrates that might influence the metabolic status of resident microbial populations. Heterotrophic growth in soil is often substrate-limited as the bulk of soil C consists of complex polymers that are resistant to decomposition. Still, in many soils, the microbial biomass maintains a high metabolic activity, driven by the need to rapidly capture discreet pulses of labile substrate that periodically become available through root exudation or lysis and/or microbial turnover. We hypothesized that the residence time of simple C substrates, such as FAA, are mechanistically linked to the turnover of endogenous soil C pools. We tested this hypothesis across a latitudinal gradient of forested ecosystems that differ sharply with regard to climate, overstory taxon, and edaphic properties. Using a combined laboratory and field approach, we compared the turnover of isotopically labeled glycine *in situ* to the turnover of mineralizable soil C

---

[2] Submitted to *Soil Biology and Biochemistry* as McFarland JW, Ruess RW, Kielland K, Hendrick R, Pregitzer K. *In situ* glycine mineralization closely correlates with soil carbon availability across six North American forest ecosystems

(C_{min}) at each site. The turnover of glycine was rapid (residence times < 2 h) regardless of soil type. However, across all ecosystems glycine turnover rates were strongly correlated with indices of soil organic matter quality. For example, C:N ratios for the upper soil horizons explained ~80% of the variability observed in glycine turnover, and there was a strong positive correlation between the turnover constants for glycine and C_{min}. The turnover of glycine *in situ* was better explained by changes in soil C availability than cross-ecosystem variation in soil temperature or concentrations of dissolved inorganic N and FAA-N. This suggests the rapid consumption of these low-molecular-weight substrates by soil microorganisms may be more a response to the overall decomposability of soil C than N limitation to microbial growth.

INTRODUCTION

Free amino acids (FAA) represent a labile pool of soil nitrogen (N) for plant and microbial uptake and play a key role in the N economy of terrestrial ecosystems (Atkin, 1996; Kaye and Hart, 1997; Kielland, 2001; Lipson and Näsholm, 2001). This recognition has led to a number of studies focusing on the turnover dynamics of amino acids in soils spanning multiple continents (Kuzyakov and Demin, 1998; Jones and Shannon, 1999; Lipson et al., 1999; Vinolas et al., 2001ab; Jones and Kielland, 2002; Bardgett et al., 2003; Jones et al., 2004; Finzi and Berthrong 2005; Kielland et al., 2007). These studies have yielded insights into the factors regulating the turnover of FAA in soils. In most instances, the residence times of soil amino acids are less than a few hours; however, they may persist for longer periods by adsorption to humic and mineral

components of soil (Gonod et al., 2005), or chemical inclusion into humic substances (Kuzyakov and Galitsa, 1993). Plants in many ecosystems take up amino acids readily (Schimel and Chapin, 1996; Schmidt and Stewart 1999; Näsholm et al.,1998; Näsholm et al., 2000), but the majority of FAA turnover is the direct result of microbial uptake and assimilation (Jones, 1999; Nordin et al., 2004). Once assimilated by microorganisms, amino acids can be channeled into growth, cell maintenance, or energy production (Kuzyakov and Demin, 1998; Vinolas et al., 2001ab) or transferred to plants via symbiotic associations.

The decomposition of FAA depends on several physical and biological processes. Microcosm experiments have found that amino acid turnover is regulated in part by soil temperature (Vinolas et al., 2001a). In addition, the molecular charge of the amino acid strongly influences the availability of amino acids for microbial uptake due to sorption to the solid phase of soil (Gonod et al., 2005). Positively charged species such as lysine are more likely to interact with cation exchange sites than net neutral (glycine) or negatively (glutamic acid) charged species. As well, the metabolic consumption of amino acids depends on microbial affinity for a particular substrate (Kielland, 1995; Lipson et al.,1999; Vinolas et al., 2001a) and the functional diversity of the microbial community (Degens, 1998), though direct evidence for the latter remains elusive (Jones et al., 2005).

While past research on FAA mineralization has focused on these compounds in the context of soil-N cycling (Jones and Kielland, 2002; Berthrong and Finzi, 2006), the importance of FAA as a carbon (C) source for microorganisms remains poorly understood. For example, there is compelling evidence that heterotrophic growth in many

soil environments is C-limited (Morita, 1988; Zak et al.,1994; De Nobili et al., 2001; Mondini et al., 2006). For most terrestrial ecosystems, the bulk of soil C consists of complex polymers that are resistant to decomposition. Despite the low bioavailability of a large percentage of soil organic matter, the microbial biomass in many soils maintains a high level of endogenous energy with adenylate charge ratios approaching those of microorganisms grown in pure culture studies (Brookes et al.,1987). The likely reason for sustaining such a high metabolic rate in an energy-poor environment stems from the need to capitalize on pulses of labile substrate. The metabolism of soil microorganisms depends heavily on the availability of low-molecular-weight substrates such as mono- and di-saccharides, peptides, and free amino acids. A large proportion of this labile C pool derives from plant exudates and fine root turnover that serves to prime decomposition processes (Kuzyakov et al., 2000). Activation of the microbial community by pulses of labile C can be nearly instantaneous (Jones and Murphy, 2007). However, evidence for this rapid response comes largely from laboratory incubation studies of processed soils where plant components are removed and microbial community structure is severely disrupted. Sample handling prior to laboratory measurements could affect biodegradation rates for FAA rendering those data inadequate for field simulations or modeling (Di et al., 1998).

Here we present a study using a nondestructive method (McFarland et al., 2002; Kielland et al., 2007) to assess the cycling dynamics of a soil amino acid *in situ* across a wide gradient of North American forest ecosystems that differed with regard to climate, plant taxa, dominant mycorrhizal association, and edaphic properties. Numerous studies

have examined turnover dynamics of soil FAA (Kuzyakov and Galitsa, 1993; Kuzyakov and Demin, 1998**;** Jones and Kielland, 2002; Jones et al., 2005; Berthrong and Finzi, 2006; Jones and Murphy, 2007, Kielland et al., 2007), but to our knowledge, this is the first attempt to use a common experimental approach to develop estimates of amino acid turnover *in situ* across multiple biomes. Our experimentation was designed to test the idea that turnover dynamics of FAA in forest soils is regulated more by C limitations than N limitations to microbial growth (Jones and Murphy, 2007). We had two objectives. First, without mechanically disturbing the soil profile, we estimated the turnover rate of uniformly labeled ^{13}C-glycine using $^{13}CO_2$ release as a proxy for residence time. Secondly, we related the *in situ* turnover dynamics of glycine to several indices of C availability determined in the laboratory. We predicted that rate constants for glycine mineralization would vary inversely with the overall decomposability of soil C across forest types (Kielland et al., 2007). Due to a high microbial demand for labile substrate, we anticipated that forest types with high-quality litter containing low concentrations of lignin, tannins, and other recalcitrant compounds (temperate deciduous) would exhibit slower rates of consumption of added FAA than forest types where plant litter chemistry and reduced soil temperatures (boreal) act as a constraint to C cycling.

MATERIALS AND METHODS

Study sites

Study sites were located across three North American biomes: boreal, northern temperate and southern temperate. We chose these sites in order to encompass the wide

range of vegetation types and environmental conditions represented by forests of this continent. Study sites selected within each biome, including the dominant forest ecosystem types were: Bonanza Creek Long-Term Ecological Research (LTER) site, AK, white spruce (*Picea glauca*) and balsam poplar (*Populus balsamifera*); Ford Forestry Center, MI, sugar maple (*Acer saccharum*); and Houghton, MI, red pine (*Pinus resinosa*); Coweeta LTER, NC, tulip poplar (*Liriodendron tulipifera*); B.F. Grant Experimental Forest, GA, white oak (*Quercus alba*). At each site we chose stands that were at or near steady-state with respect to C storage for that forest type. Relevant features for forest types are discussed below while specific stand characteristics are presented in Table 2.1.

Balsam poplar and white spruce are mid- to late-successional stands, respectively, in a primary successional sequence along the Tanana River floodplain in interior Alaska. The soils of this chronosequence are classified as Typic Cryofluvents (Viereck et al., 1993) and are predominantly silt-textured. Soils along older terraces are overlain with a well-developed organic horizon extending to 10 cm or more in depth. The climate is strongly continental, and forests are exposed to sub-freezing conditions for much of the year. Though the region is classified as semi-arid, precipitation often exceeds evapotranspiration due to low temperatures and a restricted growing season. Rates of net N mineralization are low compared to temperate forest ecosystems, so putatively the availability of labile N for plant uptake is reduced. Lower N availability reduces plant litter quality as the vegetation advances through successional development. It has been suggested that the shift to plant detritus with higher C:N ultimately reduces the overall decomposability of soil organic matter in late successional communities, thus decreasing

C turnover. Consequently, soil microorganisms in these stands are believed to become increasingly C-limited (Flanagan and Van Cleve, 1983) during the transition from deciduous tree-dominated stands to conifers.

Sugar maple is a common deciduous species in the Great Lakes and Acadian forest regions. As a habitat generalist, it is often found in mixed stands; however, our study area is located in a relatively pure stand of sugar maple that was previously managed under a selective cutting regime. The entire area was cut over 100 years ago, and most of the large overstory trees are about 95 to 100 years old. A second harvest occurred about 25 years ago, at which time approximately 2/3 of the basal area was left intact. Understory vegetation is relatively sparse and consists primarily of perennial herbaceous plants and sugar maple seedlings and saplings. Soils in this stand are well drained Typic Haplorthods, consisting of cobbly, silt and sandy loams with 2-12% clay content.

The red pine site is located at the William Payne La Croix plantation established in 1950 near Houghton, Michigan. This stand consists of evenly spaced (1.8 m x 1.8 m square) mature trees with no understory, so red pine accounts for 100% of the basal area. The overall terrain is relatively flat to gently sloping. Soils are sandy loams, classified as Entic Haplorthods, with a thin organic horizon at the surface consisting almost entirely of pine litter in various states of decomposition.

The tulip poplar stand is situated in Watershed 3 of the Coweeta LTER research site near Franklin, North Carolina. The terrain of this deciduous hardwood cove is steep (>30% slope) with deep (~1 m) well drained Humic Hapludults derived from folded

schist and gneiss. Natural reforestation began ~ 50 years ago following agricultural abandonment. The oldest trees in this stand date from that period; however, there was some underplanting of tulip poplar seedlings in the 1970s in an effort to increase stand density. The understory is sparse with scattered dogwood (*Cornus florida*) and striped maple (*Acer pennslyvanicum*) trees. The forest floor is rarely thicker than 2-3 cm except immediately following leaf fall; there is no Oe or Oa horizon. The high quality litter (C:N = 40; Lignin:N = 15) derived from this stand rapidly decomposes under the mesic conditions characteristic of this forest type.

Our oak site is located within the B.F. Grant Memorial Forest, a 5000 ha mixed-use research forest managed by the Warnell School of Forestry and Natural Resources at the University of Georgia, Athens. There is a long history of disturbance at this site beginning with Native American encampments, followed by slash and burn agriculture, and eventually cotton production. Soils, classified as Typic-Rhodic Hapludults, are well-drained, clayey, kaolinitic, and ranged in color from dark red to yellow-brown. The understory consists primarily of oak (*Quercus spp.*) and hickory (*Carya spp.*) with some maple (*Acer spp.*), beech (*Fagus grandifolia*), and dogwood (*Cornus florida*). The forest floor is relatively thick in places (12 cm) given the climate and consists primarily of an Oi layer with weakly developed Oe and Oa horizons. Overall the terrain for our study area is gently sloping with slopes of 2-6%.

Soil incubation experiment

To evaluate C availability, we performed gas flux measurements (net C mineralization) on soils from each of our research sites. In August 1998, we collected twelve 5.5 cm diameter soil cores from each of the six locations and randomly paired them to produce six laboratory replicates (Lancaster and Keller-McNulty 1998). The paired cores were stored frozen for three months, thawed and partitioned into upper (U) and lower (L) soil horizons. For the mull forest floor of red pine, sugar maple, white oak, and tulip poplar, U = 0-7 cm and L = 7-20 cm below the litter layer, while the mor forest floor of the boreal stands was separated into relatively pure organic and mineral horizons that approximated the sampling depths defined for the other stands. Subsequently, we homogenized each horizon by removing all obvious woody debris, roots, and rocks and hand-mixing the remaining soil to obtain a relatively uniform substrate. Two subsamples were taken from each homogenized core for soil moisture and other chemical measurements. Subsamples for C and N analysis were dried to a constant weight at 60°C and powdered in a modified roller mill. Soil organic C (C_{total}) and total N were determined for each horizon using combustion analysis on a LECO 2000 CNS autoanalyzer (LECO, St. Joseph, Michigan, USA). Due to the acidity (Table 2.2) of these soils, carbonate removal was not necessary prior to analysis.

In order to balance soil microbial activity against gaseous N loss, soil moisture content was adjusted to 55% water-holding capacity (Nunan et al., 2000) with distilled water after gravimetric determinations were made for each soil type. Approximately 100 g fresh weight of soil was placed into 980 ml Mason jars whose caps were fitted with

butyl rubber septa. The jars were sealed and preincubated for three days in the dark before gas measurements were initiated. Incubation temperatures varied according to the region from which the soils were collected (for tulip poplar and white oak, 19.8°C; balsam poplar and white spruce, 9°C; sugar maple and red pine, 17.0°C). These temperatures were representative of daily average soil temperatures recorded at 7 cm depth for each site 10 days prior to and following core collection in July 1998.

We sampled the headspace of each jar weekly for 16 weeks and determined the CO_2 concentration within using gas chromatography on a Shimadzu GC-8A fitted with a 200-cm Poropak column and a thermal conductivity detector (Shimadzu Corporation, Japan). In order to prevent inhibition of respiration due to excessive concentrations of CO_2 in the headspace, jars were capped for only 24 hours prior to each measurement and then aerated before being returned to the incubation chamber. Between sampling periods, each jar was covered with 0.8-ml polyethylene sheeting secured with a rubber band to prevent excessive moisture loss while still permitting gas exchange (Gordon et al., 1987). Following gas sampling, the water content of each jar was maintained at 55% WHC by adding deionized water to compensate for the measured weight loss. First order rate constants for microbial respiration were calculated using the following equation:

$$Ct = C_{\min}\left(1 - e^{-kt}\right)$$

where Ct is the cumulative carbon mineralization up to time, t (days), $C_{\min}$ is the potentially mineralizable pool of soil carbon, and k is the instantaneous rate constant describing the daily release of C from that pool (Kielland et al., 1997; Alvarez and Alvarez, 2000).

In situ glycine C mineralization experiment

The field component for this experiment was conducted during July 1999 for white spruce, July 2005 for balsam poplar, and from June to July 2000 for the remaining forest types. Randomly, we established 2 soil injection grid locations within a 9 m^2 plot. Each plot was replicated six times along a transect within each forest type. Injection grids were constructed of 3.2 mm lexan sheets that were flexible enough to mould 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 return periodically and precisely align our gas sampling chamber over the head space above each injection core (McFarland et al., 2002). Within each injection grid, we administered either U-[$^{13}C_2$]-glycine (glycine treatment) or distilled water (control). As part of a companion experiment examining plant-microbial competition for N, we added $(^{15}NH_4^+)_2SO_4$ to the glycine treatment. The fate of this N addition is discussed elsewhere (McFarland et al., *submitted*). Other amino acids have demonstrated a higher rate of microbial turnover, indicating that glycine is not necessarily the preferred substrate for microbial uptake and decomposition; however, glycine is generally intermediate with regard to microbial uptake and assimilation efficiency (Vinolas et al., 2001a). Moreover**,** glycine is a neutral amino acid and thus has a higher tendency to remain in solution compared with other species such as lysine and arginine, so its bioavailability is not limited by sorption. Injection volume was 2 ml, which delivered 0.39 g $^{13}C \cdot m^{-2}$.

We collected the $^{13}CO_2$ efflux above each injection core using a capped segment of 10.2 cm ABS pipe fitted with a #10 rubber stopper. Inserted into each stopper was a

short segment of polyethylene tubing connected to a 30 ml syringe via an air-tight stop cock. We used high-pressure vacuum grease to establish an airtight seal for the luer fitting between the stopcock and syringe as well as the connection between the polyethylene tubing and stopcock. The litter layer above each injection point was removed to reveal the partially decomposed horizons below. While eliminating the portion of the microbial community associated with litter, removing the litter layer dramatically improved our ability to seal the sampling chambers against the forest floor. Sampling chambers were then pressed firmly to the soil surface for three minutes at which time a 15 ml sample was collected. The gas in each chamber was thoroughly mixed by slowly pumping the syringe 10 times prior to sample collection. Gas samples were transferred over-pressurized to 10 ml exetainers (Labco. Ltd., United Kingdom) evacuated in our laboratory facilities to 0.007 kPa. All soils, with the exception of balsam poplar, were sampled at 6 periods (0.75, 2, 12, 24, 168, and 336 hours) following injection. Balsam poplar was sampled only at the first four sampling periods. We monitored soil temperature at a depth of 7 cm continuously throughout the experiment using HOBO temperature loggers (Onset Computer Corporation, Massachusetts, USA).

Gas samples were analyzed for ^{13}C-CO_2 using a Europa Scientific continuous flow mass spectrometer (SPEC-PDZ Europa Inc., United Kingdom). We report the data as cumulative ^{13}C atom percent excess (APE) of respired CO_2. APE was determined by subtracting the atom % ^{13}C of control samples from the atom % ^{13}C of samples treated with labeled glycine. Control values were averaged within a site prior to use in estimating enrichment. Data were fitted to the same single exponential model used for the soil

incubation study, except that all fitted curves were forced through the origin based on the assumption that ^{13}C excess was zero prior to injection.

Soil amino acid-N extraction and quantification

We randomly collected 15 soil cores along our transect using a 5.5 cm (I.D.) steel corer combusted at 450°C for 6 h prior to use. All cores were handled with nitrile gloves and stored in clean polyethylene bags on ice during transport to the laboratory. Within 4 hours, each core was gently hand-mixed and sieved (2.5 mm mesh) to remove rocks, large roots and as many small roots as possible. We took two subsamples from each homogenized core. One subsample was dried at 70°C to determine gravimetric moisture content. The other subsample (15 g wet weight) was extracted with 75 ml distilled water (15 min at 150 rev min^{-1}) and vacuum-filtered through a 0.2 μm cellulose acetate filter (Corning Inc, Corning, New York, U.S.A.). Soil extracts were stored frozen in 2 ml sterile polyethylene vials until analysis.

We analyzed soil extracts for total FAA using fluorometrics (Jones et al., 2002). Briefly, 20 μl of sample, standard, or blank was pipetted to a 96 well microplate. We used a Precision 2000 automated pipetting system (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) to add 100 μl of a working reagent consisting of a borate buffer, o-phthaldialdehyde, and β-mercaptoethanol to each well. Following derivatization (= 2 min), the fluorescence in each well was measured using a Biotek FL600 Fluorescence and Absorbance Microplate Reader (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) with excitation and emission wavelengths set to 340 and 450 nm, respectively.

Each sample was run in quadruplicate and the results are reported as μg amino acid-N per g dry soil.

Statistical analyses

We fitted the first order rate equation to CO_2 production from the microcosm study (n = 6) as well as cumulative APE ^{13}C from the glycine mineralization experiment (n = 6) for each forest type using PROC NLIN in SAS. Tukey's multiple sample *t* tests were applied to all pairwise comparisons of $k_{glycine}$, $^{13}C_{APEcum}$, and residence time for glycine *in situ* ($1/k_{glycine}$); the rate constant k_C; estimates for pool size, C_{min} ; and all measured soil variables from our laboratory incubations, including soil organic C and total N. We tested the assumption of normality for all the aforementioned parameters using PROC UNIVARIATE in SAS prior to conducting one and two-way ANOVAs to test for significant differences among forest types. When necessary, variables were log-transformed to meet the conditions for normality and constancy of variance. When log transformed values also failed to meet the assumptions for ANOVA we performed analyses on ranked values and compared those to analyses generated for unranked values. Simple linear regression analyses were used to relate $k_{glycine}$ and $1/k_{glycine}$ with soil temperature and CO_2 production for the upper soil horizon. All inferences regarding pool dynamics are made at the stand level, and significance for all statistical analyses was accepted at $\alpha = 0.05$, except $k_{glycine}$, $^{13}C_{APEcum}$, and residence time for glycine *in situ*, which were accepted at $\alpha = 0.1$.

RESULTS

Soil C, N, and mineralizable C

Soil organic C and total N varied significantly between the boreal and temperate stands, particularly for the upper soil horizon. Soil organic C in the upper horizon averaged 169 and 233 g $C \cdot kg^{-1}$ for white spruce and balsam poplar, respectively, whereas values for the temperate stands fell within a narrower range, averaging between 26 and 67 g $C \cdot kg^{-1}$ (Table 2.2). Carbon content in the lower horizon was more similar among all forest types, ranging from 45 g $C \cdot kg^{-1}$ in tulip poplar to 16 g $C \cdot kg^{-1}$ in red pine. Total soil N also differed significantly between the boreal and temperate forests with larger concentrations of N in the upper horizon for boreal than temperate stands. Average values ranged from 1.6 g $N \cdot kg^{-1}$ in white oak to 12.4 g $N \cdot kg^{-1}$ in balsam poplar. Soil N concentrations in the lower horizon were less variable, with the highest values measured in tulip polar and sugar maple (2.9 g $N \cdot kg^{-1}$) and the lowest in red pine (0.8 g $N \cdot kg^{-1}$).

Total C respired in the upper soil horizon was highest in balsam poplar and white spruce soils, least in tulip poplar and white oak soils, and intermediate in sugar maple and red pine soils (Figure 2.1a). Carbon dioxide accumulation curves constructed for each site reveal that net mineralizable C (C_{min}) ranged from 1.51 g $C \cdot kg\ soil^{-1}$ in white oak to 11.89 g $C \cdot kg\ soil^{-1}$ in white spruce, and was positively correlated with total soil organic C (C_{total}) across forest types ($C_{min} = 0.86 + 0.041 * C_{total}$; $r^2 = 0.86$, $P = 0.007$; n = 6). Though C_{min} increased with increasing latitude, this trend was no longer apparent when respired C was adjusted for C_{total} (Figure 2.1b). Normalized for C content, C_{min} ranged from 3.4% of C_{total} in the southern deciduous tulip poplar stand to 15.7% in the northern red pine plantation

(Table 3). Overall red pine soils had the highest C efflux per unit soil C in the upper horizon ($P \leq 0.05$), indicating that a higher proportion of SOM in red pine was metabolizable to soil microbes. Alternatively, differences in C_{min} could indicate that microbial C-use efficiency differed among forest types.

In the lower soil horizon, C_{min} also varied relative to C_{total} (Figure 2.2a). Stands with larger C stocks in the lower horizons generally had greater C_{min}, with the exception of red pine which had significantly smaller C_{total} than tulip poplar (Table 2.2), but similar values for C_{min}. When adjusted for C_{total} (Figure 2.2b), differences among forest types followed the same general pattern observed for the upper horizon, again suggesting differences in C-use efficiency among forest types or that a larger fraction of soil C in the temperate deciduous stands was resistant to microbial degradation compared to red pine.

Rate constants for C mineralization derived from our single exponential model varied significantly across ecosystems ($F_{5,30} = 36.58$, $P < 0.0001$). Since our intention was to estimate net C mineralization rates under near-natural environmental conditions, we remind the reader that cores from different sites were incubated under different temperatures. Therefore, net C mineralization rates reflect differences in both temperature and microbial C utilization. Highest respiration rates were observed in tulip poplar soils (0.0228 ± 0.0009 d^{-1}) and lowest in white spruce (0.0061 ± 0.0012 d^{-1}; Table 2.3). Though rate constants appeared to increase along the north-south gradient, we observed two distinct groupings between the temperate deciduous stands and the coniferous stands plus balsam poplar. These differences were reflected in the mean residence time ($1/k$) for C_{min}, where pool turnover was, on average, twice as rapid for the temperate deciduous stands

compared with the coniferous stands plus balsam poplar. Within a stand, the residence time for C_{min} was very similar between upper and lower soil horizons for all stands except red pine (Table 2.3). Mean residence time for C_{min} in red pine was 92.3 d in the lower soil horizon vs. 184.0 d in the upper soil horizon. Similarly, average C_{min}/C_{total} was almost twofold higher ($P \leq 0.05$; n= 6) for the upper soil horizon suggesting that substrate quality could be more vertically stratified in red pine than in the other forest types.

Soil FAA-N and DIN concentrations

Soil FAA concentrations differed significantly among forest types ($F_{5,84} = 15.2$, $P < 0.0001$). Most of the observed variation was attributed to soils from Alaskan stands, which in some instances had over tenfold higher concentrations of FAA than soils from the southern deciduous stands (Figure 2.3). Average values ranged from just over 0.5 mg AA-N kg^{-1} soil in tulip poplar to just over 8 mg AA-N kg^{-1} in balsam poplar (Table 2.2). Values for white spruce and balsam poplar are within the range reported for floodplain forests in interior Alaska (Kielland et al., 2006; Werdin, 2006). Across our latitudinal gradient FAA-N appears to coincide with estimates reported for a number of ecosystem types (Bardgett et al., 2003; Finzi and Schlesinger, 2003; Weintraub and Schimel 2003); however, direct comparisons with other temperate deciduous forests are complicated by differences in methodology (Berthrong and Finzi, 2006). FAA-N in red pine soils (3.25 ± 0.69 mg N kg^{-1}) were significantly greater than those of sugar maple (0.97 ± 0.14 mg N kg^{-1}; $P \leq 0.05$; n = 15) even though these forest types share a similar climate and other factors influencing soil development, e.g. topography and parent material. Conversely we

observed little variation in dissolved inorganic N (DIN) across all sites with the exception of red pine stands, which had significantly lower concentrations of inorganic N ($P \leq 0.05$) than any of the other forest types.

In situ glycine C mineralization

In situ turnover rates for glycine were rapid regardless of soil type (Table 2.4); nevertheless, rate constants for glycine turnover (k_{gly}) determined from our single exponential model were statistically different among forest types ($F_{5,30} = 3.34$, $P = 0.02$; Table 2.4). Mean residence times ($1/k_{gly}$) did not vary systematically with latitude, but there was clustering among forest types that somewhat paralleled results from the laboratory incubation (Figure 2.4). Contrary to predictions, mean *in situ* turnover rates for glycine were significantly faster in tulip poplar soils than in white spruce soils while turnover rates for the remaining forest types showed no statistical differences. However, it is worth noting that mean *in situ* glycine turnover rates were nearly identical for southern white oak and boreal balsam poplar (0.70 hr^{-1} and 0.69 hr^{-1}, respectively), whereas northern sugar maple demonstrated non-significantly higher turnover rates for glycine (0.88 hr^{-1}; Table 2.4), suggesting climatic effects alone cannot explain cross-ecosystem variation in the turnover of soil FAA.

In situ glycine turnover was correlated with indices of substrate (labile C) availability. For example, soil C:N ratio explained over 80% of the variability observed in glycine turnover rate (k_{gly}) among sites (Figure 2.5; $r^2 = 0.82$, $P = 0.01$). The highest values for k_{gly} were observed in soils with the lowest C:N ratio and vice versa. In contrast

to our original predictions concerning substrate quality, a low C:N ratio may be indicative of highly processed soil C that presents a relatively C poor substrate for microbial growth (see discussion below). Additionally, we found a strong positive correlation between rate constants for *in situ* glycine turnover (k_{gly}) and C_{min} (k_c) determined from laboratory incubations (Figure 2.6; $r^2 = 0.67$, $P < 0.05$), suggesting ecosystems with higher net mineralization rates of soil C have a higher demand for pulses of labile C, e.g. glycine. However, there was no significant relationship between C_{min} or FAA-N concentrations and k_{gly} across all ecosystems. Similarly, we observed no significant correlation between k_{gly} and soil temperature measured continuously at each site during the field experiment ($r^2 = 0.14$, $P = 0.47$). Thus it appears that among forest types, substrate quality (C_{min}/C_{total}) had a more dramatic impact on the turnover of glycine than either temperature or soil FAA concentrations.

DISCUSSION

We found that *in situ* rates of glycine turnover were rapid across all biomes, and that there was strong support for our hypothesis that consumption of soluble amino acids on a continental scale is linked to the substrate quality of soil C pools. Glycine turnover rates increased significantly with decreasing pools of C_{min}, suggesting a microbial response to C limitation. Although soil microorganisms in all forest types rapidly mineralized the glycine, neither the magnitude of response to our glycine addition nor the size of the labile fraction of SOM varied predictably along our latitudinal gradient. We

discuss these results in the context of relevant studies exploring the factors regulating the turnover of soil FAA.

Recent studies indicate that low-molecular-weight organic compounds, including FAA, play an important role in sustaining the short-term energy balance of microorganisms involved in the decomposition of SOM (De Nobili et al., 2001; Mondini et al., 2006). The more recalcitrant the SOM, the less likely SOM alone provides sufficient substrate for the basal metabolism and growth of soil microorganisms. The rapidity with which our labeled substrate appeared in soil CO_2 efflux provides further evidence that FAA represented a labile source of soil C that was rapidly metabolized by soil microbes. However, our initial ideas concerning the variability of soil C availability across forest types (increasing SOM quality and thus increasing C availability with decreasing latitude) were not supported.

Results from our laboratory incubations show that boreal forest soils yielded substantially larger pools of respired C than mid-latitude soils (Figure 2.1a) in the upper horizon, and soil C stocks explained most of the observed differences in mineralizable C (C_{min}) among forest types. This was not surprising given that decomposition is constrained in part by temperature (Hart and Perry, 1999; Garten and Hanson, 2006); stands that had a lower mean annual temperature, also had significantly higher stocks of soil C and N and thus larger pools of C_{min}. However, despite a strong correlation between latitude and total C respired, the proportion of soil C that was readily mineralizable at each site did not necessarily conform to predictions concerning soil temperature or litter quality (Figure 2.1b) particularly in the temperate regions, suggesting that neither soil

temperature nor litter quality provided an adequate explanation for FAA turnover and $C_{min.}$

Forests dominated by species producing high quality (low lignin; low C/N) aboveground (AG) litter, e.g. tulip polar and sugar maple, would be expected to have higher rates of litter decomposition and thus proportionately larger pools of labile C than forests dominated by species producing more recalcitrant litter, e.g. red pine (Moorehead et al., 1999). However, C_{min} accounted for a larger proportion of total C in stands producing lower quality AG litter. For example, soil organic matter from both horizons in red pine contained a larger proportion of labile C than either sugar maple or tulip poplar (Figures 2.1b & 2.2b), yet C turnover rates (k_C) for red pine soils were slower than for either sugar maple or tulip poplar soils. This apparent discrepancy might reflect differences in early-stage vs. late-stage decomposition of these dissimilar litter types. Field studies of litter decomposition have demonstrated a limit value for mass loss beyond which decomposition either ceases or proceeds very slowly as the remaining mass becomes part of soil humus. This limit value is highly correlated with the initial N concentration of fresh litter inputs. The higher the N levels of a litter, the faster the initial rates of decomposition, but also the more recalcitrant mass remains during the late stages of decomposition (Berg and Ekbohm, 1991; Berg and Meentemeyer, 2002; but see Hobbie, 2000). Moreover, recalcitrant residues of initially high quality AG litter, e.g., tulip poplar, can accumulate over time (Berg, 2000) becoming a larger portion of total soil C, particularly in climates more conducive to decomposition processes such as those of our southern temperate stands.

For example, we found that C_{min} represented a larger fraction of C_{total} in oak than tulip poplar soils despite that oak AG litter generally has higher lignin concentration than tulip poplar (Hunter et al., 2003). Though both forest types share a similar climate, tulip poplar soils are on average cooler, so in the field temperature effects on decomposition should favor oak. Why then was the proportion of recalcitrant soil C lower in the oak stand? More importantly, why was the rate constant for glycine turnover ~30% lower in the oak soils compared to tulip poplar soils (Table 2.4)? The answer is likely multi-faceted and includes factors such as legacies of disturbance, patterns of recovery, and differences in soil texture, and soil moisture at each site.

Both forest types established on highly eroded soils following agricultural abandonment and thus likely sustained large losses of SOM during the agricultural period. Yet, soils at the tulip poplar site contain twofold more C than the oak soils despite a 20 year offset (oak > tulip poplar) in stand initiation favoring the oak site (Table 2.2). Moreover, though the forest floor in both sites is dominated by an O_i layer, only the oak stand had subordinate organic horizons and the forest floor in oak was up to fourfold deeper than that in tulip poplar despite similar annual inputs of AG litter (Table 2.1). These observations suggest the turnover of litter and incorporation into SOM is more rapid at the tulip poplar site. Given the relatively short duration of our incubation experiment (16 weeks), our data primarily reflect the turnover dynamics of soil C pools that are less resistant to decay. Therefore, a larger fraction of SOM in our oak stand might consist of ‘less processed’ C which hasn’t undergone the degree of chemical transformation as SOM in tulip poplar.

Assuming a C-limited soil environment and a high metabolic demand, it is easier to understand why soil microorganisms in tulip poplar and sugar maple might have responded to our glycine additions with faster turnover rates than observed in oak or red pine where C_{min}/C_{total} is significantly higher. The rapid response to 'food events', e.g. pulses of FAA and other low molecular weight compounds, is likely an evolutionary mechanism for meeting the maintenance demand of C-starved cells (Mondini et al., 2006). In many soils, the microbial biomass maintains a high state of metabolic readiness (Brookes et al., 1987), even though substrate availability is usually scarce. The rationale for sustaining such a high metabolic status in an energy-poor environment stems from the need to compete effectively for temporally and spatially infrequent pulses of labile substrate.

We recognize that processes affecting the biodegradation of glycine reflect a complex of interacting factors including temperature, sorption reactions, pool size, and microbial community composition and metabolic status. For instance the community structure of soil microorganisms can strongly influence decomposition of plant litter and rates of incorporation into SOM and thus the availability of labile C (Elliot et al., 1993; but see Kemmitt et al., 2008). The size of the microbial biomass is less important than the activity of the community in predicting decomposition rates, and the metabolic activities of decomposers are often adapted to available substrates (Elliot et al.,1993; but see McClaugherty et al.,1985). Data from a companion study, (McFarland et al., *submitted*), indicate no correlation between microbial biomass N and glycine turnover rates, suggesting that patterns in glycine turnover are likely driven by differences in

heterotrophic consumption rather than the size of the microbial biomass *per se*. Similarly, other factors, some of which we measured (soil temperature, FAA pool size) and some of which we didn't (sorption reactions, microbial C substrate preference) could also have influenced FAA use among forest types. However, regardless of their contribution to the cycling dynamics of FAA, these effects were not strong enough to disrupt the strong relationship between FAA turnover and C_{min}.

CONCLUSION

Amino acids represent a significant fraction of dissolved organic N in forest soils and a number of experiments have elucidated factors controlling the production and/or turnover of these compounds. However, the primary motivation behind these research efforts has centered on issues pertaining to plant nutrition or the overall N economy of soils, not their role as a C substrate that influences the metabolic status of the soil microbial community. This study illustrates, 1) that FAA are an important substrate for soil microbial metabolism in many terrestrial forest communities, and 2) patterns of amino acid turnover *in situ* across ecosystems are closely linked to indices of SOM quality. We found that across large spatial scales, consumption of glycine by soil microorganisms is better explained by changes in soil C availability than cross-ecosystem variation in soil temperature or standing pools of FAA. This suggests the overall decomposability of native C and patterns of heterotrophic consumption of soil C influence decomposition rates for low-molecular-weight organic substrates such as amino acids.

ACKNOWLEDGEMENTS

Greg Mauer, Jennifer Mitchell, and Jason Schneider provided invaluable assistance in the field. We also wish to thank Lee Ogden of the Warnell School of Forest Resources at the University of Georgia, and Dr. Andrew Burton of the School of Forest Resources & Environmental Sciences at Michigan Technological University, for their extraordinary logistical support during our field efforts. Dr. Stephen C. Hart and Dr. F. Stuart 'Terry' Chapin III greatly improved the quality of this manuscript with their comments. This research was supported through grants from the University of Alaska's Center for Global Change, the National Science Foundation's Ecosystem program, and the Bonanza Creek LTER program.

LITERATURE CITED

Alavarez, R., Alavarez, C.R., 2000. Soil organic matter pools and their association with carbon mineralization kinetics. Soil Science Society of America Journal 64:184-189.

Atkin, O.K., 1996. Reassessing the nitrogen relations of Arctic plants: A mini-review. Plant, Cell and Environment 19:695–704.

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

Berg, B., Meentemeyer, V., 2002. Litter quality in a north European transect vs. carbon storage potential. Plant and Soil 242:83-92.

Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133:13-22.

Berg, B., Ekbohm, G., 1991. Litter mass-loss rates and decomposition patterns in some needle and leaf litter types. Long-term decomposition in a Scots pine forest. VII. Canadian Journal of Botany 69:1449-1456.

Berthrong, S.T., Finzi, A.C., 2006. Amino acid cycling in three cold-temperate forests of the Northeastern USA. Soil Biology and Biochemistry 38:861-869.

Brookes, P.C., Newcomb, A.D., Jenkinson, D.S., 1987. Adenylate energy charge measurements in soil. Soil Biology and Biochemistry 19:211-217.

Degens, B.P., 1998. Microbial functional diversity can be influenced by the addition of simple organic substrates to soil. Soil Biology and Biochemistry. 30:1981-1988.

De Nobili, M., Contin, M., Mondini, C., Brookes, P.C., 2001. Soil microbial biomass is

triggered into activity by trace amounts of substrate. Soil Biology and Biochemistry 33:1163-1170.

Di, H.J., Aylmore, L.A.G., Kookana, R.S., 1998. Degradation rates of eight pesticides in surface and subsurface soils under laboratory and field conditions. Soil Science 163:404-411.

Elliot, W.M., Elliot, N.B., Wyman, R.L., 1993. Relative effect of litter and forest type on rate of decomposition. American Midland Naturalist 129:87-95.

Finzi, A.C., Berthrong, S.T., 2005. The uptake of amino acids by microbes and trees in three cold-temperate forests. Ecology 86:3345-3353.

Finzi, A.C., Schlesinger, W.H., 2003. Soil-nitrogen cycling in a pine forest exposed to 5 years of elevated CO_2. Ecosystems 6:444-456.

Flanagan, P.W., Van Cleve, K., 1983. Nutrient cycling in relation to decomposition and organic-matter quality in taiga ecosystems. Canadian Journal of Forest Resources 13:795-817.

Garten, C.T., Hanson, P.J., 2006. Measured forest soil C stocks and estimated turnover along an elevation gradient. Geoderma 136:342-352.

Gonod, L.V., Jones, D.L., Chenu, C., 2005. Sorption regulates the fate of the amino acids lysine and leucine in soil aggregates. European Journal of Soil Science. :1-10.

Gordon, A.M., Tallas, M., Van Cleve, K., 1987. Soil incubations in polyethylene bags: effect of bag thickness and temperature on nitrogen transformations and CO_2 permeability. Canadian Journal of Soil Science 67:65-75.

Hart, S.C., Perry, D.A., 1999. Transferring soils from high- to low-elevation forests

increases nitrogen cycling rates: climate change implications. Global Change Biology 5:23-32.

Hobbie, S.E., 2000. Interactions between litter lignin and soil N availability during leaf litter decomposition in a Hawaiian montane forest. Ecosystems 3:484-494.

Hunter, M.D., Adl, S., Pringle, C., Coleman D.C., 2003. Relative effects of macroinvertebrates and habitat on the chemistry of litter during decomposition. Pedobiologia 47:101-115.

Jones, D.L., Murphy, D.V., 2007. Microbial response time to sugar and amino acid additions to soil. Soil Biology and Biochemistry 39:2178-2182.

Jones, D.L., Kemmitt, S.J., Wright, D., Cuttle, S.P., Bol, R., Edwards, A.C., 2005. Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy. Soil Biology and Biochemistry 37:1267-1275.

Jones, D.L., Farrar, J.L., Newsham, K.K., 2004. Rapid amino acid cycling in Arctic and Antarctic soils. Water, Air, and Soil Pollution: Focus 4:169-175.

Jones, D.L., Kielland, K., 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., Owen, A.G., Farrar, J.L., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biology and Biochemistry 34:1893-1902.

Jones, D.L., Shannon, D., 1999. Mineralization of amino acids applied to soils: impact of soil sieving, storage and inorganic nitrogen additions. Soil Science Society of America Journal 63:1199-1206.

Jones, D.L., 1999. Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biology and Biochemistry 31:613-622.

Kaye, J.P., Hart, S.C., 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology and Evolution 12:139–143.

Kemmitt, S.J., Lanyon, C.V., Waiteb, I.S., Wenc, Q., Addiscotta, T.M., N.R.A. Birda, N.R.A., O'Donnell, A.G., Brookes, P.C., 2008. Mineralization of native soil organic matter is not regulated by the size, activity, or composition of the soil microbial biomass—a new perspective. Soil Biology and Biochemistry 40: 61–73

Kielland, K., McFarland, J.M., Ruess, R.W., Olson, K., 2007. Rapid cycling of amino acid of organic nitrogen in taiga forest ecosystems. Ecosystems 10:360-368.

Kielland, K., McFarland, J.W., Olson, K., 2006. Amino acid uptake in deciduous and coniferous taiga ecosystems. Plant and Soil 288:297-307.

Kielland K., 2001. Short-circuiting the nitrogen cycle: Strategies of nitrogen uptake in plants from marginal ecosystems. Pages 376-398, In: Plant Nutrient Acquisition: New Perspectives, Ae, N., J. Arihara, K. Okada, and A. Srinivasan (eds.). Springer-Verlag, Berlin .

Kielland, K., Bryant, J.P., Ruess, R.W., 1997. Moose herbivory and carbon turnover of early successional stands in interior Alaska. Oikos 80:25-30.

Kielland, K., 1995. Landscape patterns of free amino acids in arctic tundra soils.

Biogeochemistry 31:85–98.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485-1498.

Kuzyakov, Y., Demin, V., 1998. CO_2 efflux by rapid decomposition of low molecular organic substances in soils. Science of Soils 3:

Kuzyakov, Y.V., Galitsa, S.V., 1993. Kinetics of [2-^{14}C] glycine decomposition and its incorporation into humus fractions of Sierozem. Eurasian Soil Science, 25:39-53.

Lancaster, V.A., Keller-McNulty, S., 1998. A review of composite sampling methods. Journal of the American Statistical Association 93:1216-1230.

Lipson, D., Näsholm, T., 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128:305-316.

Lipson, D.A., Raab, T.K., Schmidt, S.K., Monson, R.K., 1999. Variation in competitive abilities of plants and microbes for specific amino acids. Biology and Fertility of Soils 29:257-261

McClaugherty, C.A., Pastor, J., Aber, J.D., Mellilo, J.M., 1985. Forest litter decomposition in relation to soil N dynamics and litter quality. Ecology 66:266-275.

McFarland, J.W., Ruess, R., Kielland, K., Pregitzer, K., Hendrick, R., Allen, M. Cross-ecosystem comparisons of *in situ* plant uptake of amino acid-N and NH_4^+ Ecosystems, *submitted*

McFarland, J.W., Ruess, R.W., Kielland, K., Doyle, A.P., 2002. Cycling dynamics of NH_4^+ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem.

Ecosystems 5:775-788

Mondini, C., Cayuela, M.L., Sanchez-Monedero, M.A., Roig, A., Brookes, P.C., 2006. Soil microbial biomass activation by trace amounts of readily available substrate. Biology and Fertility of Soils 42:542-549.

Moorehead, D.L., Currie, W.S., Rastetter, E.B., Parton, W.J., Harmon, M.E., 1999. Climate and litter quality controls on decomposition: an analysis of modeling approaches. Global Biogeochemical Cycles 13: 575-589.

Morita, R.Y., 1988. Bioavailability of energy and its relationship to growth and starvation survival in nature. Canadian Journal of Microbiology 34:436-441.

Näsholm, T., Ekblad, A., Högberg, P., 1998. Boreal forest plants take up organic nitrogen. Nature 392:914-917.

Näsholm, T., Huss-Danell, K., Högberg, P., 2000. Uptake of organic nitrogen by four agriculturally important plant species. Ecology 81:1155–1161.

Nordin, A., Schmidt, I.K., Shaver, G.R., 2004. Nitrogen uptake by Arctic soil microbes and plants in relation to soil nitrogen supply. Ecology 85:955-962.

Nunan, N., Morgan, M.A., Scott, J., Herlihy, M., 2000. Temporal changes in nitrogen mineralization, microbial biomass, respiration and protease activity in a clay loam soil under ambient temperature. Biology and Environment: Proceedings of the Royal Irish Academy 100B:107-114.

Schimel, J.P., Chapin, F.S. III, 1996. Tundra plant uptake of amino acid and NH_4^+ nitrogen in situ: plants compete well for amino acid N. Ecology 77:2142–2147.

Schmidt, S., Stewart, G.R., 1999. Glycine metabolism by plant roots and its occurrence in

Australian plant communities. Australian Journal of Plant Physiology. 26:253-264.

Viereck, L.A., Dyrness, C.T., Foote M.J., 1993. An overview of the vegetation and soils of the floodplain ecosystems of the Tanana River, interior Alaska. Canadian Journal of Forest Research 23:889-898

Vinolas, L.C., Vallejo, V.R., Jones, D.L., 2001a. Control of amino acid mineralization and microbial metabolism by temperature. Soil Biology and Biochemistry 33:1137-1140.

Vinolas, L.C., Healey, J.R., Jones, D.L., 2001b. Kinetics of soil microbial uptake of free amino acids. Biology and Fertility of Soils.

Weintraub, M.N., Schimel, J.P., 2003. The seasonal dynamics of amino acids and other nutrients in Alaskan Arctic tundra soils. Biogeochemistry 73:359-380.

Werdin, N.R., 2006. Soil amino acid composition across a boreal forest successional sequence. MS Thesis, University of Alaska, Fairbanks 134 p.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J., Milchunas, D., Martin, C.W. 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. Ecology 75:2333-2347.

Table 2.1 Select stand characteristics for each of the six forest ecosystems employed in the study.

[a]AM = arbuscular mycorrhizae and EM = ectomycorrhizae.
[b]Overstory was determined as percentage basal area.
[c]Litterfall was collected from September 1998 to September 1999 for balsam poplar and white spruce; 1999-2000 for white oak, tulip poplar, sugar maple and red pine.

	SITE					
STAND PARAMETER	**Tulip poplar**	**White oak**	**Sugar maple**	**Red pine**	**Balsam poplar**	**White spruce**
Latitude	35° 4′ N	33° 25′ N	46° 39′ N	47° 6′ N	64° 40′ N	64° 41′ N
Dominant mycorrhizal association[a]	AM	EM	AM	EM	EM	EM
Stand age (yr)	40	>60	95-100 overstory	50	80-100	150-250
Mean annual temperature (° C)	12.7	16.5	3.8	3.8	-3.3	-3.3
Mean annual precipitation (mm)	1816	1263	841	883	287	287
Percent overstory[b]	85	68	92	100	70	98
Stem density (trees·ha^{-1})	2396	391	707	522	922	400
Basal area (m^2·ha^{-1})	34	26	33	34	37	30
Total litterfall[c] (g·m^{-2}·yr^{-1})	1468	1496	450	386	259	102
Soil classification	Humic Hapludult	Typic-Rhodic Hapludult	Typic Haplorthod	Entic Haplorthod	Typic Cryofluvent	Typic Cryofluvent
Soil texture	Sandy loam	clay loam	sandy loam	sandy loam	organic to alluvial silt	organic to alluvial silt

Table 2.2 Select soil properties for each of the six forest ecosystems employed in the study. For soil C and N (n = 6), FAA (n = 15), and DIN (n = 18), values are mean ± S.E. Letters denote significant differences ($P \leq 0.05$) between forest types.

	SITE					
SOIL PARAMETER	**Tulip poplar**	**White oak**	**Sugar maple**	**Red pine**	**Balsam poplar**	**White spruce**
Soil organic carbon ($gC \cdot kg^{-1}$)	57.4 ± 2.8^{cd}	26.1 ± 3.1^{d}	67.3 ± 5.3^{c}	38.26 ± 2.8^{cd}	232.8 ± 12.4^{a}	168.9 ± 13.8^{b}
Upper horizon[a]	44.8 ± 5.6^{a}	18.5 ± 1.6^{b}	37.3 ± 11.0^{a}	15.5 ± 0.3^{b}	19.4 ± 2.9^{b}	35.1 ± 5.6^{ab}
Lower horizon[b]						
$C:N_{UPPER}$	14.0	16.7	13.4	21.5	18.8	23.2
Total soil nitrogen ($gN \cdot kg^{-1}$)						
Upper horizon[a]	4.1 ± 0.2^{c}	1.6 ± 0.1^{d}	5.0 ± 0.3^{c}	1.78 ± 0.02^{d}	12.4 ± 0.6^{a}	7.3 ± 0.5^{b}
Lower horizon[b]	2.9 ± 0.4^{a}	1.1 ± 0.1^{b}	2.9 ± 0.8^{a}	0.8 ± 0.0^{b}	1.2 ± 0.2^{b}	1.8 ± 0.3^{ab}
$C:N_{LOWER}$	15.3	16.7	13.0	20.0	16.6	19.3
Soil pH (0-20 cm)	5.7	5.2	4.4	4.5	6.0	5.5
TFAA ($\mu g\ N \cdot g^{-1}$)	0.57 ± 0.09^{c}	1.30 ± 0.39^{c}	0.97 ± 0.14^{c}	3.25 ± 0.69^{bc}	8.61 ± 1.49^{a}	6.45 ± 1.20^{ab}
DIN ($\mu g\ N \cdot g^{-1}$)	3.24 ± 0.21^{a}	2.67 ± 0.36^{a}	3.50 ± 0.29^{a}	1.13 ± 0.11^{b}	2.80 ± 0.23^{a}	3.39 ± 0.50^{a}
TFAA:DIN	0.18	0.49	0.27	2.88	3.08	2.08

[a]Upper horizon = 0-7cm below the litter layer

[b]lower horizon = 7-20cm below the litter layer.

Table 2.3 C_{min} turnover constants, residence time for C_{min}, and C_{min} as % of total soil C for each of the six soil types used in the laboratory incubation study. C turnover constants, expressed 'per day', and residence times (inverse of turnover constant) were calculated from nonlinear single exponential models fitted to cumulative product curves generated. Mean ± S.E. Letters denote significant differences ($P \leq 0.05$) between forest types

	UPPER SOIL HORIZON			LOWER SOIL HORIZON		
STAND TYPE	***k* (d^{-1})**	**Residence time (d)**	**C_{min} as % of C_{total}**	***k* (d^{-1})**	**Residence time (d)**	**C_{min} as % of C_{total}**
Tulip poplar	0.0228 ± 0.0009^{a}	44.3 ± 1.8^{a}	3.35 ± 0.20^{a}	0.0224 ± 0.0007^{a}	45.0 ± 1.5^{a}	2.93 ± 0.27^{c}
White oak	0.0187 ± 0.0014^{a}	55.0 ± 4.5^{ab}	5.72 ± 0.65^{a}	0.0208 ± 0.0010^{a}	48.8 ± 2.7^{a}	4.47 ± 0.33^{bc}
Sugar maple	0.0140 ± 0.0005^{b}	71.8 ± 2.6^{ab}	4.92 ± 0.54^{a}	0.0131 ± 0.0008^{b}	77.8 ± 5.3^{ab}	4.75 ± 0.88^{bc}
Red pine	0.0066 ± 0.0014^{c}	184.0 ± 39.0^{bc}	15.67 ± 1.13^{b}	0.0119 ± 0.0016^{b}	92.3 ± 13.4^{b}	7.98 ± 0.74^{a}
Balsam poplar	0.0088 ± 0.0011^{c}	124.8 ± 21.2^{ac}	4.26 ± 0.44^{a}	0.0099 ± 0.0011^{bc}	107.8 ± 11.9^{b}	5.60 ± 0.72^{ab}
White spruce	0.0061 ± 0.0012^{c}	213.2 ± 59.2^{c}	7.22 ± 1.26^{a}	0.0060 ± 0.0005^{c}	171.2 ± 12.0^{c}	5.16 ± 0.51^{bc}

Table 2.4 Soil temperature and turnover parameters derived from nonlinear single exponential models fitted to cumulative production of $^{13}CO_2$ from cores treated withU-^{13}C-glycine. Values are mean ± S.E. (n = 6). Letters denote significant differences ($P \leq 0.1$) between forest types. Soil temperatures represent a temporal (n = 24) and spatial (n = 6) average of hourly values for each site collected at 7cm depth for the duration of the glycine mineralization assay.

STAND TYPE	Soil temperature (˚ C)	*k* (hr^{-1})	Residence time (hr)	APE $^{13}C_{cum}$ (24 hour)
Tulip poplar	17.0	1.03 ± 0.14^{a}	1.05 ± 0.13^{a}	1.08 ± 0.12^{a}
White oak	21.5	0.70 ± 0.04^{ab}	1.44 ± 0.08^{ab}	1.00 ± 0.11^{a}
Sugar maple	13.8	0.88 ± 0.15^{ab}	1.27 ± 0.23^{ab}	0.87 ± 0.07^{ab}
Red pine	15.7	0.64 ± 0.10^{ab}	1.71 ± 0.20^{ab}	0.53 ± 0.05^{c}
Balsam poplar	12.8	0.69 ± 0.06^{ab}	1.50 ± 0.12^{ab}	0.55 ± 0.07^{bc}
White spruce	9.3	0.53 ± 0.04^{b}	1.96 ± 0.19^{b}	0.43 ± 0.04^{c}

FIGURE LEGENDS

Figure 2.1 Cumulative CO_2 evolution expressed as (a) $C \cdot g^{-1}$ dry mass and (b) $C \cdot g^{-1}$ C, from upper soil horizons (0-7 cm depth; see methods) for six North American forest ecosystems. Values (mean ± S.E.) were fitted to a nonlinear single exponential model. Symbols are as follows: ○ Tulip poplar (*Liriodendron tulipifera*), ● White oak (*Quercus alba*), □ Sugar maple (*Acer saccharum*), ■ Red pine (*Picea resinosa*), △ Balsam poplar (*Populus balsamifera*), and ▯ White spruce (*Picea glauca*).

Figure 2.2 Cumulative CO_2 evolution expressed as (a) $C \cdot g^{-1}$ dry mass and (b) $C \cdot g^{-1}$ C, from lower soil horizons (7-20 cm depth; see methods) for six North American forest ecosystems. Values (mean ± S.E.) for A were fitted to a nonlinear single exponential model. Symbols are as follows: ○ Tulip poplar (*Liriodendron tulipifera*), ● White oak (*Quercus alba*), □ Sugar maple (*Acer saccharum*), ■ Red pine (*Picea resinosa*), △ Balsam poplar (*Populus balsamifera*), and ▯ White spruce (*Picea glauca*).

Figure 2.3 Concentration of soil free amino acid-N across a latitudinal gradient of six forest ecosystems. Data are means (n = 15) ± S.E.

Figure 2.4 The time dependent mineralization of ^{13}C-labelled glycine *in situ* across six North American forest ecosystems. Values are expressed as atom% enrichment of ^{13}C-CO_2. CO_2 efflux above each injection area was sampled at 45 min, 2, 12, and 24 h. Symbols are as follows: ○ Tulip poplar (*Liriodendron tulipifera*), ● White oak (*Quercus alba*), □ Sugar maple (*Acer saccharum*), ■ Red pine (*Picea resinosa*), △ Balsam poplar (*Populus balsamifera*), and ▯ White spruce (*Picea glauca*). Data are means (n = 6) ± S.E.

Figure 2.5 Relationship between the rate constants for *in situ* glycine mineralization ($k_{glycine}$) and the soil C:N (upper horizon; see methods). Mineralization constants are means (n = 6) calculated from nonlinear single exponential models fitted to cumulative product curves. The line is a linear regression fitted to the data [$r^2 = 0.82$].

Figure 2.6 Relationship between the rate constants for *in situ* glycine mineralization ($k_{glycine}$) and the decomposition potential for soil C (upper horizon; see methods) expressed per unit C (k_C) among all forest types. Values are means (n = 6) calculated for each constant from nonlinear single exponential models fitted to cumulative product curves generated for each data set. The line is a linear regression fitted to the data [$r^2 = 0.67$].

Figure 2.1a

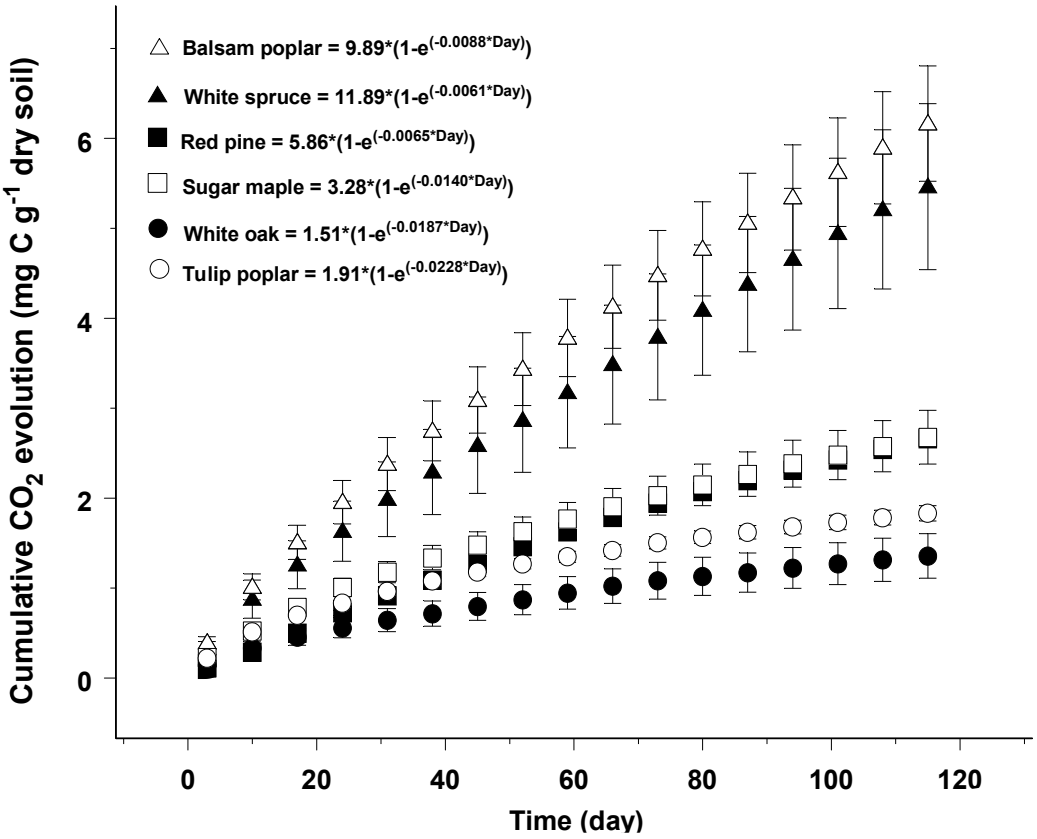

Balsam poplar = 9.89*(1-e(-0.0088*Day))
White spruce = 11.89*(1-e(-0.0061*Day))
Red pine = 5.86*(1-e(-0.0065*Day))
Sugar maple = 3.28*(1-e(-0.0140*Day))
White oak = 1.51*(1-e(-0.0187*Day))
Tulip poplar = 1.91*(1-e(-0.0228*Day))
Cumulative CO2 evolution (mg C g-1 dry soil)
0
2
4
6
0
20
40
60
80
100
120
Time (day)

Figure 2.1b

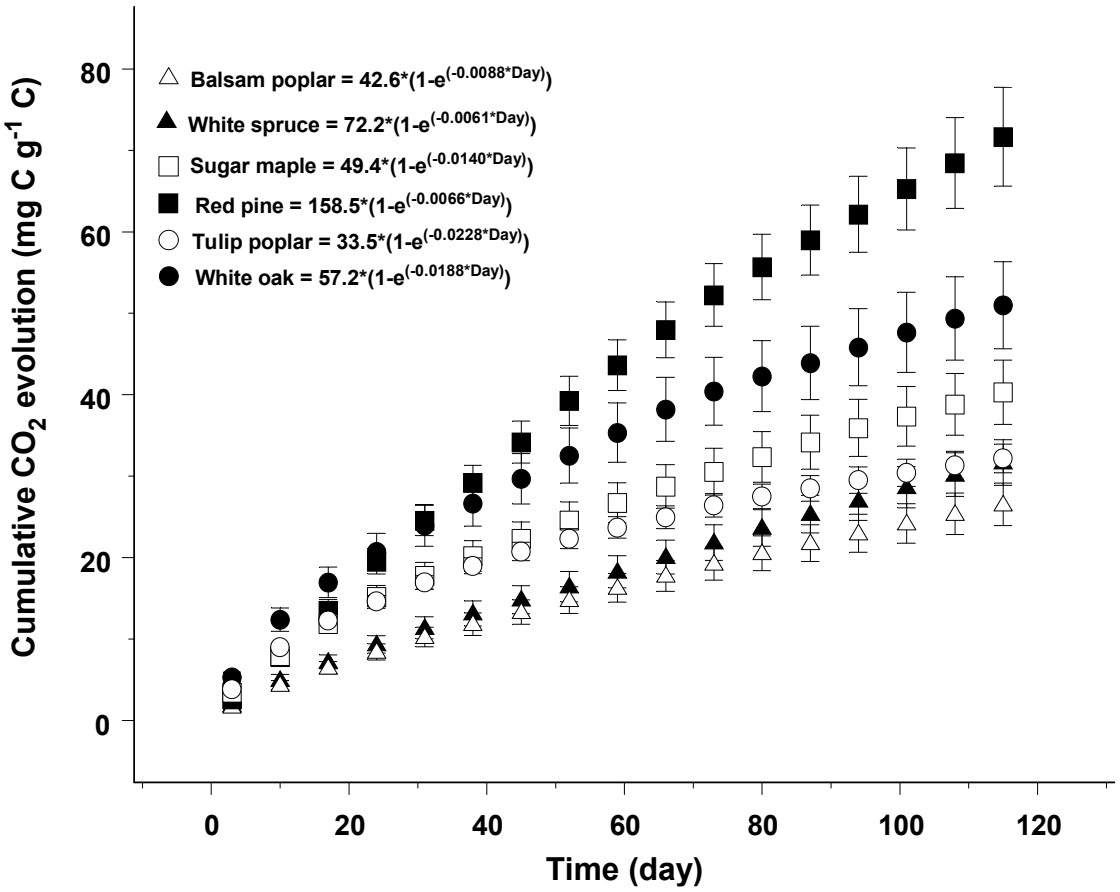

Balsam poplar = 42.6*(1-e(-0.0088*Day))
White spruce = 72.2*(1-e(-0.0061*Day))
Sugar maple = 49.4*(1-e(-0.0140*Day))
Red pine = 158.5*(1-e(-0.0066*Day))
Tulip poplar = 33.5*(1-e(-0.0228*Day))
White oak = 57.2*(1-e(-0.0188*Day))
Cumulative CO2 evolution (mg C g-1 C)
Time (day)
0
20
40
60
80
100
120

Figure 2.2a

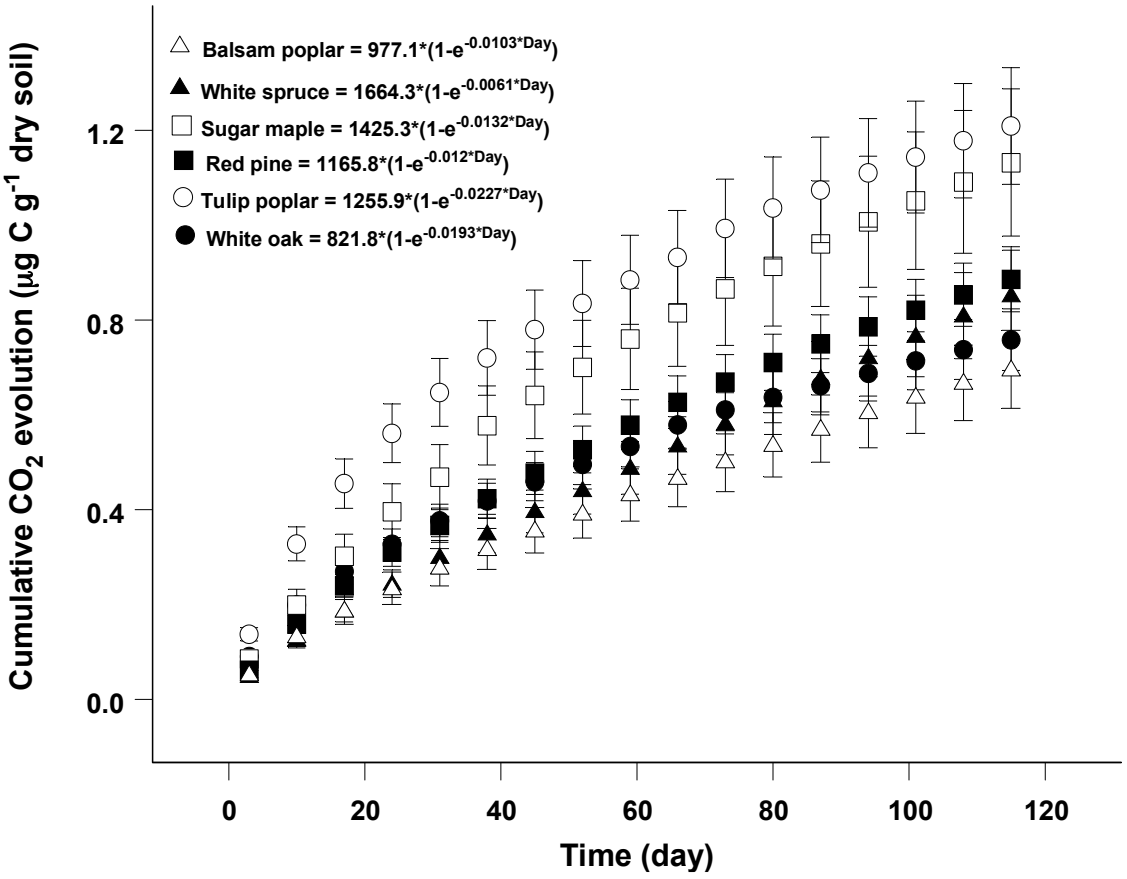

Balsam poplar = 977.1*(1-e-0.0103*Day)
White spruce = 1664.3*(1-e-0.0061*Day)
Sugar maple = 1425.3*(1-e-0.0132*Day)
Red pine = 1165.8*(1-e-0.012*Day)
Tulip poplar = 1255.9*(1-e-0.0227*Day)
White oak = 821.8*(1-e-0.0193*Day)
Cumulative CO2 evolution (μg C g-1 dry soil)
0.0
0.4
0.8
1.2
0
20
40
60
80
100
120
Time (day)

Figure 2.2b

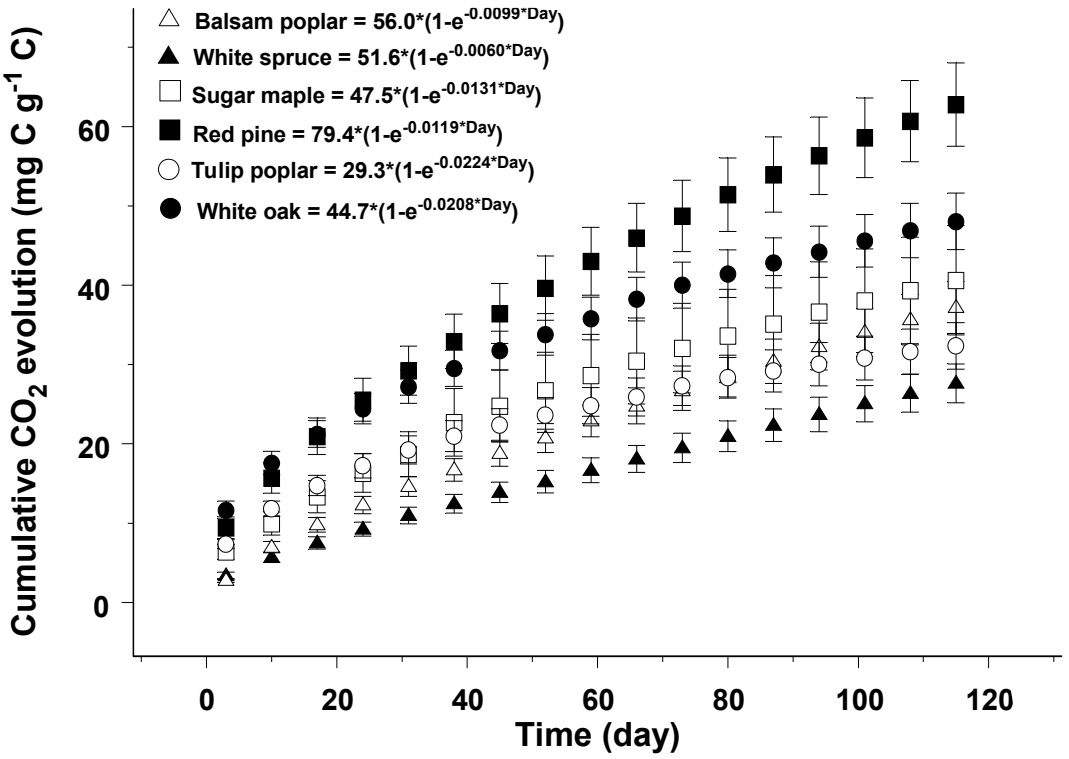

Balsam poplar = 56.0*(1-e-0.0099*Day)
White spruce = 51.6*(1-e-0.0060*Day)
Sugar maple = 47.5*(1-e-0.0131*Day)
Red pine = 79.4*(1-e-0.0119*Day)
Tulip poplar = 29.3*(1-e-0.0224*Day)
White oak = 44.7*(1-e-0.0208*Day)
Cumulative CO2 evolution (mg C g-1 C)
0
20
40
60
0
20
40
60
80
100
120
Time (day)

Figure 2.3

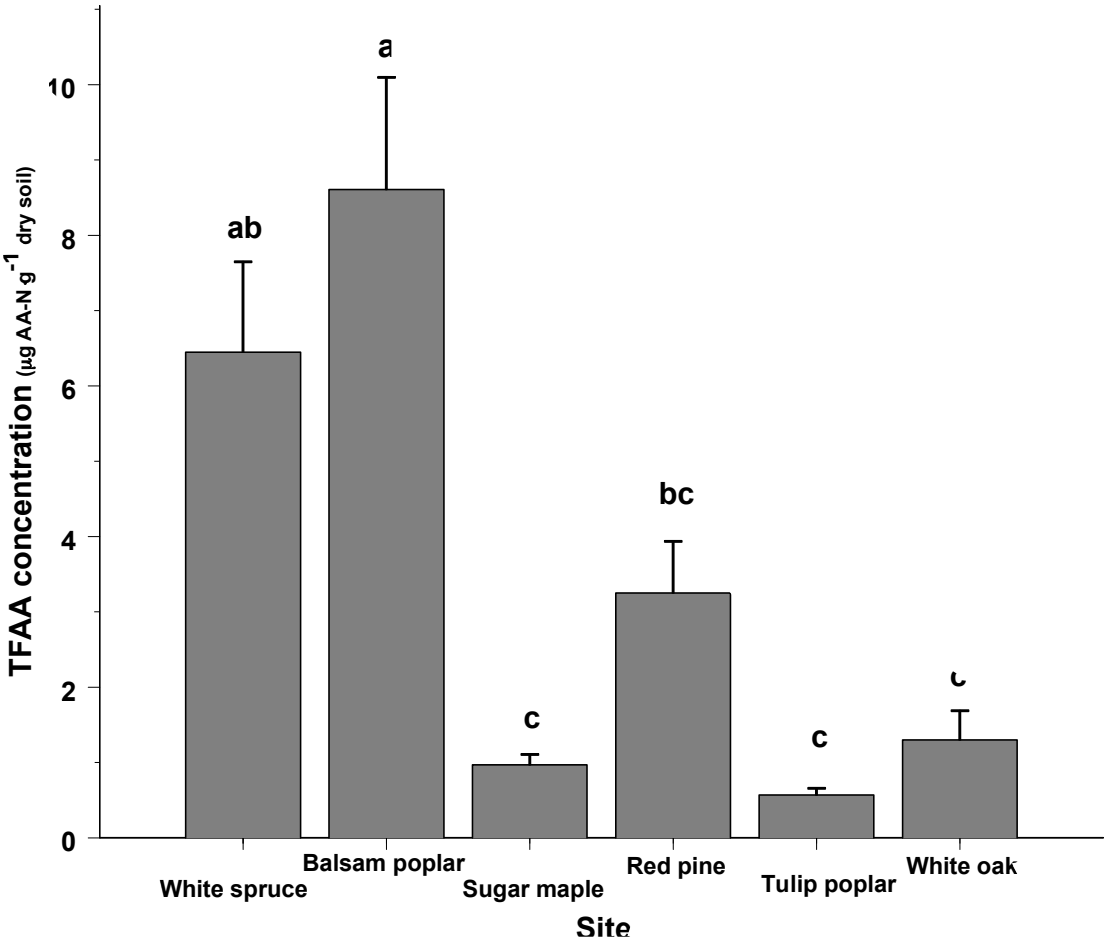

TFAA concentration (μg AA-N g-1 dry soil)
0
2
4
6
8
10
ab
a
c
bc
c
c
White spruce
Balsam poplar
Sugar maple
Red pine
Tulip poplar
White oak
Site

Figure 2.4

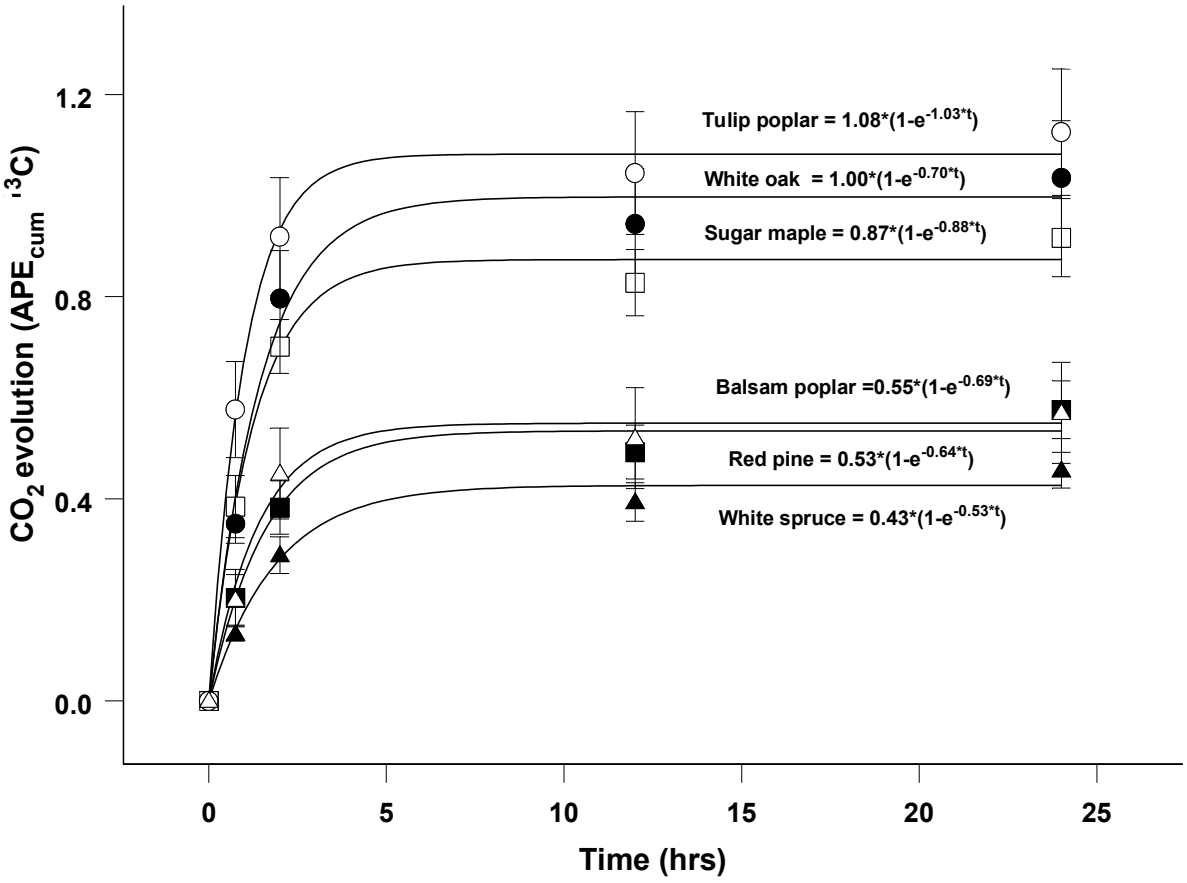

1.2
0.8
0.4
0.0
CO_2 evolution (APE_{cum} ^{13}C)
0
5
10
15
20
25
Time (hrs)
Tulip poplar = 1.08*(1-e$^{-1.03*t}$)
White oak = 1.00*(1-e$^{-0.70*t}$)
Sugar maple = 0.87*(1-e$^{-0.88*t}$)
Balsam poplar =0.55*(1-e$^{-0.69*t}$)
Red pine = 0.53*(1-e$^{-0.64*t}$)
White spruce = 0.43*(1-e$^{-0.53*t}$)

Figure 2.5

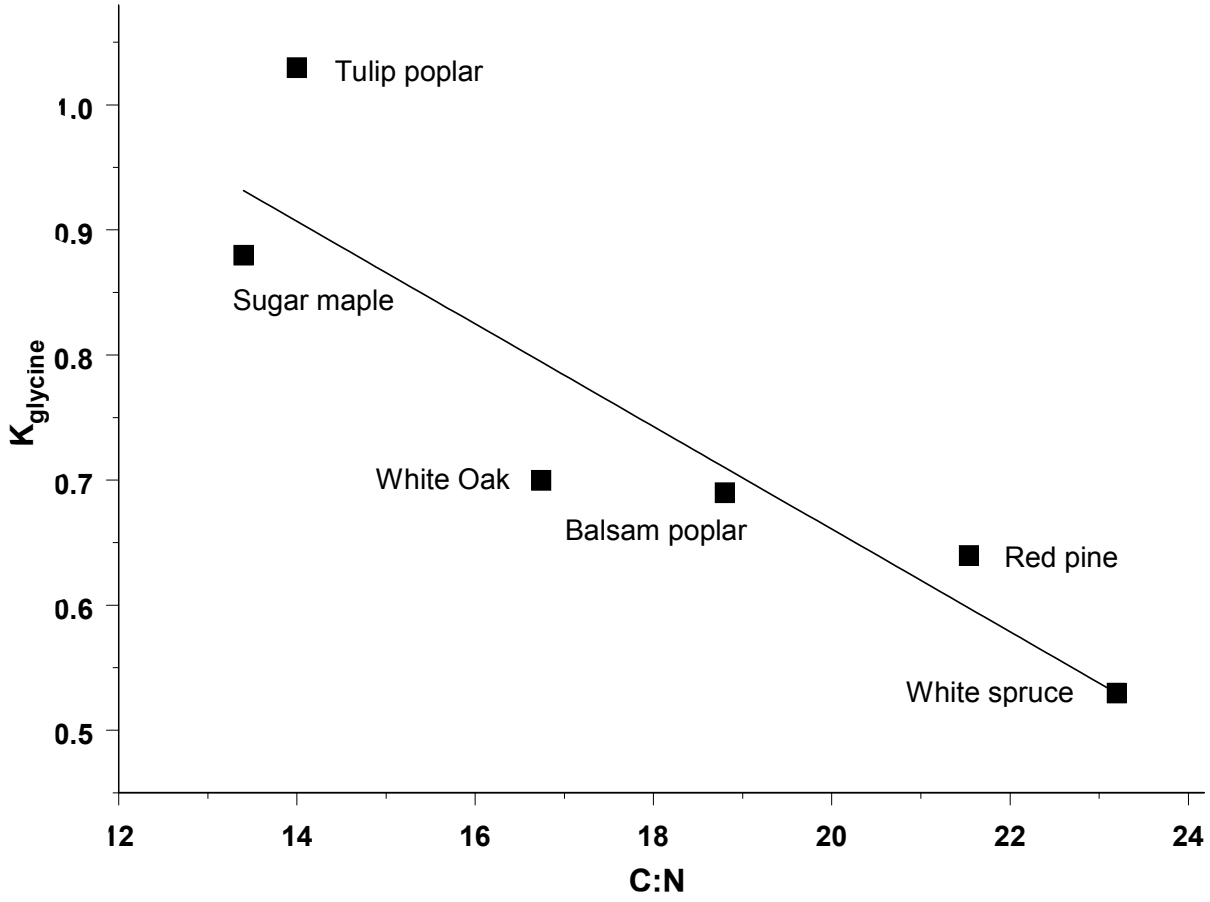
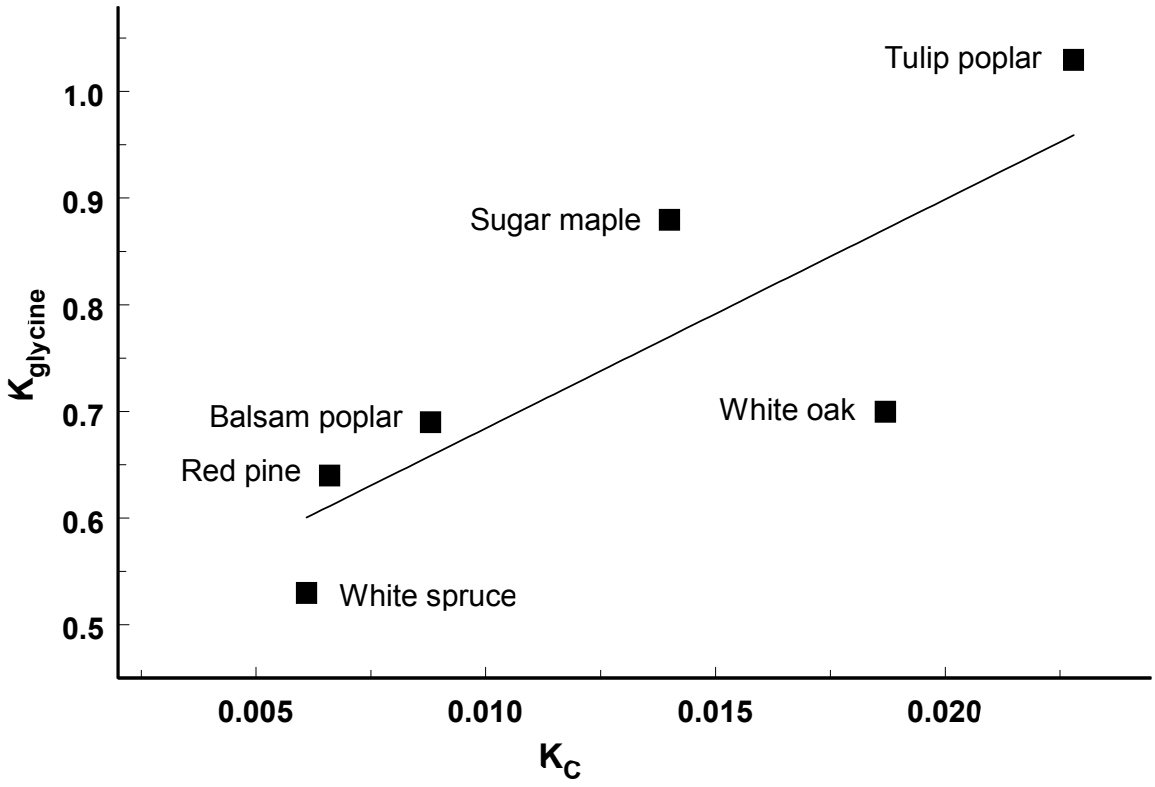

Tulip poplar
Sugar maple
White Oak
Balsam poplar
Red pine
White spruce
$K_{glycine}$
1.0
0.9
0.8
0.7
0.6
0.5
12
14
16
18
20
22
24
C:N

Figure 2.6


Tulip poplar
Sugar maple
White oak
Balsam poplar
Red pine
White spruce
1.0
0.9
0.8
0.7
0.6
0.5
$K_{glycine}$
0.005
0.010
0.015
0.020
K_C

CROSS-ECOSYSTEM COMPARISONS OF *IN SITU* PLANT UPTAKE OF AMINO ACID N AND NH_4^+[3]

ABSTRACT

The failure of net nitrogen (N) mineralization estimates to account for a large proportion of annual plant N supply in a number of ecosystems has focused attention on the importance of plant and microbial capacity to directly absorb amino acids. However, plant and microbial use of N can be simultaneously mutualistic and competitive, particularly in ecosystems dominated by mycorrhizal fungi. The goal of our research was to quantify plant uptake of organic and inorganic-N across a broad latitudinal gradient of forest ecosystems that varied with respect to overstory taxon, edaphic characteristics, and dominant mycorrhizal association. Using a multiple-isotope (^{13}C, ^{15}N) tracer design we followed *in situ* the cycling dynamics of NH_4^+ and glycine through various soil pools and fine roots over 14 days. Recovery of ^{15}N as soil N varied with respect to N form, forest type, and sampling period; however, there were similarities in the cycling dynamics of glycine and NH_4^+ among all forest types. ^{15}N enrichment of soil dissolved inorganic N (DIN) and dissolved organic N (DON) declined sharply following injections for both NH_4^+ and glycine-treated cores at all sites. Within 45 minutes we recovered ~60% of the NH_4^+ label as DIN and ~38% of the glycine label as DON. These values declined to <5%

[3] Submitted to *Ecosystems* as McFarland JW, Ruess R, Kielland K, Pregitzer K, Hendrick R, Allen M. Cross-ecosystem comparisons of *in situ* plant uptake of amino acid-N and NH_4^+.

for both pools in the subsequent two weeks. Microbial immobilization of ^{15}N was immediately apparent for both treatments and represented the largest sink (~25%) for ^{15}N among extractable soil N pools during the first 24h. In contrast, fine roots were a relatively small sink (<10%) for both N forms, but fine root ^{13}C enrichment indicated that plants in all forest types absorbed glycine intact, suggesting that plants and microbes effectively target the same labile soil N pools. Relative uptake of amino acid-N versus NH_4^+ varied significantly among sites and approximately half of this variation was explained by mycorrhizal association. Estimates of plant uptake of amino acid-N relative to NH_4^+ were 3x higher in EM-dominated stands (1.6 ± 0.2) than AM-dominated stands (0.5 ± 0.1). We conclude that free amino acids are an important component of the N economy in all stands studied; however, in these natural environments plant uptake of organic N relative to inorganic N is explained as much by mycorrhizal association as by the availability of N forms *per se*.

INTRODUCTION

The majority of soil N is closely associated with soil organic matter (SOM) (Schulten and Schnitzer 1998), and in many forested ecosystems the mean residence time for SOM fractions may vary from several years to several thousand years (Stevenson and Cole 1999). The slow turnover of this N pool has been cited as a major constraint to plant nitrogen (N) supply. However, the operational definition for organic N is broad, encompassing both labile and recalcitrant forms. Polymeric molecules are often too large to cross membranes, but monomeric forms of organic N, e.g. amino acids and amino

sugars, are readily absorbed by plants (Schobert and others 1988; Kielland 1994; Raab and others 1999; Weigelt and others 2003; Finzi and Berthrong 2005). Though these N forms often represent only a small proportion of total soil organic N, the turnover of these substrates has important implications for plant-microbial competition for N, effectively functioning as an alternative pathway for plant N supply in some ecosystems (Chapin 1995; Kaye and Hart 1997).

For example, laboratory incubations of late successional black spruce soils from interior Alaska have demonstrated that gross rates of N flux throughout the soil free amino acid (FAA) pool are substantially greater than gross rates of N mineralization (Jones and Kielland 2002). Correspondingly, plants in black spruce ecosystems absorb amino acids to a much greater extent than ammonium, roughly in proportion to their availability in the soil (Kielland and others 2006). Measures of amino acid turnover *in situ* reveal that FAA cycling rates are rapid across a variety of forest ecosystems as well as across broad latitudinal gradients (Kielland and others 2007; McFarland and others, unpublished manuscript). Residence times for FAA in some temperate forest soils are less than two hours (McFarland and others, unpublished manuscript), further supporting the notion that the cycling dynamics other labile N forms may be as significant to the N economy of terrestrial plants as the turnover of soluble inorganic N.

Our incomplete understanding of the mechanisms controlling turnover and subsequent plant uptake of soil organic N is a major obstacle in modeling the N cycling dynamics of terrestrial forest ecosystems. This recognition has led to a revision of the traditional view of soil N cycling which emphasized the importance of net N

mineralization in regulating plant N availability (Schimel and Bennett 2004). Under the 'new paradigm' of N cycling, plant preference for N reflects the relative dominance of a particular N form, e.g. NO_3^-, NH_4^+, or FAA-N, which is in turn mediated by microbial activity. In extremely N-limited environments, plants and microorganisms alike rely largely on monomers of organic-N, e.g. amino acids, to satisfy their N requirements. Since soil microorganisms have higher substrate affinities and larger surface-to-volume ratios than roots, plant N uptake under these conditions is considered to be 'opportunistic' as soluble organic N diffuses past the fine root complex from N-rich to N-poor microsites. At the other end of the N availability gradient, high rates of N mineralization coupled with a relatively homogenous resource distribution, reduces microbial competition for N; nitrifiers flourish in the presence of excess NH_4^+, and plants rely primarily on inorganic N forms.

Though conceptually simple, this model of terrestrial N cycling has not fully evolved to encompass the role of plant and associated symbionts in mediating N bioavailability. Plant-microbial interactions for N can be simultaneously mutualistic and competitive, particularly in forest ecosystems where the microbial community structure is dominated by fungi. In some floodplain stands of interior Alaska, it is estimated that 85-90% of soil microbial biomass is fungal (Flanagan and Van Cleve 1983) and over 60% of fungal richness is represented by root-associated taxa (D.L. Taylor *personal communication*). How much fungal biomass is actually in symbiosis with plants remains uncertain for many forest ecosystems, but from at least one account we know that extramatrical mycelia of ectomycorrhizal (EM) fungi can contribute up to one third of the

soil microbial biomass (Högberg and Högberg 2002). This has several important implications with regard to plant nutrition as there is some evidence that organic matter quality, mineral N turnover, the type of mycorrhizal association, and organic N uptake by plants are intercorrelated across a broad range of tropical, temperate, and boreal forests (Allen and others 1995).

Expanding on Moser's (1967) global map of forest mycorrhizal types, Read (1983) proposed a geographical distribution of mycorrhizal associations based on their use of organic N forms. This hypothesis was based on observations that soils high in surface organic matter have ecto- and ericoid mycorrhizae, while environments with low accumulation of surface organic matter are dominated by arbuscular mycorrhizal (AM). Direct uptake of labile organic N has been demonstrated for many plant species across a range of biomes irrespective of mycorrhizal association (Chapin and others 1993; Kielland 1997; Näsholm and others 1998; Raab and others 1999; Schmidt and Stewart 1999; Streeter and others 2000; Näsholm and others 2000; Henry and Jefferies 2003; Nordin and others 2004; Xu and others 2004; Finzi and Berthrong 2005). However, ericoid and some EM fungal associations are physiologically capable of directly hydrolyzing certain components of SOM and transferring the resultant N to the host plant (Bajwa and Read 1985; Abuzinadah and others 1986; Finlay and others 1992). Work in stands of *Pinus muricata* in northern California suggests a coevolved strategy for N acquisition whereby exoenzyme production by EM symbionts facilitates host access to recalcitrant soil organic N derived from its own litter (Northrup and others 1995). In contrast, AM associations appear to have a more limited ability to decompose organic

matter (Read 1991; but see Hodge and others 2001). Still, AM fungi possess permeases for amino acid uptake (Cappellazzo and others 2008), and recent experiments of plant amino acid uptake indicate that both AM plants and some non-mycorrhizal plants absorb amino acids under conditions where soil concentrations of FAA are relatively high (Näsholm and others 1998; Raab and others 1999; Hawkins and others 2000). Whereas the importance of mycorrhizal associations in nutrient acquisition for vascular plants is well established (Allen 1991), the aforementioned studies pose several challenging questions regarding N cycling dynamics in terrestrial ecosystems. To what extent is plant potential for DON uptake realized in the field? Do mycorrhizal types differ in their capacity to sequester organic N for their host? If not, what are the principal factors controlling plant N source selectivity in terrestrial forests? These linkages between plant N uptake and mycorrhizal type have never been explicitly tested across ecosystems that vary in climate, soil organic matter quality, or dominant forest species.

The objective of this project was to evaluate plant uptake of organic and inorganic-N in forest ecosystems across a broad latitudinal gradient of forest ecosystems. We hypothesized that plant preference for FAA across this range of forest ecosystems was inversely correlated with rates of inorganic N mineralization. Thus, in N-limited boreal ecosystems where low soil temperatures constrain N mineralization rates, we predicted that FAA would represent a greater proportion of the annual N requirements of vascular plants. Secondly, we hypothesized that mycorrhizal association plays a major role in determining the ratio of organic to inorganic N uptake among ecosystem types. Since EM-dominated soil communities generally exhibit more extensive hyphal networks

and a greater capacity to degrade complex soil organic N than AM-dominated communities (Smith and Read 1997), we predicted that plant species from predominantly EM colonized stands would have higher uptake of organic relative to inorganic N forms in the field.

METHODS AND MATERIALS

Study sites

We chose a series of forest ecosystems that differed in climate, soil type, overstory taxon, and dominant mycorrhizal association (EM vs. AM), including stands from three regions in North America: southern temperate, northern temperate, and boreal. In the southeastern United States, we sampled from two deciduous hardwood stands: AM-dominated tulip poplar (*Liriodendron tulipifera*) at the USDA Forest Service Coweeta Research Station in North Carolina and EM-dominated white oak (*Quercus alba*) at the B. F. Grant Experimental Forest in central Georgia. In northern Michigan we sampled in an AM-dominated sugar maple (*Acer saccharum*) stand near the Ford Forestry Center, as well as an EM-dominated red pine (*Pinus resinosa*) plantation just outside Houghton. Our boreal site was EM-dominated white spruce (*Picea alba*) stand located within the floodplain portion of the Bonanza Creek Experimental Forest near Fairbanks, AK. Two of these sites, Bonanza Creek and Coweeta belong to the Long-Term Ecological Research (LTER) network while the B.F Grant and upper Michigan stands include areas where studies on C and N cycling dynamics have been conducted for several decades. Select site and soil properties are presented in Table 3.1; specific

characteristics for these stands are described in detail elsewhere (Pregitzer and others 2002; McFarland and others, unpublished manuscript).

Field sampling

Previous experiments in floodplain willow, balsam poplar, and black spruce communities along a primary successional gradient in interior Alaska, have demonstrated the effectiveness of using a multiple isotope (^{13}C, ^{15}N) tracer approach (McFarland and others 2002; Kielland and others 2007), to quantify plant and microbial uptake of NH_4^+ and amino acid-N *in situ*. The transient nature of inter-pool transfers of soil N as well as plant-microbial interactions during N acquisition makes it difficult to accurately evaluate any particular pool or flux in the laboratory, where plant and microbial uptake processes are often measured separately. Moreover, intact hyphal networks representative of the mycorrhizal community structure naturally occurring in forest ecosystems are nearly impossible to recreate under controlled environmental conditions. Therefore, we chose to conduct as much of our experimentation as possible in the field in order to directly observe linkages between plant N uptake and microbial turnover of soil N.

The field component for our project was conducted in July 1999 for white spruce and from June to July 2000 for the remaining forest types. Our sampling protocol was similar to that of previous work conducted in floodplain balsam poplar stands of interior Alaska (McFarland and others 2002). Using a randomized complete block design, we established 3 replicate soil injection grids within a 9 m^2 subplot. For the temperate forest stands, this design was replicated six times along a transect so that a minimum of 30 m

separated each subplot. In Alaska our experiment was conducted within three mature white spruce stands scattered along a 10 km stretch of the Tanana River. Within each stand, we established 3 subplots (n = 9) separated by a minimum of 20 m. For all forest types, the three grids in each subplot were injected with one of 2 treatment solutions or a control (distilled water). Treatment solutions were (1) $(^{15}NH_4^+)_2SO_4$ plus U-[^{13}C]-glycine or (2) $(NH_4^+)_2SO_4$ plus U-[^{13}C][^{15}N]-glycine. Unlabeled ammonium was added to the dually-labeled glycine treatment to mirror any fertilization effect brought about by the addition of glycine to the ^{15}N-labeled ammonium treatment. Soils were injected to a depth of 10 cm beginning at the L-F interface and cored to 12 cm in order to account for any vertical leaching of the label and to stay well within the zone for fine root development (Hendrick and Pregitzer 1996; Ruess and others 2006). We removed all leaf litter prior to injection, but added it back following treatment application to maintain moisture and temperature constancy in the soil environment around the injection area. Total injection volume was 37 ml (~1 ml·cm^{-2} for each injection area along our treatment grid), which delivered approximately 0.39 g ^{13}C m^{-2} and 0.22 g ^{15}N m^{-2} for both labeled solutions. Injecting a complete subplot with all treatments required approximately 20 min. Cores within each grid were harvested sequentially at 45 min, 2, 12, and 24 h, and 7 and 14 d.

Our coring grids consisted of 6 holes large enough to allow a soil corer with an inside diameter of 5.5 cm to pass through unobstructed (see McFarland and others 2002 for grid design). Harvest periods were randomized within a grid. For each core the areal extent of our injections at 1 cm resolution was twice that of the area harvested. Since the center of each soil core removed matched exactly the center of its respective treatment

area, we were able to remove a soil core with a theoretically known amount of added label (600 μg ^{15}N and 1040 μg ^{13}C).

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

We collected additional cores (n = 15) within each forest type for determination of amino acid-N concentrations using fluorometrics (Jones and others 2002). Sampling and laboratory protocols for handling and analyzing these samples are discussed elsewhere (McFarland and others, unpublished manuscript).

Laboratory analyses

Root samples were thawed and hand sorted to remove any residual organic matter. We separated roots into two size classes. Roots over 1 mm diameter were classified as coarse, while those 1 mm or less were classified as fine with the exception of tulip poplar which had much thicker fine roots than the other forest types. The threshold for fine root classification in tulip poplar was set at 2 mm. Fine root samples were freeze-dried and subsequently powdered using a modified roller mill to ensure complete homogenization. Soil moisture content was determined by drying subsamples at 60 ˚C for 48 h, and subsamples were ground to a powder using the same roller mill design. Both roots and soils were analyzed for C, N, ^{13}C, and ^{15}N using a Europa Scientific continuous flow mass spectrometer (PDZ Europa, Inc.). All isotope values obtained for root and soil C and N were normalized using standards derived from NBS-19 or IAEA ammonium sulfate, respectively.

Fumigated and nonfumigated soil extracts were digested using a modified micro-Kjeldahl procedure (Bremner and Mulvaney 1982). All soil extracts were analyzed for either available NH_4^+ or NO_3^- (DIN) or total Kjeldahl N (TKN) by flow injection colorimetry using a Technicon autoanalyzer (Whitledge and others 1981). DON was calculated as the difference between TKN and DIN in unfumigated extracts, while MBN was calculated as the difference in TKN between fumigated and unfumigated digests. We did not use a conversion factor to correct the extraction efficiency of N (K_N) for microbial biomass determinations. A conversion factor is highly dependent on edaphic

characteristics within each forest type, including microbial composition, and thus likely to be quite variable among the stands used in this study. Moreover, a conversion factor could over-represent the amount of label sequestered within microbial biomass, as freshly assimilated N is more chloroform labile than the more recalcitrant components of MBN.

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

Calculations and statistical analyses

We report all isotope values for soil and root C and N pools as percent recovery of added label. Recovery of isotopic labels was calculated for individual cores by multiplying pool size (μg C or $N \cdot g^{-1}$ dry root or soil) by the respective ^{13}C or ^{15}N atom % enrichment (APE) and dividing by the amount of label added. APE was determined by subtracting the atom % ^{13}C or ^{15}N of control cores from the atom % ^{13}C or ^{15}N of treated cores. Control values were averaged within stands prior to use in estimating isotopic enrichment. To economize our analyses we randomly selected one control core from each subplot within a stand (n = 6). For each core the areal extent of our injections (59.7 cm^2) was ~2.5x the areal extent of the core harvested (23.7 cm^2; total core volume = 285 cm^3); we used this ratio to estimate the amount of isotope (~600 μg ^{15}N and 1040 μg ^{13}C) applied to each core.

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

RESULTS

Soil and root pools of N

We observed several quantitative and qualitative differences in soils along our latitudinal transect. For instance, though we did not measure soil bulk density, we did calculate the average mass of cores harvested at each site. White spruce cores averaged less than half the mass (90 g) of cores collected from the temperate biomes (200-300 g), reflecting the high organic matter content in the upper horizons. Values derived from control samples revealed that soluble N pools also differed significantly among sites; however, most of this variation was attributable to comparisons between boreal white spruce and the temperate forest types (Table 3.1). DIN concentrations were lowest in red pine (1.1 ± 0.1 $\mu g \cdot g^{-1}$) and highest in white spruce (5.5 ± 0.5 $\mu g \cdot g^{-1}$), although temperate forest soils generally had higher rates of net mineralization. DON was also lowest in red pine (13.6 ± 0.9 $\mu g \cdot g^{-1}$) and highest in white spruce (42.6 ± 4.0 $\mu g \cdot g^{-1}$), with the two southern temperate stands having average DON concentrations that were almost twice those observed in the northern temperate stands. Soil concentrations of FAA-N varied more predictably with latitude, increasing over tenfold from just over 0.5 μg AA-N g^{-1} in tulip poplar to 6.5 μg AA-N g^{-1} in white spruce. Together, these data indicate that the N additions associated with our treatment applications enhanced the DIN and DON concentrations differently at each site. Though white spruce soils had the highest concentrations of DIN, enhancement of DIN was lowest in sugar maple (68 %) due to substantially higher masses for the temperate soil cores. In contrast, we increased DIN

concentrations to the greatest degree in red pine (185%) due to relatively low standing stocks of NH_4^+ and NO_3^-. Augmentation of DON was comparatively even across forest types ranging from 10% in white oak to 17% in sugar maple; although, when considering only the FAA fraction of DON, our amino acid amendment represented a two to fivefold increase in FAA-N for the temperate deciduous stands, but less than a doubling of FAA-N for either coniferous stand.

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

Partitioning of $^{15}NH_4^+$ and $^{13}C^{15}N$-glycine in soil pools

Total recoveries of the ^{15}N-labeled tracers are summarized for all sampling periods and soil N pools in Table 3.2. In bulk soil samples (soil from which only roots are removed), recovery of ^{15}N varied significantly among forest types for both NH_4^+ ($F_{4,193} = 16.07$; $P < 0.0001$) and glycine-amended ($F_{4,193} = 24.40$; $P < 0.0001$) cores. Forty-five minutes following injections average recovery of label in bulk soil ranged from 63 ± 3% (sugar maple) to 108 ± 5% (tulip poplar) for the NH_4^+ treatment, and from 60 ± 5% (white spruce) to 89 ± 6% (tulip poplar) for the glycine treatment (Figure 3.1). Mean recovery of ^{15}N declined over the next two weeks, albeit slightly for some stands (Table

3.2); however, this trend was significant only for sugar maple. We observed no significant treatment effect on recovery at any site with the exception of the white spruce stands where total recovery of ^{15}N in NH_4^+-amended cores (73 ± 2%) cores was approximately 13% higher than cores receiving the glycine treatment (60 ± 2%; $F_{5,40}$ = 3.60; P = 0.001). Still, comparison of treatment differences at each sampling period indicate that treatment effects in white spruce were significant for only two sampling periods (12 and 24 h), largely due to anomalously high recoveries in cores receiving $^{15}NH_4^+$.

Recovery of ^{13}C in bulk soil declined significantly at all sites throughout the experiment (P < 0.05; Figure 3.2). Initial recoveries in the temperate stands ranged from 78 ± 7% in red pine to 90 ± 7% in sugar maple. After two weeks, overall recovery of labeled glycine dropped to ~50% for all stands. In boreal white spruce, temporal patterns of ^{13}C recovery resembled those for the temperate stands, but the proportional recovery of label at any sampling period was approximately half that of the temperate stands (Figure 3.2). This discrepancy between temperate and boreal stands might be attributable to rapid mineralization of glycine label to CO_2 in white spruce soils by C-stressed microorganisms; however, the more likely explanation is a dilution of our label by high background levels of ^{13}C in these organic matter-rich soils (Näsholm and others 1998). We have observed a similar response in floodplain stands of balsam poplar and black spruce where high SOM content and thus high background ^{13}C can mask tracer additions of labeled-C (Kielland and others 2006; McFarland and others 2002).

Recovery of ^{15}N in soluble soil N pools varied with respect to N form, forest type, and sampling period (Figure 3.3; Table 3.2), yet we also observed similarities in the cycling dynamics of both N forms among forest types. Recovery of our $^{15}NH_4^+$ label as DIN declined sharply during the initial hours of the experiment at all sites. Less than one hour following treatment application, we recovered ~60% (averaged across sites) of $^{15}NH_4^+$ label as DIN. This value declined to 20% at 24 h, and to 4% two weeks following injection. This pattern was mirrored by a significant enrichment of the DON pool at all sites, suggesting rapid assimilation and release of $^{15}NH_4^+$ as organic N by the microbial biomass. Recovery of $^{15}NH_4^+$ as DON-^{15}N increased from 3% at 45 min to 14% at 24 h before declining to near background levels at the conclusion of the 14-d experiment. In glycine-treated cores, we found a similar decline in the recovery of label as DON throughout the experiment. Recovery of ^{15}N-glycine as DON-^{15}N averaged 38% across all sites at the first sampling period, less than 10% at 24 h, and only 5% at the final sampling period. Some of the glycine ^{15}N label was quickly mineralized as we noticed a significant enrichment of the DIN pool at the first sampling period. Average recovery of glycine label in the DIN pool peaked between 12 and 24 h before declining to less than 6% of total ^{15}N addition two weeks later.

Immobilization of ^{15}N label by the microbial biomass was apparent for both treatments within the first sampling period and represented the largest sink for ^{15}N among extractable soil N pools (Table 3.2). In cores receiving $^{15}NH_4^+$, recovery of ^{15}N averaged 14% across all stands at the first sampling period. Recovery peaked 1 d later when microbial immobilization accounted for 21% of the added $^{15}NH_4^+$ before declining to

~11% at 14 d (Figure 3.3). The amount of ^{15}N recovered in MBN for glycine-amended cores closely mirrored that of cores treated with NH_4^+. Average recovery increased from 15% at 45 min to 25% at 24 h before declining to 14% at 14 d. With the exception of sugar maple, we observed no significant time-dependent differences in MBN recovery between treatments for any stand type, indicating that both N forms represent a labile N source for microbial assimilation. Total recovery of ^{15}N in the microbial N pool was significantly higher for glycine than NH_4^+ in sugar maple soils ($F_{5,25} = 10.34$; $P = 0.024$). However this effect disappeared within 12 h of treatment application as ^{15}N recoveries among cores receiving different treatments varied more or less in concert for subsequent sampling periods.

Plant N uptake in situ

Net accumulation of ^{15}N in fine roots increased throughout the two week experiment for both treatments. After 14 d, average recovery in fine roots ranged from 1.9 ± 0.3% (white spruce) to 8.2 ± 1.1% (tulip poplar) for the NH_4^+ treatment, and from 1.6 ± 0.1% (white spruce) to 5.1 ± 1.5% (tulip poplar) for the glycine treatment. For all stands and sampling periods, ^{15}N recovery in fine roots was higher for cores receiving $^{15}NH_4^+$; however, the significance of this effect was dependent on stand type and sampling period. For example, 12hrs into the experiment, root uptake of $^{15}NH_4^+$ was significantly higher than that of ^{15}N-glycine for all temperate deciduous stands, but not for either of the conifers (Figure 3.4a). Two weeks later, we observed a treatment effect only in tulip poplar fine roots (Figure 3.4b). The fact that glycine-^{15}N and $^{15}NH_4^+$ were not

taken up by fine roots at the same rate in some stands signifies differences either in plant physiological capacity for uptake, or availability the two N forms, or both (see Discussion).

We observed enrichment of fine root ^{13}C above background levels for all forest types at the first sampling period suggesting that a portion of our glycine label was taken up intact. However, in contrast to fine root N, recovery of ^{13}C in fine roots was in most instances less than 1% of additions and demonstrated no significant increase beyond the first few sampling periods. This suggests a substantial fraction of the glycine label was not absorbed intact, but rather much of the ^{15}N sequestered by fine roots was derived from microbial turnover of glycine. To test this idea, we regressed molar excess ^{13}C against molar excess ^{15}N in fine roots from each core receiving the doubly-labeled glycine treatment. To expand our comparison, we included data from three balsam poplar stands along the Tanana River in interior Alaska (McFarland and others 2002). Slopes generated from these regressions indicate a decrease in fine root ^{13}C enrichment relative to ^{15}N enrichment throughout the experimental period for all forest types (Figure 3.5), confirming our assumption that direct uptake of glycine by fine roots was limited to the initial 2 h following treatment application.

DISCUSSION

Microbial mediation of soil N availability

Soil microorganisms at all sites rapidly incorporated both forms of added N, with up to 36% and 47% of our ^{15}N amendments recovered in MBN within 24 h of soil injections for ammonium and glycine-treated cores, respectively (Table 3.2). Microbial immobilization of ^{15}N coincided with a rapid depletion of original labeled forms over the same time period (Figure 3.3). Redistribution patterns of added ^{15}N into other soluble N pools and retention within bulk soil suggests that microbial N turnover was a function of soil C to N balance at each site. Generally, soils with narrow C:N ratios are thought to promote higher rates of N processing due to C limitation, while microbial function is generally considered more N-limited in soils with a wider C:N ratio. Amino acids differ from DIN in their dual function as a source of both metabolic C and N. Therefore, we predicted stands with narrow soil C:N ratios would transform a greater proportion of glycine-N to DIN, compared to N-limited ecosystems where soil microorganisms would retain amino acid-N and C. Data from this experiment are consistent with that prediction. For example, soil N availability was relatively high in sugar maple and tulip poplar, where average C:N ratios in the top 7 cm (13-14) were the lowest recorded among forest types and significantly lower than those of red pine (22) or white spruce (23). Corresponding to these differences, we found that initial recovery of ^{15}N-glycine as DON was 41-56% lower than recovery of $^{15}NH_4^+$ as DIN for the two AM-dominated forest types (Figures 3.3a,c). Moreover, microbial-^{15}N immobilization in sugar maple was significantly higher during the first 12 h of sampling for cores receiving ^{15}N-glycine. In contrast, we observed no significant difference in recovery between ^{15}N-glycine and

$^{15}NH_4^+$ as DON and DIN, respectively, at the first sampling period for any of the EM-dominated stands (Figure 3.3b,d,e).

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

Ecosystem N retention

With the exception of sugar maple, the majority of our NH_4^+ and glycine label remained in the non-biomass fraction of soil N after cycling through the microbial biomass. After 14 d, retention of ^{15}N in root-free bulk soil ranged from 41 ± 3% (sugar maple) to 83 ± 10% (tulip poplar) for the NH_4^+ treatment, and from 47 ± 7% (sugar maple) to 90 ± 6% (tulip poplar) for the glycine treatment. We have no explanation for relatively poor recovery of both labels in sugar maple soils at the first sampling period, other than mass flow away from the injection site due to steady precipitation shortly following the onset of our treatment applications. Nevertheless, sugar maple was the only site where we witnessed a significant decline in label over time (Figure 3.1).

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

in sugar maple was either exported from the coring area or resided in lower, unsampled soil horizons.

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

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

and abiotic N immobilization obviously has important implications for plant nutrition and N acquisition strategies.

Plant N uptake

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

resulting analytical bias against plant uptake is proportional to the magnitude of this experimental error.

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

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

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

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

N forms only becomes relevant to plant nutrition when examined in view of soil N availability as mediated by microbial, including mycorrhizal, competition for these N forms.

Uptake data of tracer ^{15}N alone may be misleading in assessing the relevance of different N forms to plant nutrition due to differences in availability of endogenous N (*sensu* Kielland and others 2006). Therefore, we adjusted our estimates of fine root N uptake to account for differential isotope dilution between treatments based on soil concentrations of DIN and free amino acid-N at each site (Figure 3.6). In order to broaden the scope of our comparison, we included plant uptake data from EM-dominated balsam poplar stands growing in the same landscape as our white spruce stands (McFarland and others 2002). We found that relative uptake of amino acid-N versus DIN was significantly ($P \leq 0.05$) lower than 1:1 in tulip poplar and significantly higher than 1:1 in red pine and balsam poplar; however, white oak, sugar maple, and white spruce were statistically near unity with respect to the two N forms. Additionally, both EM-dominated white oak and AM-dominated tulip poplar had higher uptake rates for DIN than amino acid-N. Though these observations appear to indicate lack of physiological preference for amino acids based on mycorrhizal association, pool adjusted uptake was significantly different among forest types ($F_{5,34} = 6.02$, $P < 0.001$), and a substantial fraction of this variation was explained by mycorrhizal association. Plant uptake of amino acid-N vs. DIN was threefold higher in EM-dominated stands (1.6 ± 0.2) than AM-dominated stands (0.5 ± 0.1). Thus, FAA appears be an important component of the N economy in all these stands. What remains unclear is whether plant uptake of N is

determined more by the availability of N forms or an evolved physiological preference for a particular N form linked to mycorrhizal association (AM vs. EM). This uncertainty stems largely from our ignorance of the true extent of the involvement of mycorrhizal association in determining the patterns in N uptake observed in this study. Regardless, our data suggest that the relative importance of inorganic and organic N forms to the N economy of plants could be connected in part to the distribution of these major mycorrhizal types.

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

We are equally cautious about our interpretations concerning plant access to both N treatments in the field. In theory, enhanced N availability associated with label additions could have temporarily overwhelmed microbial transporter systems, giving

plants access to N outside their usual resource niche for N nutrition (McKane and others 2002). However, in this study we don't feel our N additions were excessive. K_m values for uptake kinetics of soil amino acids, for instance, suggest that microorganisms typically have low-affinity transport systems. In general, soil concentrations of FAA or NH_4^+ required to saturate the potential for soil microbial uptake are in the millimolar range (Vinolas and others 2001) while our N additions were significantly less.

CONCLUSION

Quantitative relationships between production and the availability of limiting resources cannot be established until reliable and accurate estimates of organic N cycling and uptake by plants are obtained. Our research provides important insights into the cycling dynamics of labile N in forested ecosystems. To our knowledge this study is the first to use a common experimental approach to develop quantitative patterns of microbial utilization and plant uptake of inorganic and organic N across a broad geographic and taxonomic range of forest ecosystem species. We found that while plant N uptake was low in comparison to microbial immobilization. Accumulation of our ^{15}N tracer in plant tissue over several weeks indicated that they are effective long term sinks. Plant apparent N preference is governed to a large extent by N availability as mediated by patterns of microbial utilization, and immobilization within abiotic soil pools; however, we could not discount the influence of distinctive plant-fungal symbioses in regulating plant N nutrition. Our data suggest plant capacity to directly absorb amino acids is a

pervasive characteristic for a variety of forest types across a large latitudinal gradient, regardless of dominant mycorrhizal association. Moreover, the finding that the ratio of fine root amino acid uptake was threefold higher for stands dominated by EM fungi than AM fungi suggests that mycorrhizal type mediates plant uptake for different N forms. This discrimination of N form by mycorrhizal type is probably due to unique functional attributes of AM vs. EM fungi in adaptation to physiochemical properties of the soil environments in which they have evolved.

ACKNOWLEDGEMENTS

We are very thankful to Greg Mauer for all his cheerful effort in the field. We also appreciate the expert technical assistance from Lola Oliver of the Forest Soils Lab at the University of Alaska, Fairbanks. This project would not have been possible without the logistical support provided by Dr. Andrew Burton of the School of Forest Resources and Environmental Science at Michigan Technological University, and Lee Ogden of the Warnell School of Forest Resources at the University of Georgia. Our research was supported through grants from the National Science Foundation's Ecosystem program (DEB 966537), and the Bonanza Creek LTER program (NSF DEB-0137896).

LITERATURE CITED

Abuzinadah RA, Finlay RD, Read DJ. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. II. Utilization of protein by mycorrhizal plants of *Pinus contorta*. New Phytologist 103:495-506.

Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R, and Rincon E. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. pp 47-62. The significance and regulation of soil biodiversity. Kluwer Academic Publishers, The Netherlands.

Allen MF. 1991. The Ecology of Mycorrhizae. Cambridge University Press, Cambridge.

Bajwa R, Read DJ. 1985. The biology of mycorrhiza in the Ericaceae IX. Peptides as nitrogen sources for the ericoid endophyte and for mycorrhizal and non-mycorrhizal plants. New Phytologist 101:459–467

Bremner JM, Mulvaney CS. 1982. Nitrogen—total. Page AL, editor. Methods of soil analysis. Part 2. Chemical and microbiological properties. Madison (WI):American Society of Agronomy. p595-622.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17:837-842.

Cappellazzo G, Lafranco L, Fitz M, Wipf D, Bonfante P. 2008. Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*. Plant Physiology *in press*.

Chapin FS III, Moilanen L, Kielland K. 1993. Preferential use of organic nitrogen for growth by a nonmycorrhizal arctic sedge. Nature 631:150-153.

Chapin FS, III. 1995. New cog in the nitrogen cycle. Nature 377: 199-200.

Clemmensen KE, Sorensen PL, Michelsen A, Jonasson S, Ström L. 2007. Site-dependent N uptake from N-form mixtures by arctic plants, soil microbes, and ectomycorrhizal fungi. Oecologia.

Fahey TJ, Yavitt JB. 2005. An *in situ* approach for measuring root-associated respiration and nitrate uptake of forest trees. Plant and Soil 272:125-131.

Finlay RD, Frostegard A, Sonnerfelt AM. 1992. Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl ex Loud. New Phytologist 120:105-115.

Finzi AC, Berthrong ST. 2005. The uptake of amino acids by microbes and trees in three cold-temperate forests. Ecology 86:3345-3353.

Flanagan PW, Van Cleve K. 1983. Nutrient cycling in relation to decomposition and organic-matter quality in taiga ecosystems. Canadian Journal of Forest Resources 13:795-817.

Hawkins H-J, Anders J, Eckhard G. 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizae. Plant and Soil 226:275-285.

Hendrick RL, Pregitzer KS. 1996. Applications of minirhizotrons to understand root function in forests and other natural ecosystems. Plant and Soil 185:293–304.

Henry HAL, Jefferies RL. 2003. Interactions in the uptake of amino acids, ammonium, and nitrate ions in the Arctic salt-marsh grass, *Puccinellia phryganodes*. Plant,

Cell, and Environment 26:419-428.

Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297-299.

Högberg M and Högberg P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytologist 154:791-795.

Jackson LE, Schimel JP, Firestone MK. 1989. Short-term partitioning of nitrate and ammonium between plants and microbial biomass in annual grassland. Soil Biology and Biochemistry 21: 409–415.

Jones DL, Kielland K. 2002. Soil amino acid turnover dominates the nitrogen flux in permafrost dominated taiga forest soils. Soil Biology and Biochemistry 34:209-219.

Jones DL, Owen AG, Farrar JF. 2002. A simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biology and Biochemistry 34:1893-1902.

Kaye JP, Hart SC. 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology and Evolution 12:139-143.

Kaye JP, Binkley D, and Rhoades C. 2003. Stable soil nitrogen accumulation and flexible organic matter stoichiometry during primary floodplain succession. Biogeochemistry 63:1-22.

Khan SA, Mulvaney RL, Mulvaney CS. 1997. Accelerated diffusion methods for inorganic nitrogen analysis of soil extracts and water. Soil Science Society of America Journal 61:936-942.

Kielland K. 1994. Amino acid absorption by Arctic plants: implications for plant nutrition and nitrogen cycling. Ecology 75:2373-2383.

Kielland K. 1997. Role of free amino acids in the nitrogen economy of arctic cryptograms. Ecoscience 4:75-79.

Kielland K, McFarland JW, Olson K. 2006. Amino acid uptake in deciduous and coniferous taiga ecosystems. Plant and Soil 288:297-307.

Kielland K, McFarland JM, Ruess RW, Olson K. 2007. Rapid cycling of amino acid of organic nitrogen in taiga forest ecosystems. Ecosystems 10:360-368.

Krab EJ, Cornelissen JHC, Lang SI, van Logestijn RSP. 2008. Amino acid uptake among wide-ranging moss species may contribute to their strong position in higher latitude ecosystems. Plant and Soil 304:199-208.

Lovett GM, Weathers KC, Arthur MA. 2002. Control of nitrogen loss from forested watersheds by soil carbon:nitrogen ratio and tree species composition. Ecosystems 5:712-718.

Lovett GM, Mitchell MJ. 2004. Sugar maple and nitrogen cycling in the forests of eastern North America. Frontiers in Ecology and the Environment 2:81-88.

McFarland JW, Ruess RW, Kielland K, Doyle AP. 2002. Cycling dynamics of NH_4^+ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. Ecosystems 5:775–788.

McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA, Murray G. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415:68–71.

Miller AE, Bowman WD. 2003. Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. Plant and Soil 250:283–292

Moser M. 1967. Die ectotrophe Ernahrungsweise an der Waldgrenze Mitteilungen Forstl. Bundesversuchsanst. Wien 75:357-380.

Näsholm T, Ekblad A, Högberg P. 1998. Boreal forest plants take up organic nitrogen. Nature 392:914-917.

Näsholm T, Huss-Damell K, Högberg P. 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81:1155-1161.

Nordin A, Schmidt IK, Shaver G. 2004. Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. Ecology 85:955-962.

Northrup RR, Yu Z, Dahlgren RA, and Vogt KA. 1995. Polyphenol control of nitrogen release from pine litter. Nature 377:227-229

Persson J, Näsholm T. 2001. Amino acid uptake: a widespread ability among boreal forest plants. Ecology Letters 4:434–438

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, and Hendrick RL. 2002 Fine root architecture of nine North American trees. Ecological Monographs. 72: 293-309.

Raab TK, Lipson DA, Monson RK. 1999. Soil amino acid utilization among the

Cyperaceae: plant and soil processes. Ecology 80:2408-2419.

Read, D.J. 1983. The biology of mycorrhiza in the Ericales. Canadian Journal of Botany. 61:985-1004.

Read DJ. 1991. Mycorrhizas in ecosystems. Experientia 47:376-391.

Ruess, RW, Hendrick RL, Vogel JG, and Sveinbjornsson, B. 2006. The role of fine roots in the functioning of boreal forests. In: F.S. Chapin, III, M.W. Oswood, K. Van Cleve, L. Viereck, and D. Verbyla (editors), Alaska's changing boreal forest. Oxford University Press, New York, NY.

Schimel, JP, Bennett J. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591-602.

Schmidt S, Stewart GR. 1999. Glycine metabolism by plant roots and its occurrence in Australian plant communities. Australian Journal of Plant Physiology 26:253-264.

Schobert C, Köckenberger W, Komor E. 1988. Uptake of amino acids from the soil: a comparative study with castor bean seedlings grown under natural and axenic conditions. Plant and Soil 109:181-188.

Schulten HR, Schnitzer M. 1998. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26:1-15.

Sinsabaugh RL, Moorhead DL. 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26:1305–1311.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. Academic Press, London.

Stevenson FJ and Cole MA. 1999. Cycles of soil: carbon, nitrogen, phosphorus, sulfur,

micronutrients. John Wiley and Sons, Inc., New York

Streeter TC, Bol R, Bardgett RD. 2000. Amino acids as a nitrogen source in temperate upland grasslands: the use of dual labelled (13C, 15N) glycine to test for direct uptake by dominant grasses. Rapid Communications in Mass Spectrometry 14:1351-1355.

Svennerstam H, Ganeteg U, Bellini C, Näsholm T. 2007. Comprehensive screening of Arabidopsis mutants suggests the lysine histidine transporter 1 to be involved in plant uptake of amino acids. Plant Physiology 143:1853-1860.

Templer PH, Dawson TE. 2004. Nitrogen uptake by four tree species of the Catskill Mountains, New York: implications for nitrogen cycling. Plant and Soil 262:251-261.

Vinolas LC, Healey JR, Jones DL. 2001. Kinetics of soil microbial uptake of free amino acids. Biology and Fertility of Soils. 33:67-74.

Virtanen AI, Linkola H. 1946. Organic nitrogen compounds as nitrogen nutrition for higher plants. Nature 158: 515

Weigelt A, King R, Bol R, Bardgett RD. 2003. Interspecific variability in organic nitrogen uptake of three temperate grassland species. Journal of Plant Nutrition and Soil Science 166:606-611.

Whitledge T, Malloy S, Patton C, Wirick C. 1981. Automated nutrient analysis in seawater. Brookhaven National Laboratory Tech. Rep. BNL-51398.

Xu X, Ouyang H, Cao G, Pei Z, Zhou C. 2004. Uptake of organic nitrogen by eight

dominant plant species in *Kobresia* meadows. Nutrient Cycling in Agroecosystems 69:5-10.

Xu X, Ouyang H, Kuzyakov Y, Richter A, Wanek W. 2006. Significance of organic nitrogen acquisition for dominant plant species in an alpine meadow on the Tibet plateau, China. Plant and Soil 285:221-231.

Zak DR, Groffman PM, Pregitzer KS, Christensen S, Tiedje JM. 1990. The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. Ecology 71:651-656.

Zogg GP, Zak DR, Pregitzer KS, Burton AJ. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81:1858-1866.

Table 3.1. Select site and soil properties of forest ecosystems used in cross-ecosystem evaluation of plant uptake of amino acid-N vs. NH_4^+-N. AM = arbuscular mycorrhizae; EM = ectomycorrhizae; DIN = dissolved inorganic N; DON = dissolved organic N; FAA-N = free amino acid N. [a]Mycorrhizal root tips are live AM or EM root tips per meter fine root of dominant overstory taxa and [b]percent mycorrhizal infection is the number of healthy AM or EM tips per total tips counted. Dead tips or tips that were older or not easily distinguishable as mycorrhizal were not counted (Lansing *unpublished data*). [c]Soil temperatures represent daily average calculated from hourly measurements collected during the first 24 hours of the tracer experiment. Soil and fine root data are mean ± SE, n = 6-9. Letters denote significant differences ($P \leq 0.05$) between sites. Fine root mass estimates are reported on an areal basis to a soil depth of 12 cm.

	SITE				
Parameter	**Tulip poplar**	**White oak**	**Sugar maple**	**Red pine**	**White spruce**
Latitude	35° 4′ N	33° 25′ N	46° 39′ N	47° 6′ N	64° 41′ N
Dominant mycorrhizal association	AM	EM	AM	EM	EM
Mycorrhizal root tips[a]					
Percent mycorrhizal infection[b]	30	>90	42	>90	>90
Percent overstory	85	68	92	100	98
Mean annual temperature (°C)	12.7	16.5	3.8	3.8	-3.3
Mean annual precipitation (mm)	1816	1263	841	883	287
Soil classification	Humic Hapludult	Typic-Rhodic Hapludult	Typic Haplorthod	Entic Haplorthod	Typic Cryofluvent

Table 3.1 (cont.)

Soil texture	sandy loam	clay loam	sandy loam	sandy loam	organic to alluvial silt
DIN ($\mu gN \cdot g^{-1}$)	3.24 ± 0.21^{a}	2.67 ± 0.36^{a}	3.50 ± 0.29^{a}	1.13 ± 0.11^{b}	3.39 ± 0.50^{a}
DON ($\mu Gn \cdot g^{-1}$)	23.8 ± 1.6^{a}	24.7 ± 1.2^{a}	14.0 ± 1.8^{b}	13.6 ± 0.9^{b}	42.6 ± 4.0^{c}
FAA-N ($\mu gN \cdot g^{-1}$)	0.57 ± 0.09^{c}	1.30 ± 0.39^{c}	0.97 ± 0.14^{c}	3.25 ± 0.69^{bc}	6.45 ± 1.20^{ab}
Fine root mass ($g \cdot m^{-2}$)	237.8 ± 12.7^{c}	323.9 ± 14.3^{b}	323.3 ± 11.0^{b}	275.0 ± 14.2^{bc}	350.0 ± 14.9^{a}
Fine root N ($mgN \cdot g^{-1}$)	16.5	7.6	12.3	11.4	8.7
Average core dry mass (g)	202 ± 4	230 ± 4	264 ± 5	285 ± 4	90 ± 2
Soil temperature (°C)[c]	17.0	21.5	13.8	15.7	9.3

Table 3.2. Percent recovery of added ^{15}N within plant and soil pools at each sampling period. TP = tulip poplar; WO = white oak; SM = sugar maple; RP = red pine; WS = white spruce; Gly = glycine. Mean ± SE, n = 6-9.

			Recovery of added ^{15}N (%)					
Site	**N form**	**Pool**	45 min	2 hr	12 hr	24 hr	168 hr	336 hr
TP	NH_4^+	DIN	87.27 ± 13.76	48.21 ± 10.56	23.07 ± 5.45	24.18 ± 5.24	6.36 ± 1.96	5.59 ± 1.88
		DON	1.12 ± 0.51	2.68 ± 0.9	7.62 ± 5.30	6.93 ± 3.13	1.67 ± 0.43	2.18 ± 0.54
		Microbial N	20.21 ± 7.62	32.25 ± 5.83	35.49 ± 6.81	30.57 ± 8.70	32.17 ± 4.75	22.58 ± 5.15
		Fine Root N	0.41 ± 0.05	0.43 ± 0.15	1.68 ± 0.53	2.21 ± 1.25	3.73 ± 0.54	8.18 ± 1.13
		Bulk soil N	108.32 ± 5.29	96.97 ± 11.77	94.17 ± 4.57	101.83 ± 8.13	88.80 ± 10.33	82.58 ± 9.67
	Gly	DIN	11.32 ± 2.68	16.86 ± 4.05	19.84 ± 7.53	12.79 ± 3.67	8.08 ± 1.52	5.85 ± 1.64
		DON	31.26 ± 2.08	15.00 ± 3.34	6.19 ± 1.66	3.74 ± 0.83	3.10 ± 0.40	2.94 ± 0.90
		Microbial N	28.66 ± 7.96	26.88 ± 4.35	28.48 ± 4.76	34.29 ± 3.32	35.17 ± 3.54	22.56 ± 5.08
		Fine Root N	0.28 ± 0.07	0.25 ± 0.06	0.67 ± 0.24	0.45 ± 0.09	3.24 ± 0.72	5.06 ± 1.54
		Bulk soil N	89.01 ± 10.18	104.25 ± 7.79	102.00 ± 10.43	95.64 ± 11.11	87.36 ± 14.31	89.94 ± 5.85
WO	NH_4^+	DIN	55.25 ± 7.52	34.26 ± 12.03	15.43 ± 3.65	12.15 ± 1.86	10.84 ± 2.02	9.53 ± 2.39
		DON	3.02 ± 1.38	5.56 ± 2.68	4.98 ± 2.10	7.59 ± 2.40	8.61 ± 1.48	9.61 ± 3.55
		Microbial N	8.70 ± 4.08	11.77 ± 3.45	27.92 ± 7.05	24.13 ± 5.60	25.24 ± 6.92	13.72 ± 4.96
		Fine Root N	0.26 ± 0.04	0.33 ± 0.04	0.76 ± 0.13	0.86 ± 0.14	1.33 ± 0.37	3.42 ± 1.27
		Bulk soil N	91.79 ± 7.99	85.80 ± 10.49	95.84 ± 9.65	80.95 ± 5.06	90.24 ± 10.33	88.63 ± 11.33
	Gly	DIN	7.80 ± 0.83	10.39 ± 3.78	10.12 ± 2.65	10.45 ± 2.16	6.05 ± 2.22	5.94 ± 1.47
		DON	41.20 ± 7.39	41.24 ± 6.92	15.86 ± 1.37	12.47 ± 3.33	8.01 ± 1.86	12.00 ± 5.21
		Microbial N	9.87 ± 4.51	11.60 ± 5.33	29.66 ± 8.65	21.11 ± 4.87	22.95 ± 4.04	16.99 ± 5.46
		Fine Root N	0.36 ± 0.09	0.23 ± 0.05	0.35 ± 0.08	0.84 ± 0.17	1.17 ± 0.20	1.55 ± 0.40
		Bulk soil N	85.86 ± 3.58	90.98 ± 11.55	89.49 ± 6.40	92.21 ± 6.32	92.02 ± 4.25	76.80 ± 9.68
SM	NH_4^+	DIN	66.75 ± 7.99	80.11 ± 6.91	49.00 ± 5.48	36.70 ± 6.81	8.52 ± 3.04	3.04 ± 0.94
		DON	2.95 ± 2.05	2.17 ± 1.43	3.88 ± 1.95	16.05 ± 7.58	2.96 ± 1.03	4.10 ± 1.73
		Microbial N	10.76 ± 1.59	13.96 ± 5.30	17.70 ± 7.81	9.24 ± 1.91	13.07 ± 2.50	13.93 ± 2.28
Table 3.2 (cont.)								
		Fine Root N	0.44 ± 0.04	0.80 ± 0.20	1.53 ± 0.21	2.59 ± 0.29	6.08 ± 0.74	7.27 ± 1.59
		Bulk soil N	63.63 ± 2.58	65.35 ± 4.81	70.09 ± 6.94	66.92 ± 7.44	50.96 ± 5.10	40.90 ± 3.38
	Gly	DIN	6.30 + 1.55	8.49 ± 1.67	16.15 ± 4.11	25.52 ± 4.28	6.88 ± 1.78	6.10 ± 0.81
		DON	25.79 ± 3.29	23.51 ± 4.01	15.62 ± 4.20	4.45 ± 1.85	2.19 ± 0.91	5.94 ± 1.77
		Microbial N	23.51 ± 3.63	28.79 ± 2.45	34.54 ± 6.28	19.52 ± 3.64	18.51 ± 5.56	15.14 ± 5.57
		Fine Root N	0.59 ± 0.07	0.64 ± 0.10	0.82 ± 0.08	1.39 ± 0.18	4.21 ± 0.20	4.93 ±0.81
		Bulk soil N	63.13 ± 7.02	67.23 ± 11.45	64.16 ± 8.82	71.27 ± 6.74	41.18 ± 6.61	46.71 ± 6.90
RP	NH_4^+	DIN	72.48 ± 6.65	70.69 ± 2.91	28.80 ± 6.10	18.64 ± 4.03	3.39 ± 0.71	1.36 ± 0.78
		DON	5.36 ± 1.19	9.53 ± 2.71	24.46 ± 9.22	23.59 ± 3.96	5.44 ± 2.51	6.74 ± 2.12
		Microbial N	23.06 ± 5.72	18.37 ± 3.97	20.60 ± 3.81	35.84 ± 9.84	28.51 ± 16.43	5.98 ± 1.94
		Fine Root N	0.76 ± 0.17	0.61 ± 0.13	1.64 ± 0.52	2.08 ± 0.57	6.68 ± 2.57	5.50 ± 0.91

		Bulk soil N	100.49 ± 14.26	90.55 ± 9.79	63.04 ± 6.56	69.74 ± 8.18	67.95 ± 15.17	68.55 ± 8.11
	Gly	DIN	4.82 ± 1.31	5.74 ± 0.71	11.88 ± 2.11	11.41 ± 2.29	4.91 ± 1.62	2.57 ± 0.40
		DON	59.04 ± 16.20	43.68 ± 9.57	20.95 ± 4.93	15.24 ± 3.02	5.76 ± 2.17	3.01 ± 0.69
		Microbial N	12.64 ± 1.95	31.28 ± 5.54	39.61 ± 4.84	46.91 ± 7.62	28.44 ± 9.51	16.97 ± 3.31
		Fine Root N	0.42 ± 0.11	0.64 ± 0.13	0.98 ± 0.14	1.36 ± 0.25	2.28 ± 0.65	4.54 ± 0.88
		Bulk soil N	78.20 ± 12.20	70.24 ± 8.50	76.94 ± 10.54	63.27 ± 10.40	56.36 ± 9.77	55.02 ± 12.22
WS	NH_4^+	DIN	33.39 ± 3.73	19.52 ± 3.56	10.77 ± 2.17	13.99 ± 2.69	2.98 ± 1.01	2.15 ± 0.61
		DON	2.74 ± 1.15	6.65 ± 2.79	8.17 ± 3.09	16.94 ± 6.23	2.88 ± 0.77	2.38 ± 0.61
		Microbial N	9.17 ± 3.54	11.24 ± 2.82	15.77 ± 4.58	11.13 ± 5.08	8.56 ± 2.90	4.60 ± 1.81
		Fine Root N	0.55 ± 0.12	0.60 ± 0.11	1.25 ± 0.23	1.22 ± 0.17	2.08 ± 0.34	1.89 ± 0.28
		Bulk soil N	80.13 ± 6.25	73.16 ± 6.52	68.06 ± 3.04	86.02 ± 3.58	67.41 ± 6.84	64.46 ± 5.71
	Gly	DIN	20.25 ± 2.58	12.66 ± 2.69	12.79 ± 1.75	11.63 ± 1.95	2.61 ± 0.51	3.09 ± 0.79
		DON	34.12 ± 3.98	14.23 ± 2.54	5.01 ± 1.58	2.70 ± 0.94	1.73 ± 0.32	1.35 ± 0.45
		Microbial N	4.94 ± 2.53	8.05 ± 4.01	13.13 ± 2.50	11.29 ± 2.54	6.51 ± 2.48	4.31 ± 2.43
		Fine Root N	0.26 ± 0.05	0.43 ± 0.07	0.71 ± 0.11	0.85 ± 0.11	2.02 ± 0.38	1.63 ± 0.12
		Bulk soil N	60.43 ± 5.04	63.11 ± 4.91	55.62 ± 2.46	66.68 ± 4.44	63.18 ± 6.78	53.70 ± 4.52

FIGURE LEGENDS

Figure 3.1 Percent recovery of added ^{15}N in bulk soil N for cores receiving NH_4^+ (white bar) and glycine (black bar) treatments at a) 45min and b) 14d following injection. Letters above the bars indicate differences ($P \leq 0.05$) among sites within NH_4^+ (normal text) and glycine (*italic*) treated cores. Symbols (*) denote differences ($P \leq 0.05$) in recovery between sampling periods within treatment and forest type. Bars are mean ± SE, n = 6-9.

Figure 3.2 Time dependent recovery of added ^{13}C in bulk soil pooled across treatments within a forest type. Symbols: ○ tulip poplar; ● white oak; □ sugar maple; ■ red pine; and white spruce. Mean ± SE, n = 12-18.

Figure 3.3 Percent recovery of added ^{15}N within DIN, DON, or microbial N pools over time for a) tulip poplar; b) white oak; c) sugar maple; d) red pine; and e) white spruce. Symbols are as follows: ● microbial N pool; DIN pool; and ■ DON pool. Open and solid symbols represent ammonium and doubly-labeled glycine treatments respectively. Data are means ± SE.

Figure 3.4 Recovery of ^{15}N in fine roots receiving NH_4^+ (white bar) and glycine (black bar) treatments at a) 12hrs and b) 14d following injection. Symbols (*) denote significant differences ($P \leq 0.05$) between treatments. Note differences in Y-axis scales between the two graphs. Bars are mean ± SE, n = 6-9.

Figure 3.5 Time dependent plot of slopes generated from linear regression of molar excess ^{13}C:excess ^{15}N in fine roots of all forest types from the glycine treatment. Horizontal line represents the 2:1 injection ratio of C:N administered with the doubly

labeled glycine treatment.

Figure 3.6 Relative uptake of free amino acid-N vs. DIN for all forest types. Horizontal stippled line represents the 1:1 uptake ratio between amino acid-N and DIN. Mean ± SE, n = 6–9. Calculations for plant N uptake are based on total fine root ^{15}N accumulation at the second sampling period, two hours following treatment application. Letters above the bars indicate differences ($P \leq 0.05$) among sites. Symbols (*) indicate ratios for which 95% C.I. were not found to include 1:1.

Figure 3.1a

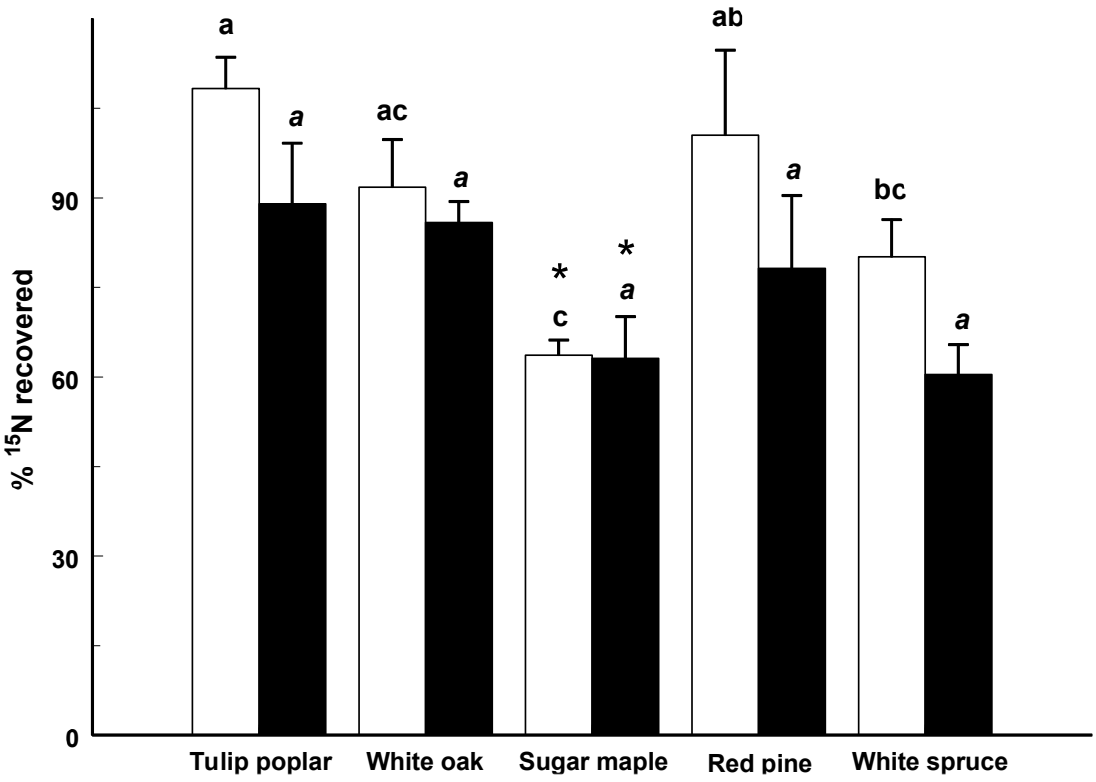

a
a
ac
a
*
c
*
a
ab
a
bc
a
% 15N recovered
0
30
60
90
Tulip poplar
White oak
Sugar maple
Red pine
White spruce

Figure 3.1b

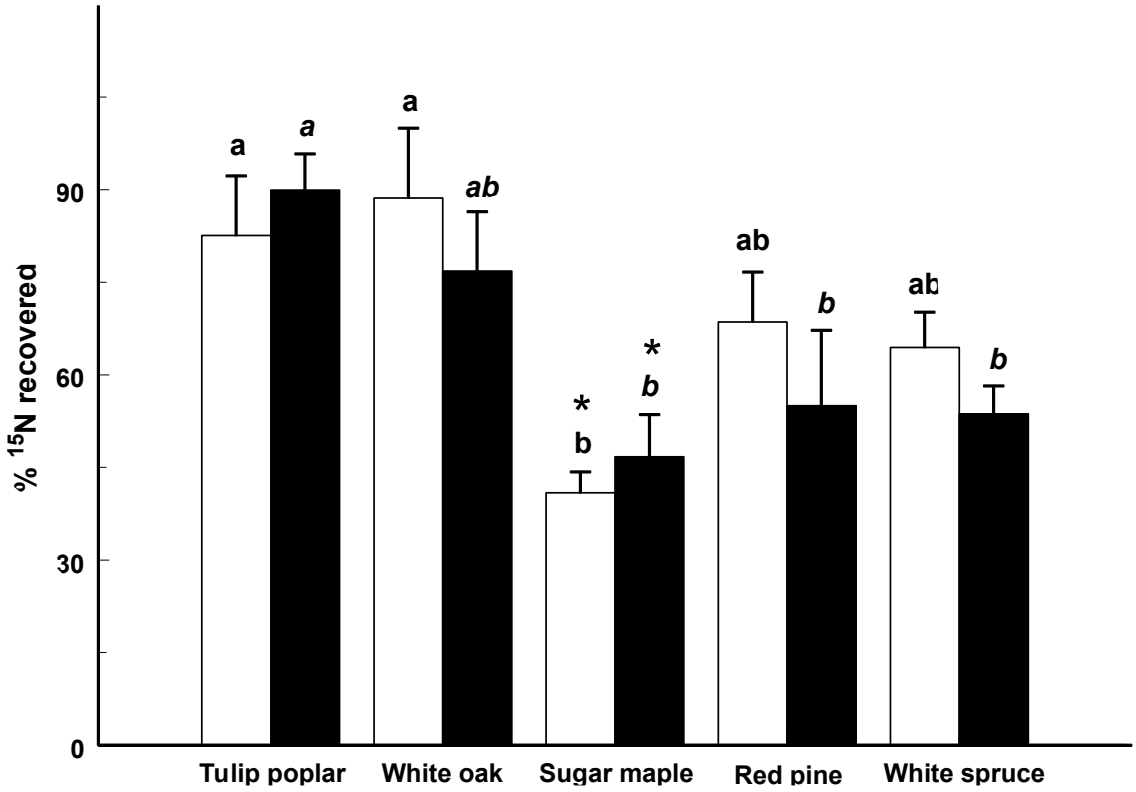

% 15N recovered
0
30
60
90
a
a
a
ab
*
b
*
b
ab
b
ab
b
Tulip poplar
White oak
Sugar maple
Red pine
White spruce

Figure 3.2

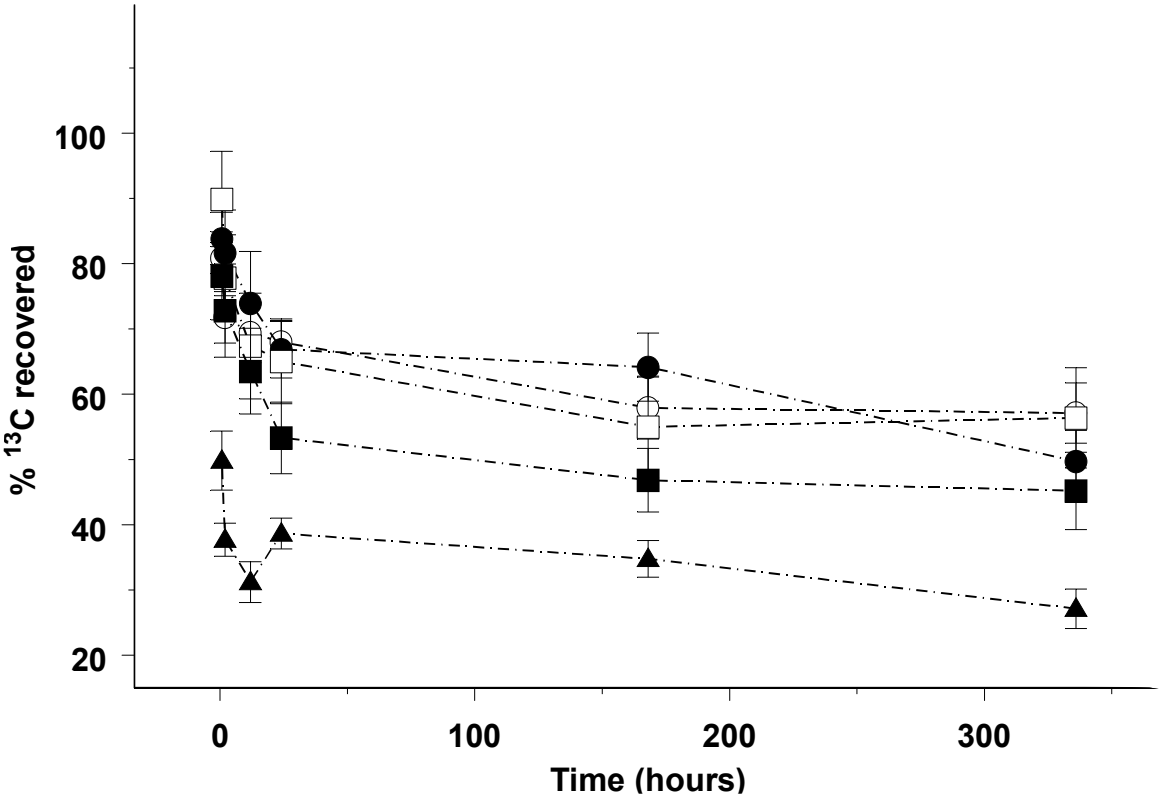


Figure 3.3a

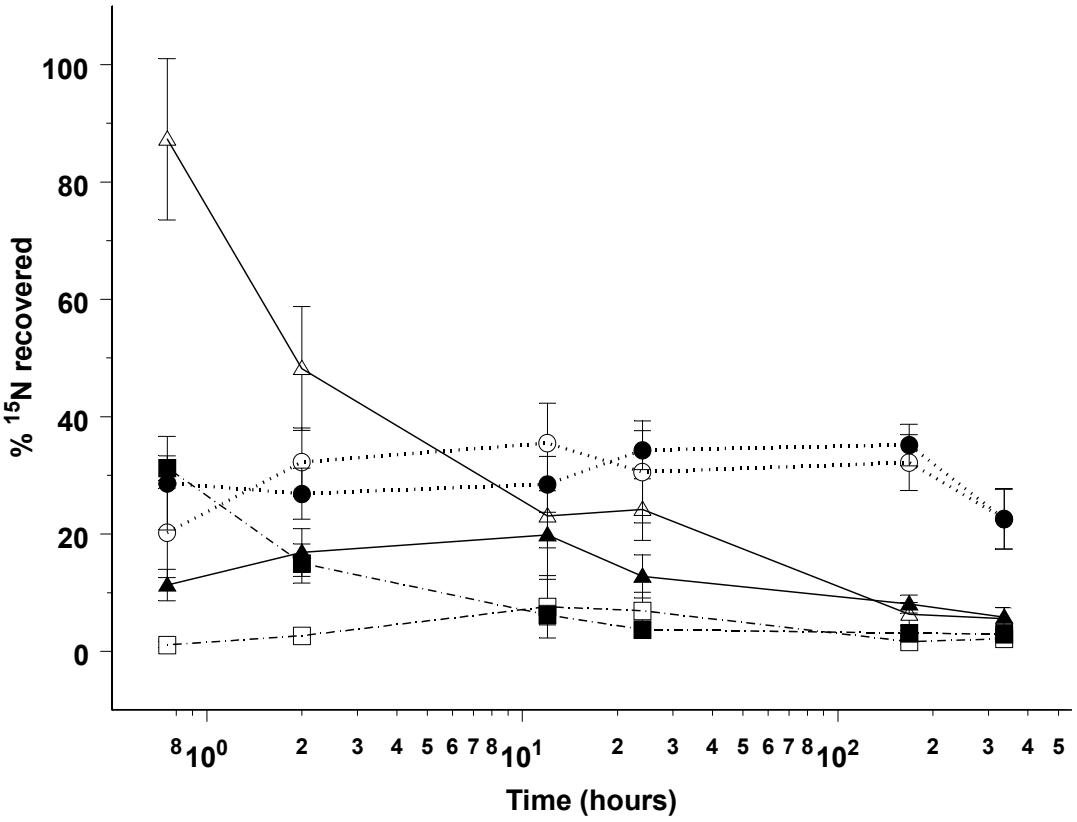

100
80
60
40
20
0
% 15N recovered
8 10^0 2 3 4 5 6 7 8 10^1 2 3 4 5 6 7 8 10^2 2 3 4 5
Time (hours)

Figure 3.3b

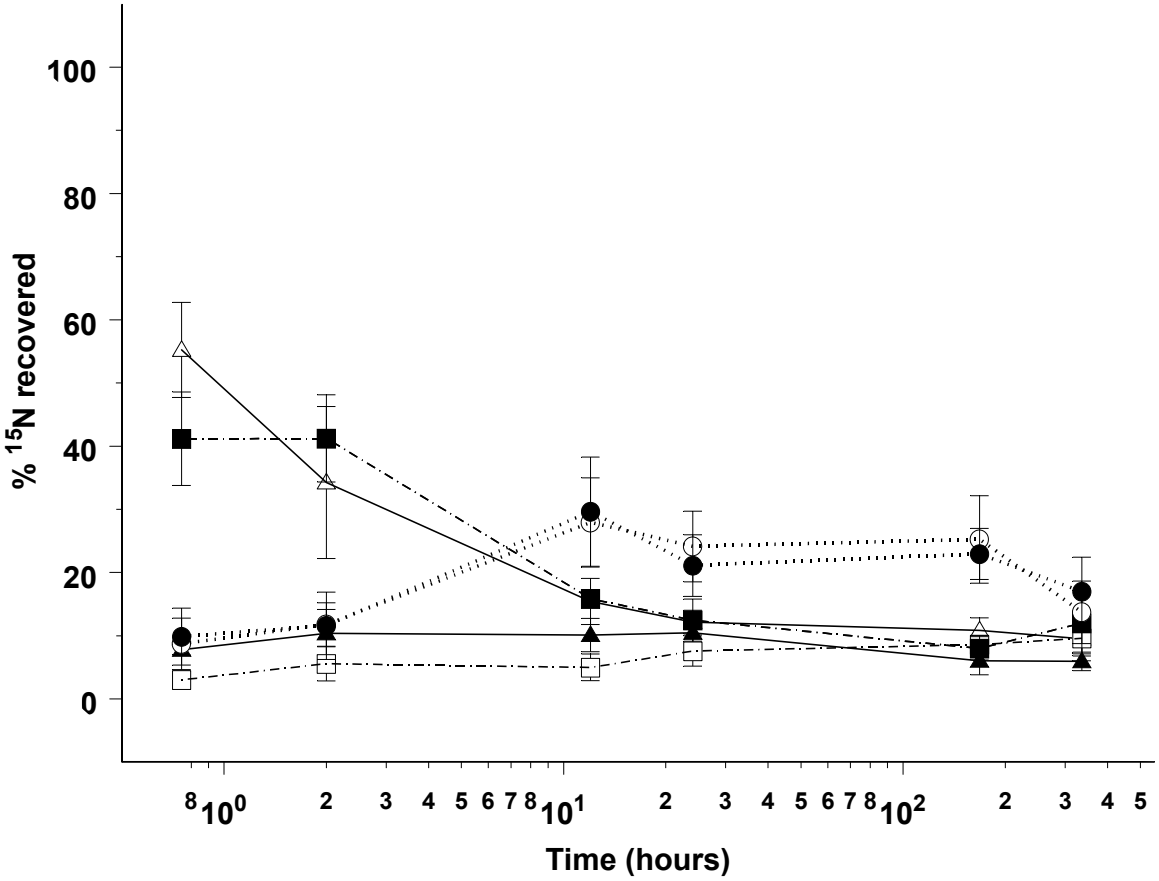

100
80
60
40
20
0
% 15N recovered
8 10^0 2 3 4 5 6 7 8 10^1 2 3 4 5 6 7 8 10^2 2 3 4 5
Time (hours)

Figure 3.3c

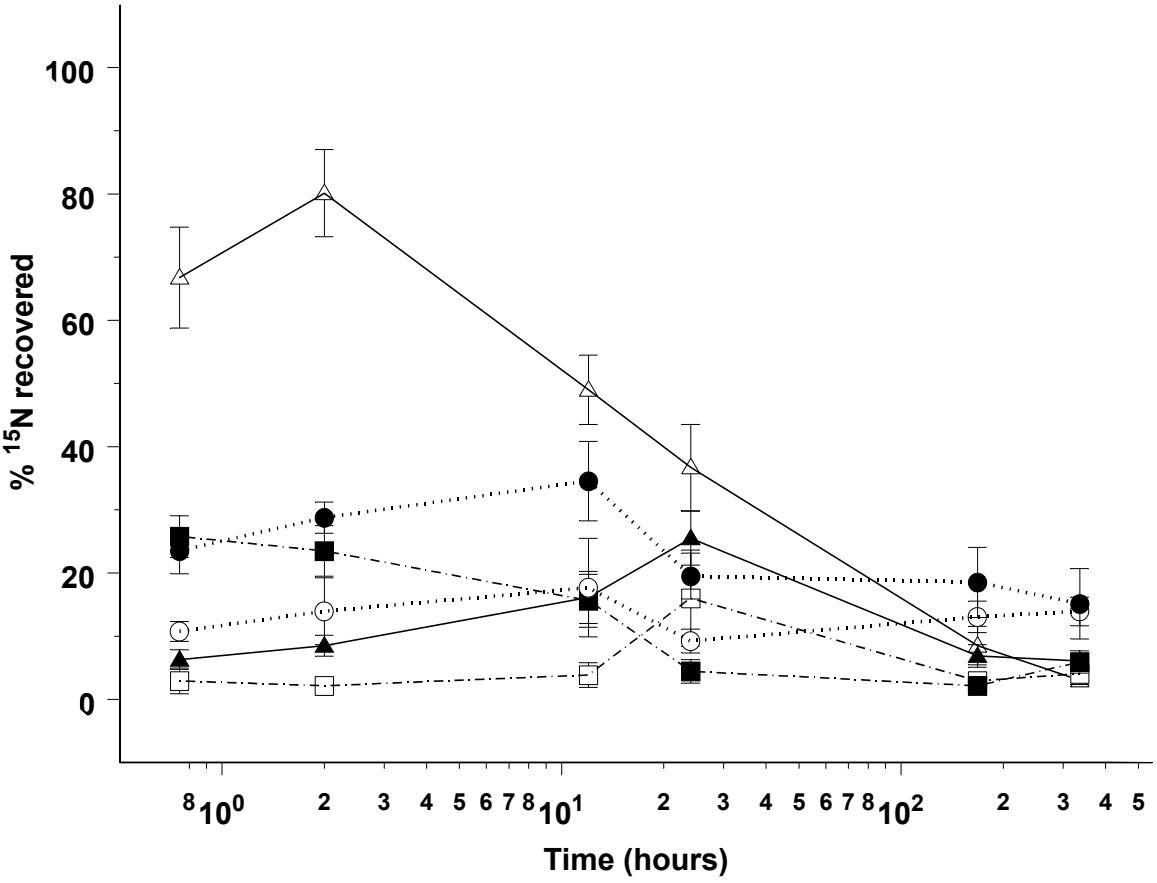

100
80
60
40
20
0
% 15N recovered
8 10^0 2 3 4 5 6 7 8 10^1 2 3 4 5 6 7 8 10^2 2 3 4 5
Time (hours)

Figure 3.3d

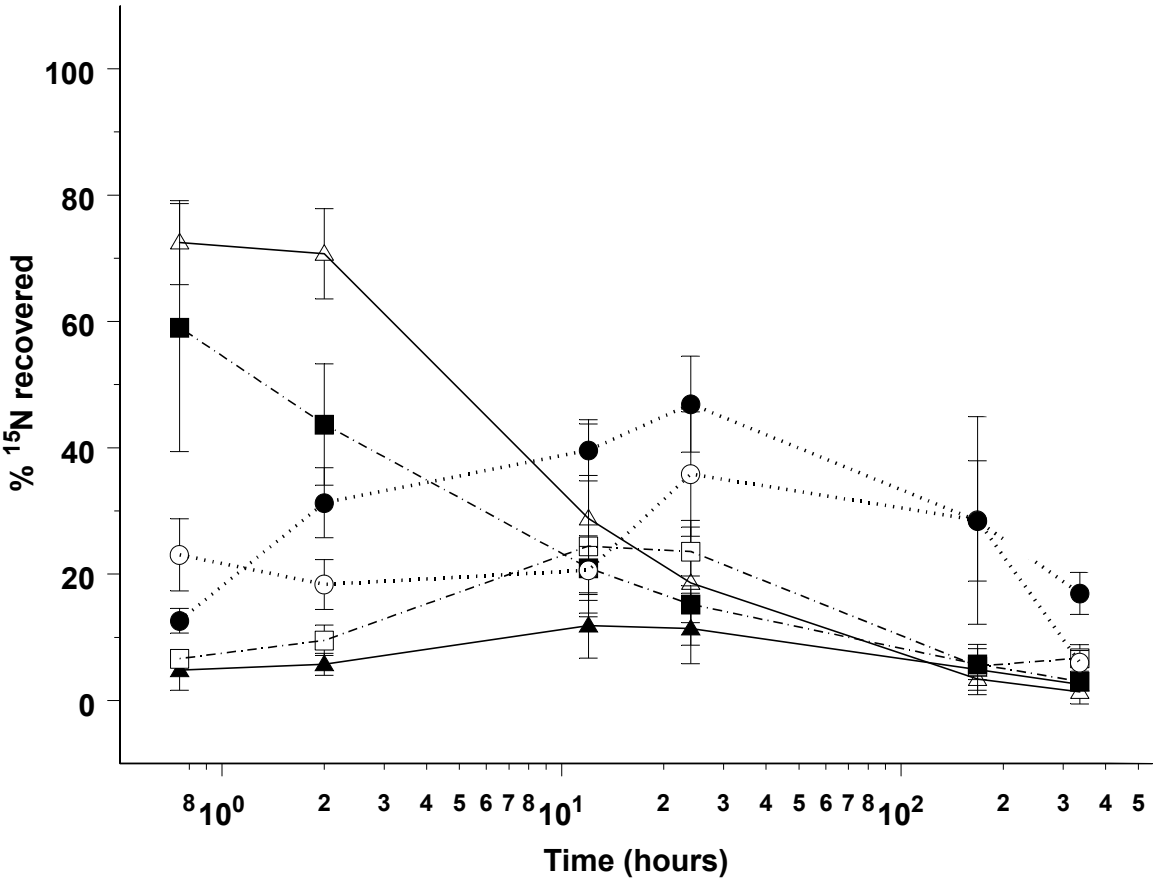

100
80
60
40
20
0
% 15N recovered
8 10^0 2 3 4 5 6 7 8 10^1 2 3 4 5 6 7 8 10^2 2 3 4 5
Time (hours)

Figure 3.3e

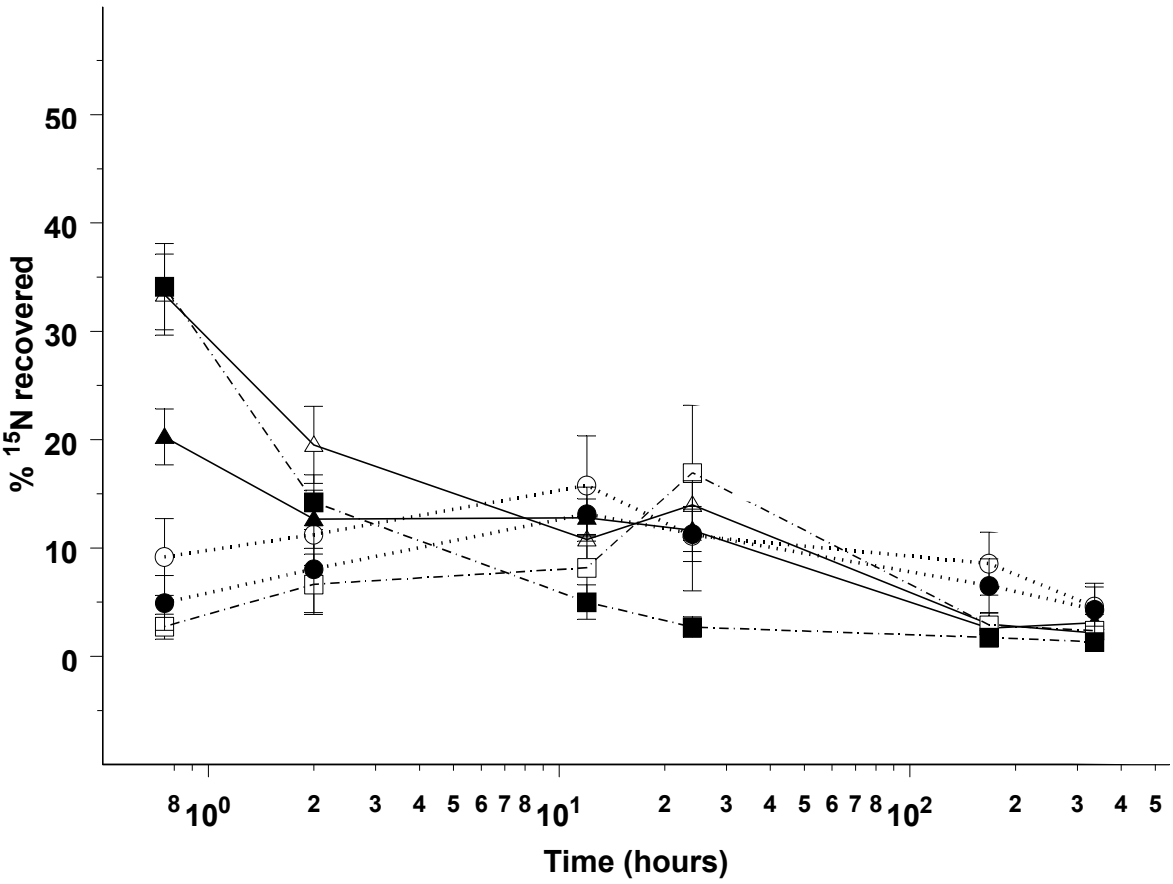

% 15N recovered
50
40
30
20
10
0
8 10^0 2 3 4 5 6 7 8 10^1 2 3 4 5 6 7 8 10^2 2 3 4 5
Time (hours)

Figure 3.4a

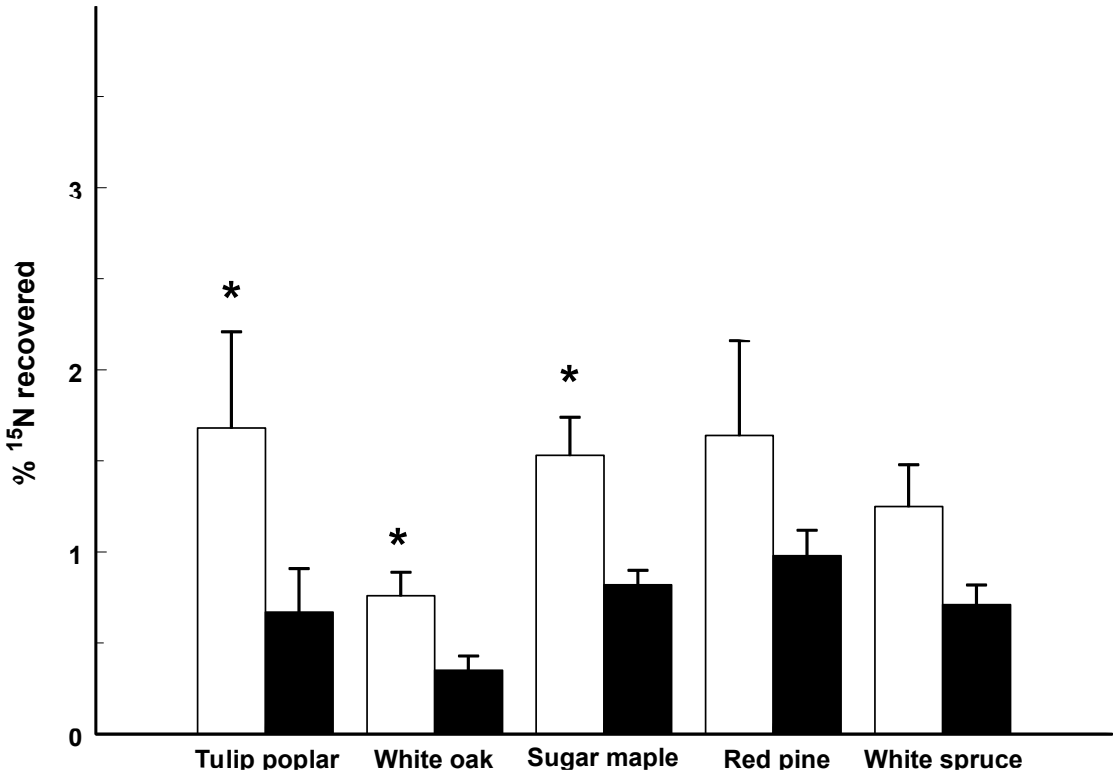

% 15N recovered
0
1
2
3
*
*
*
Tulip poplar
White oak
Sugar maple
Red pine
White spruce

Figure 3.4b

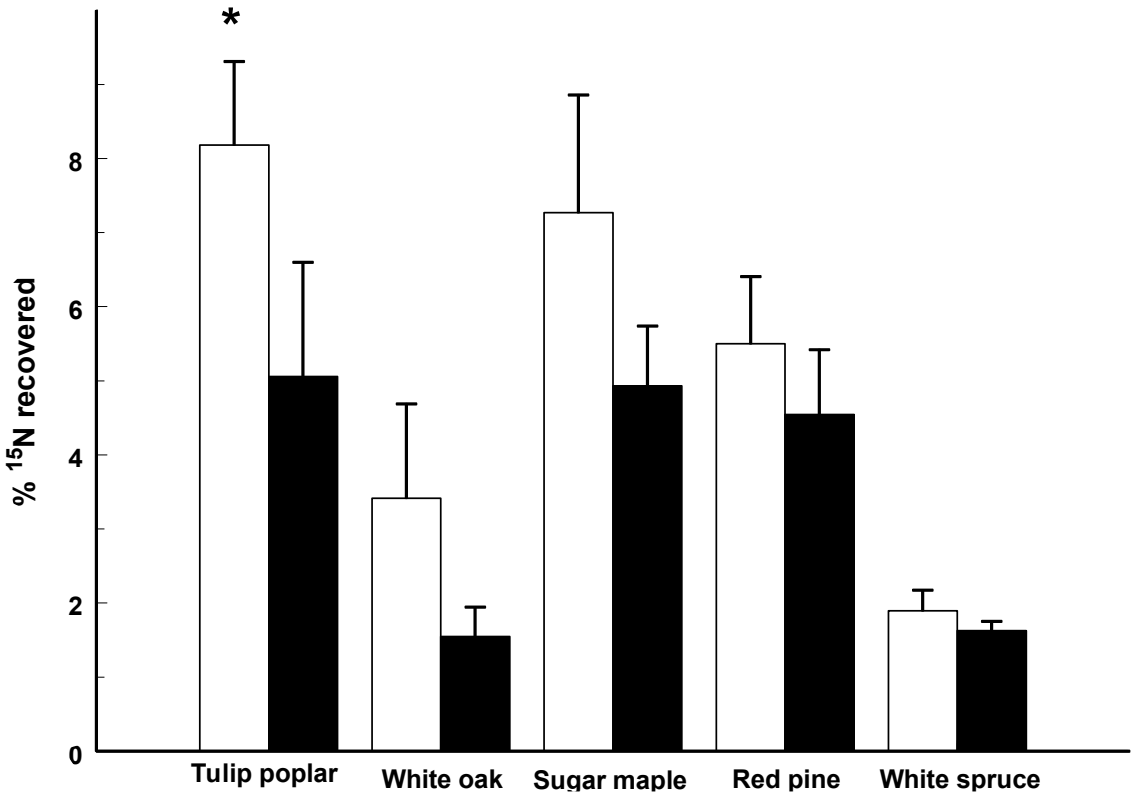

*
8
6
4
2
0
% 15N recovered
Tulip poplar
White oak
Sugar maple
Red pine
White spruce

Figure 3.5

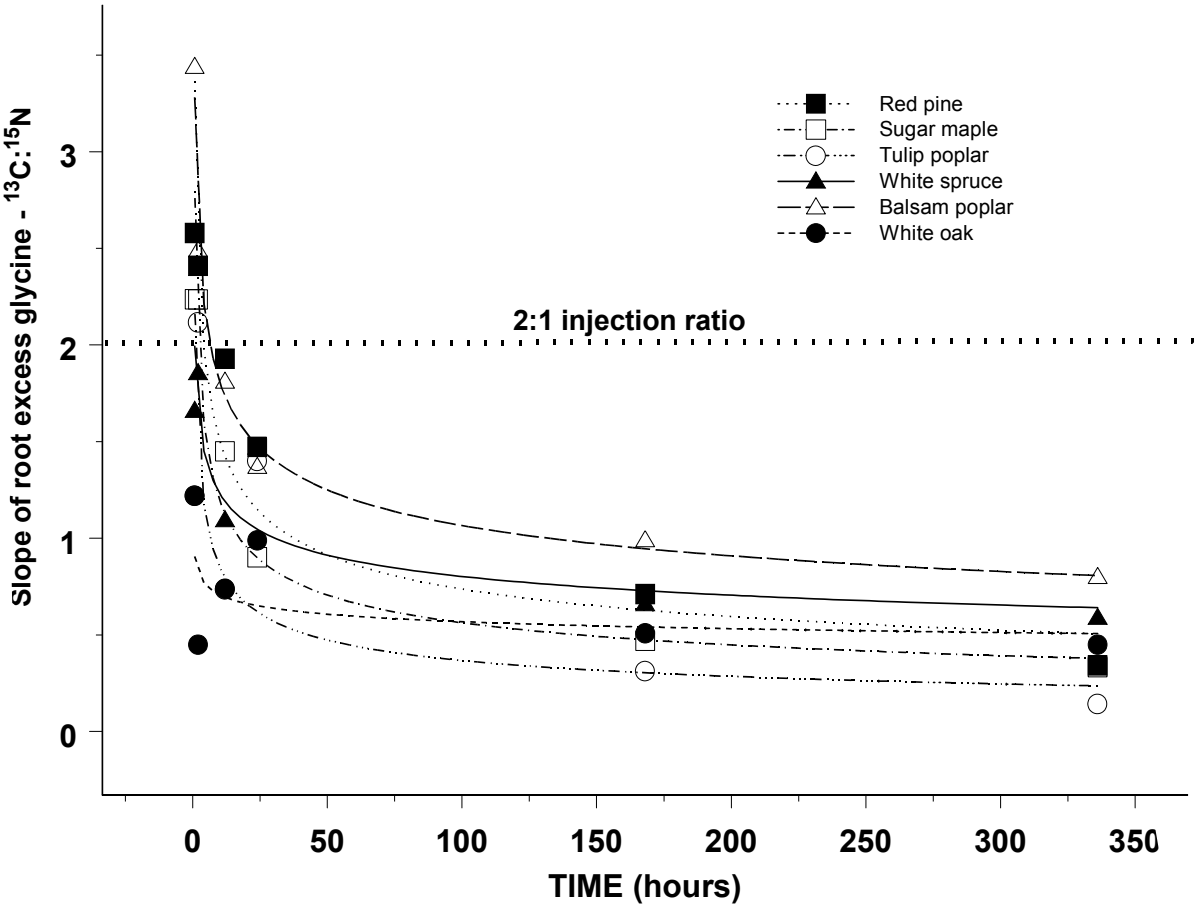
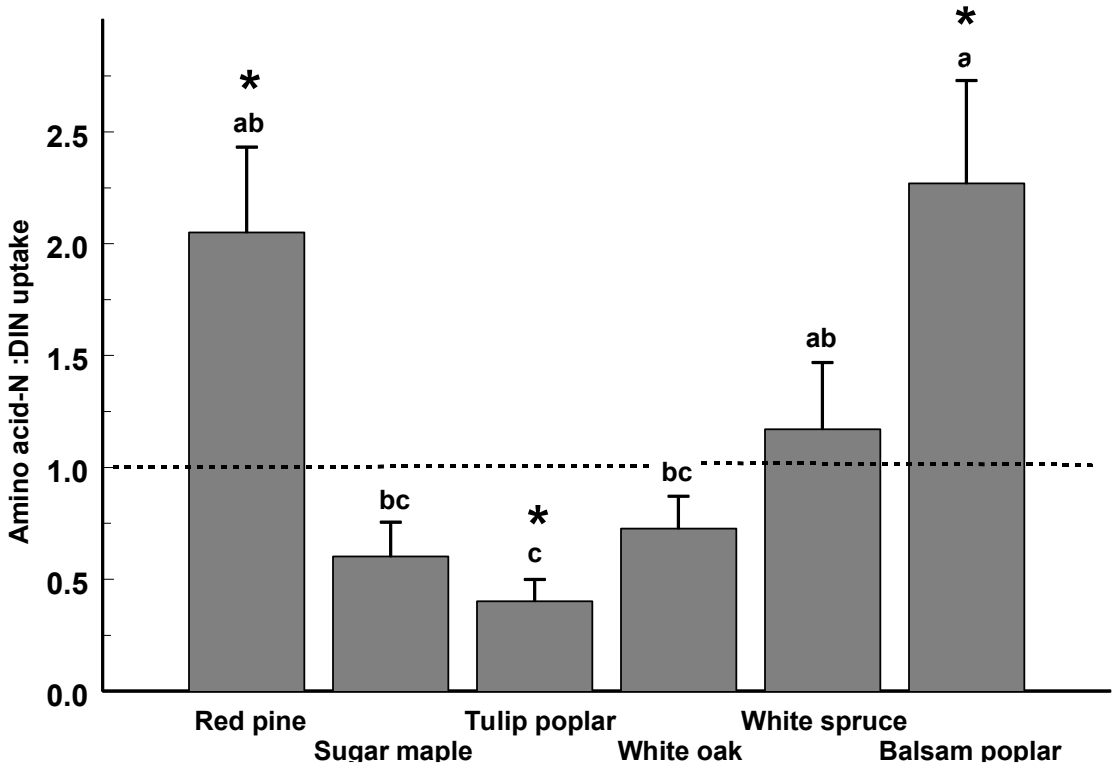

Red pine
Sugar maple
Tulip poplar
White spruce
Balsam poplar
White oak
2:1 injection ratio
Slope of root excess glycine - $^{13}C:^{15}N$
TIME (hours)
0
1
2
3
0
50
100
150
200
250
300
350

Figure 3.6


Amino acid-N :DIN uptake
0.0
0.5
1.0
1.5
2.0
2.5
*
ab
bc
*
c
bc
ab
*
a
Red pine
Sugar maple
Tulip poplar
White oak
White spruce
Balsam poplar

GENERAL CONCLUSIONS

Conventional studies of terrestrial N cycling have focused almost exclusively on inorganic N uptake as the primary source of N for plant metabolism. This characterization has become universal to most regional and global models predicting forest ecosystem response to changing climate or altered land-use patterns (Bonan 1990, Running et al. 1993, Melillo et al. 1993) despite evidence that direct uptake of organic N by plants or the indirect access to various forms of organic N via mycorrhizal connections may constitute a large proportion of total plant N uptake in some ecosystems. The research presented in this dissertation was designed to quantify rates of organic vs. inorganic N cycling and uptake by vegetation across a range of temperate and boreal forest ecosystems. There were two central hypotheses at the inception of this research. First, plant preference for soil organic N is linked directly to soil organic matter quality and inversely correlated with rates of inorganic N mineralization. Second, mycorrhizal type plays an important role in determining the relative importance of organic vs. inorganic N turnover to plant N nutrition. These hypotheses were developed under the assumption that net mineral N availability generally increases with increasing rates of SOM turnover. The results and conclusions generated from this cross-site characterization of soil amino acid cycling and plant N uptake are summarized below.

In the first study, $^{13}C^{15}N$-glycine and $^{15}NH_4^+$ were directly injected into the soil environment of 3 floodplain balsam poplar stands in interior Alaska to track microbial and plant utilization of labile organic and inorganic N *in situ*. Short term patterns (<24 h) of recovered ^{15}N indicated that soil microorganisms represented the largest biotic sink for

both N forms. Immobilization of the ^{15}N tracers within microbial biomass N (MBN) pools ranged from 12 to 64% of experimental additions depending on treatment and sampling period. Subsequent sampling at 7 and 14 days following injection revealed that microbial N turnover had transferred the majority of immobilized ^{15}N to non-extractable soil pools. In contrast, total recovery of ^{15}N in fine root biomass was small, averaging < 2% after 14 days for both glycine and NH_4^+. Additionally, regression analysis of fine root excess ^{13}C vs. ^{15}N demonstrated that plants were taking up glycine intact, though direct uptake was limited to the early hours of the experiment. Consecutive sampling revealed decreasing ^{13}C:^{15}N ratios in fine roots, pointing to increased consumption of mineralized ^{15}N by plants in later periods. However, the fact that plants in this boreal forest ecosystem initially (0-12 h) absorbed glycine and NH_4^+ at approximately the same rate suggested that, 1) plants are targeting the same N resources as the soil microbiota, and 2) amino acids represent an important component of the N economy in boreal balsam poplar forests as predicted.

Based on these results, the study was expanded to include boreal white spruce stands (EM), as well as temperate ecosystems with both AM- and EM-dominated forest types (tulip poplar, AM; white oak, EM; sugar maple, AM; red pine, EM). In general, patterns of plant and microbial utilization of NH_4^+ and amino acid-N were similar to those observed for balsam poplar forests. First, microbial immobilization again represented the largest short-term biotic sink for both N forms. Second, long-term measurements of MBN turnover indicated that the majority of the ^{15}N tracers were ultimately transferred to stabile soil N pools. Third, plant uptake of ^{15}N, though small, increased throughout the

experimental period for both N forms and all forest types demonstrated some capacity to directly absorb amino acids based on linear regressions of excess fine root ^{13}C:^{15}N. However, there were deviations in the cycling patterns for glycine and NH_4^+ for some forests types that warrant further consideration. For example, in sugar maple and tulip poplar, microbial immobilization was 41-56% higher for glycine than NH_4^+ at the first sampling period. In contrast, immobilization was similar for the two N forms for the other forest types. Similarly, the transformation (presumably microbial) of glycine-N to dissolved inorganic N (DIN) was higher than the conversion of NH_4^+ to dissolved organic N (DON) under sugar maple and tulip poplar. Combined these results indicated that soil microorganisms in the two AM-dominated stands were utilizing glycine primarily as a C source.

Recovery of ^{13}C in bulk soil declined significantly at all sites throughout the experiment concomitant with a measurable release of $^{13}CO_2$ to the atmosphere above each injection area, signifying that the soil heterotrophic complex was using glycine-C for metabolism. *In situ* turnover rates for glycine were estimated for each forest type by fitting rate equations to the $^{13}CO_2$ efflux data. The most rapid rates were noted in the two AM stands, tulip poplar and sugar maple while the slowest rates were noted in the EM-dominated coniferous stands. Moreover, rate constants for glycine *in situ* correlated inversely with soil C:N ratios, or in other words, forests with higher soil C:N had longer residence times for glycine. These results suggested that the cycling dynamics of amino acids was linked to soil substrate quality, but patterns of amino acid turnover did not fit

predictions that cycling rates would be higher in forests presumed to have lower SOM quality (white spruce, red pine, or white oak).

Results from a laboratory soil incubation study helped resolve the disparity between predictions concerning amino acid turnover and perceptions of SOM quality among sites. Mineralizable C pools were highest for the Alaskan sites and decreased with latitude, largely due to differences in SOM quantity between boreal and temperate stands. However, when mineralizable C was adjusted for soil C content, pools of labile C were high for stands producing relatively low quality (high lignin, high C:N) litter. In opposition to expectations, the decomposability of soil C was higher for coniferous red pine than, for example, deciduous tulip poplar. Moreover, rates constants for glycine mineralization *in situ* and net C mineralization *in vitro* were linearly correlated across forest types, but measures of microbial biomass were not. These results suggest that cross-site variation soil amino acid turnover is driven by differences in heterotrophic consumption.

This has important implications with respect to plant N nutrition in terrestrial forest ecosystems. If microbial consumption of amino acids for some ecosystems is regulated more by C availability than N-limitation to microbial growth, then competition for labile organic N would presumably be higher in forests where labile C availability is low. Consequently the N economies of plants in those forests should rely more upon net N mineralization to sustain primary production. Data from this study fit that presumption. When plant ^{15}N uptake was adjusted for cross-site availability of DIN and soil FAA, forest type explained a significant proportion of the variation in plant preference for

inorganic vs. organic N. More importantly, sites dominated by EM fungi (white spruce, balsam poplar, red pine and white oak) demonstrated a three fold higher rate of amino acid:DIN uptake than sites dominated by AM fungi (sugar maple and tulip poplar). Assuming that amino acid utilization is higher for EM- than AM-dominated forest ecosystems, what is the mechanism underlying this characteristic?

There is emerging evidence that mycorrhizae and ecosystem function are tightly correlated. For example, mycorrhizal fungi can influence belowground litter quality by regulating the availability and turnover of soil C (Langley and Hungate 2003). Both EM and AM hyphae can comprise a substantial portion of soil microbial biomass, but their respective effect on rhizosphere processes differ substantially. EM fungal associations have the potential to reduce both the size and activity of bacterial biomass in the mycorrhizosphere by channeling plant C into recalcitrant EM structures rather than labile exudates (Olsson et al. 1996a). In contrast AM roots are known to promote both the composition and activity of rhizobacteria through root or fungal exudation into mycorrhizospheric soil (Olsson et al. 1996b, Andrade et al. 1997). Carbon-use efficiency is reportedly higher for fungi than bacteria (Hodge et al. 2000, Six et al. 2006). Therefore, if AM associations tend to enrich bacterial flora while EM associations render the rhizosphere and surrounding soil less hospitable to bacterial growth, the predominance of one mycorrhizal type over the other, among the forest types used in this study, could partially explain differences in turnover dynamics for soil free amino acids among these stands as well as plant preference for inorganic vs. organic N.

In terrestrial ecosystems soil microorganisms play a critical role in structuring N cycling dynamics, both as mediators of plant N availability as well as processes regulating ecosystem N retention. The research presented in this dissertation supports that role of microbes in ecosystem function; however, the notion that mycorrhizal plants also exert an influence on N pools and fluxes cannot be discounted. Feedback effects of plant litter inputs to decomposition and nutrient cycling have been recognized for some time (Melin 1930), but only recently have ecologists started to appreciate belowground controls that the plant-complex can exert on N cycling. While the results discussed here do not demonstrate the direct effect of mycorrhizal type on N cycling and plant N uptake in terrestrial forest ecosystems, they do suggest that plants and associate fungal symbionts probably have greater dominion over their N nutrition than previously thought.

LITERATURE CITED

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and Soil 192:71-79.

Bonan, G.B. 1990. Carbon and nitrogen cycling in North American boreal forests. I. Litter quality and soil thermal effects in interior Alaska. Biogeochemistry 10:1-28.

Hodge A, Robinson D, Fitter A. 2000. Are microorganisms more effective than plants at competing for nitrogen? Trends in Plant Science 5:304-308.

Langley JA, Hungate BA. 2003. Mycorrhizal controls on belowground litter quality. Ecology 84:2302-2312

Melillo JM, McGuire AD, Kicklighter DW, Moore B III, Vorosmarty CJ, Schloss AL. 1993. Global climate change and terrestrial net primary production. Nature 363:234-240.

Melin E. 1930. Biological decomposition of some types of litter from North American forests. Ecology 11:72-101

Olsson PA, Chalot M, Baath RD, Soderstrom B. 1996a. Ectomycorrhizal mycelia reduce bacterial activity in a sandy soil. FEMS Microbial Letters. 21:77-86

Olsson PA, Baath E, Jakobsen I, Soderstrom B. 1996b. Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. Soil biology and Biochemistry 28:463-470

Running SW, Hunt RE. 1993. Generalization of forest ecosystem process model for other biomes, BIOME-BGC, and an application for global-scale models. In Scaling physiological processes: leaf to globe. Eds. J.R. Ehleringer and C.B. Field. Academic Press, San Diego, CA, USA. Pp 141-158.

Six J, Frey SD, Thiet RK, Batten KM. 2006.Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70:555-569.