Biogeochemistry (2009) 92:201–215
DOI 10.1007/s10533-009-9284-1

Soil amino-acid availability across a temperate-forest fertility gradient

David E. Rothstein

Received: 13 May 2008 / Accepted: 2 January 2009 / Published online: 24 January 2009

Abstract Despite increasing recognition that free amino acids can be an important source of N for plant uptake, we have a poor understanding of environmental variation in the availability of amino-acid N in soils outside of arctic, alpine and boreal regions. I investigated patterns of amino-acid availability along a temperate forest fertility gradient ranging from low mineral N availability, oak-dominated forests to high mineral N availability, maple-basswood forests (5 sites). I measured standing pools of free amino acids, soluble peptides, ammonium and nitrate, rates of amino acid production (native proteolysis activity) and rates of consumption of a ^{15}N-labeled leucine tracer. Standing pools of amino acid N decreased consistently along the fertility gradient from the low fertility black oak/white oak system to the high fertility sugar maple/basswood system, with a 25-fold difference in pool sizes between the poorest and richest sites. Standing pools of soluble peptides varied little among sites, instead, the relationship between free amino acids and peptides changed markedly across the gradient. At low fertility sites free amino acids were positively correlated with soluble peptides, whereas free amino acid pools were universally low at high fertility sites, regardless of peptide pools. Assays for native proteolysis activity indicated that amino acid production did not vary significantly among sites. Recovery of leucine tracer in inorganic (NH_4^+ and NO_3^-) pools and in residual soil organic matter both increased with increasing soil fertility; however, total consumption of the added amino-acid tracer did not vary among sites. Results from this study demonstrate that free amino acids can make an important contribution to potentially plant-available N pools in temperate forest soils, particularly at low fertility sites.

Keywords Soil amino acids · Organic nitrogen · Nitrogen availability · Proteolysis · Temperate forest soils

D. E. Rothstein (✉)
Forestry Department, Michigan State University, 126 Natural Resources, East Lansing, MI 48824-1222, USA
e-mail: rothste2@msu.edu

Introduction

The traditional view of nitrogen (N) cycling in terrestrial ecosystems has focused almost exclusively on the production, consumption, and loss of inorganic N. Plants have been widely assumed to be unable to acquire organic N and to be poor competitors for inorganic N relative to soil microorganisms. As a result, only amounts of ammonium (NH_4^+) and nitrate (NO_3^-) in excess of microbial demand were thought to be available for plant uptake, and the net production of inorganic N in the absence of live roots (net N mineralization) was considered an adequate metric of plant available N (reviewed by Schimel and Bennett 2004). Over the last 10–15 years a new

paradigm of terrestrial N cycling has begun to emerge which recognizes the potential for plants to acquire N from organic sources (Chapin et al. 1993; Lipson and Näsholm 2001; Schimel and Bennett 2004). In particular, a series of studies have documented that plants can take up free amino acids at rates that equal or exceed those for inorganic forms of N (e.g. Chapin et al. 1993; Kielland 1994; Raab et al. 1996, 1999; Näsholm et al. 1998). Decades of study of soil N cycling processes have resulted in well-developed conceptual models for understanding how plant–soil–microorganism feedbacks drive environmental variation in inorganic N availability (Hobbie 1992; Aerts and Chapin 2000). In contrast, we are just beginning to describe environmental variation in amino acid availability, and conceptual models describing amino acid N availability remain largely untested.

Early attempts to incorporate organic N cycling into conceptual models of terrestrial N cycling have viewed amino acids as making substantial contributions to plant-available pools of N only in soils from very low fertility sites (Aber and Melillo 2001; Schimel and Bennett 2004). Both Aber and Melillo (2001) and Schimel and Bennett (2004) propose versions of a general model in which the dominant form of plant-available N shifts from amino acids to NH_4^+ to NO_3^- along a low to high N availability gradient (Fig. 1). The mechanistic basis for this

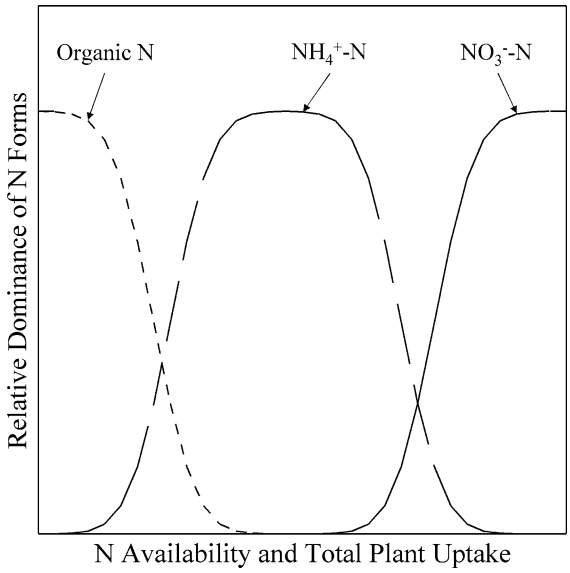

Fig. 1 Hypothesized shifts in the dominant form of available N along a hypothetical gradient of N availability. Adapted from Aber and Melillo (2001) and Schimel and Bennet (2004)

model rests primarily on microbial demand for N relative to overall N status of the ecosystem: in very N-poor soils microorganisms incorporate amino acid N into biomass rather than mineralizing it, at intermediate N status mineralization ensues but competition for NH_4^+ is strong enough to prevent net nitrification, and in the most N-rich soils competition for NH_4^+ is low enough to support substantial net nitrification (Schimel and Bennett 2004).

While this model fits well with existing data on soil amino acid availability, it is also clear that most of the intensive studies of amino acid availability and plant uptake have been conducted in arctic, boreal and alpine ecosystems (e.g. Chapin et al. 1993; Kielland 1994; Näsholm et al. 1998; Lipson et al. 2001; Jones and Kielland 2002; McFarland et al. 2002; Weintraub and Schimel 2005; Kielland et al. 2007) where N mineralization is constrained by cold temperatures. In contrast, very little attention has been given to amino acid cycling in temperate regions where N mineralization is limited by soil mineralogy, disturbance regime and litter quality. In one notable exception, Finzi and Berthrong (2005) quantified potassium-chloride extractable pools of free amino acids, NH_4^+ and NO_3^- from soils of two low-fertility and one high-fertility deciduous forest in Connecticut, USA. Their results were generally consistent with the model of shifting dominance from organic to inorganic forms; however, they did find that free amino acid N remained an important component of available N pools at the N-rich site (23%).

In order to advance our knowledge of amino acid cycling in temperate forest ecosystems we also need to understand underlying mechanisms controlling the availability of these molecules in soil. A priori, we know that availability of free amino acids is likely to result from the balance between productive processes (proteolysis, exudation by roots and microbes, and leaching of amino acids from organic materials) and consumptive processes (i.e. assimilation by microorganisms and plants, mineralization by microorganisms, and sorption to soil colloids). The depolymerization of proteins and peptides by extracellular proteolytic enzymes is thought to be the most important process by which free amino acids are produced in soil (Lipson and Näsholm 2001; Schimel and Bennett 2004). Jones (1999) has argued strongly that rapid microbial consumption and/or mineralization of free amino acids are a dominant force keeping soil solution concentrations

low, and, along with several colleagues, has demonstrated substantial potential for microbial biodegradation of amino acids in a variety of soils (Jones 1999; Jones and Hodge 1999; Jones and Kielland 2002; Jones et al. 2005).

We have almost no data on either the composition or seasonal variability of free amino acid pools in temperate forest soils. Variability in amino acid composition among ecosystems could have important implications for plant utilization of this N form, as different amino acid species are not all equally suitable for either plant uptake or microbial metabolism. Glycine, in particular is thought to be not well utilized by microorganisms, but highly suitable as a source of N for plants (Lipson et al. 1999; Raab et al. 1999). Finally, an understanding of any seasonal variations in amino acid availability is essential to evaluate their potential to serve as a source of plant-available N in temperate forest ecosystems. To add to our understanding of organic N cycling in temperate forests, I investigated the production, consumption and availability of free amino acids in soils across a well-studied landscape fertility gradient in northern Lower Michigan, USA. This gradient ranges from oak-dominated forests on coarse, sandy outwash deposits to maple-basswood forests on finer-textured morainal landforms. I used this system to address the following questions:

1. Do standing pools of free amino acids decrease from low to high fertility sites?
2. Does the composition of free amino acids change across the fertility gradient?
3. What are the seasonal patterns of free amino acid availability, and do they differ across the fertility gradient?
4. Does the dominant form of plant-available N shift from amino acids to NH_4^+ to NO_3^- with increasing fertility?
5. To what degree do productive versus consumptive processes explain spatial patterns in free amino acid pools?

Materials and methods

Site selection and characterization

I studied soil amino acid dynamics along a five-site fertility gradient in the Manistee National Forest in northwestern Lower Michigan, USA. The five study sites were selected from a pool of 56 forest stands originally described as part of an ecosystem classification study conducted in the mid 1980s (Host et al. 1988). Upland forest ecosystems of this area were classified into nine ecosystem types using an integrated multifactor approach incorporating glacial landform, soils, overstory vegetation and ground flora indicator species. Numerous follow-on studies demonstrated that these ecosystem types varied systematically in inorganic N cycling and productivity with oak-dominated forests on coarse sands exhibiting low N mineralization with negligible NO_3^- production, maple-oak forests on finer-textured soils exhibiting rapid N mineralization with little NO_3^- production, and maple-basswood stands on finer-textured soils exhibiting rapid N cycling with high NO_3^- production (Zak et al. 1986, 1989; Holmes and Zak 1994, 1999). This gradient appears to be representative of associations between soil properties, species composition, inorganic N availability, and productivity throughout the Upper Midwest (Pastor et al. 1984; Reich et al. 1997; Morris and Boerner 1998). The five stands for this study were selected systematically from the pool of 56 original stands in order to span the range of soil, vegetation and inorganic N cycling rates described in previous studies (Table 1).

In late March 2005, I selected study areas within each stand on areas of uniform terrain, with no evidence of recent disturbance, and as far as possible from roads or vegetative or physiographic boundaries. Within each study area, I located a 100-m × 40-m rectangular plot which was used both for initial site characterization and all subsequent soil sampling. All trees >5-cm diameter in each plot were inventoried for species identity and diameter at breast height (DBH). On April 24, 2005 I collected five soil cores (5.08 cm diameter × 15 cm depth) per plot, with one core located randomly within each 20-m interval along the long axis of the plot. These samples were used for initial soil characterization including: soil pH ($CaCl_2$), total carbon (C) and N (Carlo Erba NA 1500 elemental analyzer), potential N mineralization and nitrification (28-days lab incubation) and microbial biomass (chloroform-fumigation extraction). Additional soil samples were collected with a bucket augur to a depth of 2 m at two points within each plot, and these samples were used for particle size analysis. Silt and clay content were determined using the Bouyocous hydrometer method and the fine sand

Table 1 Landform, soil, vegetation and ecosystem characteristics of the five study sites

	Site 1	Site 2	Site 3	Site 4	Site 5
Location					
North latitude	44.24	44.28	44.19	44.25	44.23
West longitude	85.94	85.90	85.67	85.76	85.74
Vegetation characteristics					
Stand age (2005)	74	85	83	104	97
Stand basal area (m^2/ha)	21.2	29.7	32.6	33.5	36.1
% Oak (*Q. rubra*, *Q. velutina*, *Q. alba*)	99	83	54	15	0
% Maple (*A. saccharum*, *A. rubrum*)	0	17	46	41	66
% Basswood	0	0	0	19	22
Leaf litterfall (g/m^2)	313	444	533	509	491
Leaf litterfall N (g/m^2)	2.4	3.1	4.6	4.5	4.7
Leaf litterfall C:N ratio	67	65	53	48	45
Soil characteristics					
Bulk density (g/cm^3)[a]	1.16	1.06	1.17	1.01	1.08
Soil pH ($CaCl_2$)[a]	4.4	4.2	4.2	4.5	5.0
Silt + clay content (%)[b]	2.5	5.5	5.0	5.0	8.5
Fine sand content (%)[b]	28	22	36	39	47
Soil N (mg/g)[a]	0.86	0.71	0.61	0.97	2.04
Soil C:N ratio[a]	23.3	25.3	20.6	18.6	14.5
N mineralization (μg N g^{-1} day^{-1})[a,c]	0.61	0.70	0.60	1.04	1.32
Nitrification (μg N g^{-1} day^{-1})[a,c]	0.01	0.01	0.04	0.16	1.10
Microbial biomass C (ug/g)[a,d]	400	580	671	1,037	1,169

[a] 0–15 cm depth

[b] 50–100 cm depth

[c] Potential, net rates from aerobic lab incubations, 2 M KCl

[d] $CHCl_3$-fumigation extraction, correction factor = 0.45

fraction (50–250 μm) was determined by dry sieving. Finally, five 0.37 m^2 littertraps were placed at stratified random points in each stand and leaf litterfall collected every 2 weeks from August 15 through December 1 in both 2005 and 2006. Characteristics of soil and vegetation for all five study sites are summarized in Table 1.

Soil soluble N pools

In order to characterize seasonal and landscape variability in the size and composition of available N pools, I conducted monthly measurements of soluble peptides, free amino acids, NH_4^+ and NO_3^- at all five sites throughout the 2005 growing season. Five soil cores (5.08 cm diameter × 15 cm depth) were collected from stratified random points in each plot on April 24, May 23, June 27, July 27, August 21 and September 25, 2005. Cores were immediately packed on ice for transport to the laboratory where they were kept refrigerated prior to processing. Laboratory processing was blocked by site in order to avoid confounding effects of handling delays and all extractions were completed within 24 h of collection. Each core was passed through a 4-mm sieve to remove rocks, roots and other debris and then homogenized. Eighteen-gram subsamples of fresh soil were weighed into 50-ml polypropylene centrifuge tubes to which 30 ml of 4 mM $CaCl_2$ was added. I chose a dilute $CaCl_2$ solution in order to best approximate the fraction of labile N that could enter soil solution in situ (Zsolnay 2003). Tubes with $CaCl_2$ plus soil were shaken on an end-over-end shaker for 10 min, centrifuged at 182 g for 5 min and the supernatant passed through a 0.4 μm polycarbonate filter and frozen until analysis.

I used a modification of the *o*-phthaldialdehyde (OPA) and *β*-mercaptoethanol (bME) derivation method of Jones et al. (2002) to determine total free amino acids in $CaCl_2$ extracts. This method has been shown to have a lower detection limit and fewer interferences than the traditional ninhydrin assay (Jones et al. 2002). One hundred microliter subsamples of each extract were pipetted into 3 wells of a black 96-well microplate. Each plate also contained a standard curve (in triplicate) of 5, 2, 1, 0.4 0.2 and 0 μM glycine in 0.4 mM $CaCl_2$. Two wells for each

sample or standard received 100 µl of a derivation solution containing 20 µM OPA and 1 mM bME in 0.2 M potassium tetraborate buffer (pH 9.3), while the remaining well served as a negative control and received 100 µl of buffer alone. After a 1-h incubation fluorescence was read on a FLUOstar OPTIMA microplate reader (BMG LABTECH Inc., Durham, NC) set at 355 nm excitation and 440 nm emission wavelengths.

In order to validate the accuracy of the OPA–bME derivation method, as well as to compare amino acid composition among sites, subsamples of 37 $CaCl_2$ extracts (i.e. 12 from April 2005 + 25 from July 2005) were sent to the Macromolecular Structure Facility at Michigan State University for high-performance liquid chromatography (HPLC) analysis. There 1 ml subsamples were dried in a speed-vac concentrator and then brought up in 50 µl of 20 mM HCl. Twenty microliter of the concentrated sample was then derivatized with 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate and run on a 3.9 mm × 150 mm Nova-Pak C18 column using a Nova-Pak C18 guard column. Amino acid derivatives were detected in a Waters model 247 fluorescence detector with an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Twelve of the April, 2005 samples spanning the range of observed concentrations, plus all of the July, 2005 samples, were analyzed by HPLC. I compared HPLC measures of free amino acids and OPA–bME estimates using simple linear regression and found excellent agreement (OPA–bME Concentration (mM) = 1.235 * HPLC Concentration (mM) + 1.481; $r^2 = 0.866$; $P < 0.001$). The positive intercept and slope >1 suggest that the OPA–bME overestimates amino acid concentration relative to HPLC, and is likely explained by two factors: (1) some overestimation of actual free amino acids by the OPA–bME method due to limited reactivity with other compounds in soil extracts (e.g. peptides, NH_4^+, humic acids; Jones et al. 2002), and (2) some underestimation of actual free amino acids by HPLC where less common amino acids occurred at or below the detection limit (50 pM in final solution). In addition to method validation, HPLC data from the July sample date were used to compare the composition of soluble amino acids among sites.

Total amino acid content of each $CaCl_2$ extract was determined by first acid hydrolyzing a 1 ml aliquot (6 N HCl, 120°C; Paul and Williams 2005) and then analyzing the resulting solution for free amino acids using the OPA–bME procedure described above. Soluble peptides were then determined as the difference between total amino acids (hydrolyzed subsample) and free amino acids (unhydrolyzed subsample). I used an average ratio of 1.4 mole N per mole of amino acids to convert free amino acid concentrations to N concentrations. This ratio was calculated using chemical formulae for individual amino acids and molar composition of extractable amino acid pools (HPLC analysis above) averaged across all sites. Ammonium and NO_3^- in $CaCl_2$ extracts were quantified using automated colorimetry on a Flow Solution IV (OI Analytical, College Station, TX). Soluble N concentrations were converted to an area basis using bulk density measures from each replicate core.

Amino acid fluxes

I used laboratory assays of native proteolysis activity in order to quantify the capacity for soils from all five sites to contribute free amino acids to the soluble N pool. This assay measures the accumulation of free amino acids produced by proteolytic enzymes acting on native soil peptides. Native proteolysis assays were conducted on the May, July and September sampling dates in 2005, and again on August, 2006 in conjunction with the amino acid consumption assay (see below). Two-gram subsamples of homogenized fresh soil were weighed into 15-ml polypropylene centrifuge tubes to which 10 ml of 50 mM sodium acetate buffer and 0.4 ml of toluene was added to inhibit microbial uptake of amino acids (Watanabe and Hayano 1995). Two separate batches of acetate buffer were used in order to match differences in soil pH among sites: pH 4.25 for Sites 1–4 and pH 5 for Site 5. At the May and July sample dates in 2005 these slurries were incubated for 12 h at 20°C on an end-over-end shaker. At that point, 2 ml of trichloroacetic acid solution (0.11 M trichloroacetic acid; 0.22 M sodium acetate; 0.33 M acetic acid) was added to terminate the reaction, tubes were spun, filtered and extracts analyzed for free amino acids as described above. Rates of amino acid production were calculated as the difference between incubated and unincubated subsamples. Due to concerns that rates of amino acid production might be non-linear over a 12-h incubation, on the September sample date, I incubated subsamples for 0, 2, 4, 8 and 12 h.

Some samples showed saturation of amino acid accumulation curves beginning at the 8 h point; therefore, the slope from 0 to 4 h was used to calculate production rates at this sample date. A single point, 4-h incubation was used at the August 2006 sample date in order to ensure that sampling was done within the linear range. An additional 4-h incubation was performed at the August, 2006 sample date to estimate potential proteolysis activity. All assay conditions and analyses were the same except that the buffer solutions also contained 0.6% casein in order to alleviate any substrate limitations to proteolysis activity.

Potential amino acid consumption was assayed by adding a ^{15}N-labeled leucine tracer to soils and tracking the flow of tracer into dissolved inorganic N ($NH_4^+ + NO_3^-$; DIN), dissolved organic N (DON) microbial N (MN) and soil organic N (SON) following a 4-h incubation. Leucine was selected as a tracer because it is neutral, non-polar and has been shown to be readily metabolized by soil microorganisms (Lipson et al. 1999) and less strongly sorbed by soil solids compared to amino acids with a positive charge (Gonod et al. 2006). Thus I reasoned that differences in ^{15}N-leucine consumption among sites should primarily reflect differences in microbial demand and activity rather than differences in pH or surface-charge characteristics of the various soils. Five cores per plot were collected in the afternoon of August 14, 2006 and transported on ice to Michigan State University where they were processed the following morning. Initial processing was as described above for monthly sampling in 2005. Twelve-gram subsamples of each soil were weighed into 50 ml polypropylene centrifuge tubes and then injected with 1 ml of a 29 μg leucine–N/ml solution (98 atom.% ^{15}N). Labeling solutions were injected into the soil in five 0.2-ml aliquots, with solution dispensed evenly as the needle was removed from the soil. Soil samples were incubated at 20°C for 4 h after which I utilized a sequential extraction procedure (Holmes et al. 2003) to quantify tracer ^{15}N in DIN, DON, MN, and SON.

Ammonium, NO_3^- and DON were extracted in the first step in which 25 ml of 0.5 M K_2SO_4 was added to each tube. The vials were capped and shaken for 20 min on an end-over-end rotisserie shaker, and then centrifuged at 182 g for 15 min. This was repeated with a second 25-ml aliquot of K_2SO_4 and the two supernatants combined in a 60-ml syringe equipped with a 0.4 μm filter apparatus. Microbial cells and particulate matter were trapped on the filter, which was placed back in the original vial of soil. After removal of inorganic N, tubes containing soils + filters were fumigated with chloroform ($CHCl_3$) for 5 days to liberate microbial-incorporated ^{15}N. Fumigated soils + filters were extracted twice with 0.5 M K_2SO_4 as described above and soil remaining in the vials was dried at 65°C and pulverized in a ball mill for analysis of residual N.

Extracts of unfumigated soil were analyzed for NH_4^+ and NO_3^- using the Flow Solution IV autoanalyzer and then NH_4^+ was diffused onto acid traps for ^{15}N analysis following the methods of Hart et al. (1994). Following NH_4^+ diffusion, Devarda's alloy was added to diffuse NO_3^- onto acid traps via reduction to NH_4^+. After diffusion of NH_4^+ and NO_3^-, DON in a 15 ml subsample of the unfumigated soil extracts was converted to NO_3^- by alkaline persulfate digestion (Cabrera and Beare 1993). DON digests and digests of fumigated soil extracts were analyzed for NO_3^- using the Alpkem autoanalyzer and NO_3^- diffused onto acid traps for ^{15}N analysis. Subsamples of pulverized residual soil were analyzed for total N concentration on a Carlo-Erba NA 1500 elemental analyzer (Carlo-Erba, Milan, Italy). Acid traps and additional subsamples of pulverized residual soil were folded into tin capsules and analyzed for atom.% ^{15}N on a Europa Scientific Tracermass mass spectrometer at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley. Microbial ^{15}N was estimated simply as the amount of ^{15}N excess recovered in the 2nd K_2SO_4 extract following $CHCl_3$ fumigation. I did not apply a correction factor to quantify microbial biomass per se (Brookes et al. 1985), because of concern that no single correction factor could be applied to soils from sites that vary strongly in texture, chemistry and organic matter quality.

Data analysis

I analyzed standing pools of free amino acids, soluble peptides, NH_4^+ and NO_3^- using two-way analysis of variance (ANOVA) with site (5 levels) and sampling date (6 levels) as main effects. Abundance of individual amino acids, native and potential protease activity, and ^{15}N tracer recovery were analyzed using one-way ANOVA with site as the main effect. In cases where there was a main effect of site or date on

a parameter with $P \leq 0.1$, I then used trend contrast analysis to examine patterns of change across the landscape-fertility gradient. I used trend contrast analysis rather than typical pairwise comparisons because I was more interested in patterns of change in individual parameters across the gradient than in differences among individual sites. In this case I was interested in the direction of change (increasing or decreasing) as well as the pattern of change (linear, quadratic, cubic). Because the fertility gradient encompasses variation in multiple ecosystem parameters (Table 1), I made the simplifying assumption for trend contrast analysis of equal intervals between sites. I used simple linear regression to analyze the relationship between pools of free amino acids and soluble peptides across dates, within each site. Values for standing pools of free amino acids, NH_4^+ and NO_3^- were log-transformed for statistical analyses. All statistical analyses were performed using SYSTAT for personal computers. Statistical significance was accepted at $\alpha = 0.05$.

Results

Soil soluble N pools

Changes in standing pools of $CaCl_2$-extractable N forms across the gradient conformed well to theoretical predictions of shifting relative abundance from amino acids to NH_4^+ to NO_3^- with increasing fertility (compare Fig. 1 vs. Fig. 2). Averaged across all sample dates, free amino acid N made up 39%, 31%, 17%, 8% and 2% of the potentially plant-available N forms in Sites 1, 2, 3, 4 and 5 respectively. There was a significant main effect of site on free amino acid pools ($P < 0.001$), as well as a significant linear trend of decreasing amino acid N from low to high fertility sites ($P < 0.001$). Differences in standing pools of free amino acids were quite large, with Site 1 having 25 times the pool size of Site 5 (averaged across seasons). There were also significant main-effects of site on standing pools of NH_4^+ ($P < 0.001$) and NO_3^- ($P < 0.001$). Although changes in NH_4^+ pools across the gradient conformed visually to the hump-shaped pattern predicted by theory (Fig. 1), the quadratic trend was not statistically significant ($P = 0.105$). The pattern of NO_3^- pool size across the gradient was the exact opposite of that for free amino acids, increasing linearly from low to high fertility ($P < 0.001$).

I also observed strong seasonal variation in potentially plant-available N pools (Fig. 2) such that sampling date was a statistically-significant main effect for free amino acids ($P < 0.001$), NH_4^+ ($P < 0.001$) and NO_3^- ($P < 0.001$). Whereas free amino acid concentrations appeared to decrease from spring to summer to autumn, the overall trend was not statistically significant ($P = 0.120$; linear contrast). Contributing to this was a nearly-significant site by sampling date interaction ($P = 0.079$), whereby the seasonal decline in free amino acid pools was greater at low fertility sites then at high fertility sites. For example, from April through September, free amino acid N declined from 136 to 33 mg/m^2 at Site 1 compared to a decline from 4 to 2 mg/m^2 at Site 5. Across all sites, extractable NH_4^+ pools were generally greater in the late summer and autumn compared to spring and early summer, resulting in a significant linear trend ($P = 0.021$) and a nearly-significant quadratic trend ($P = 0.061$). Extractable NO_3^- pools also tended to increase through the growing

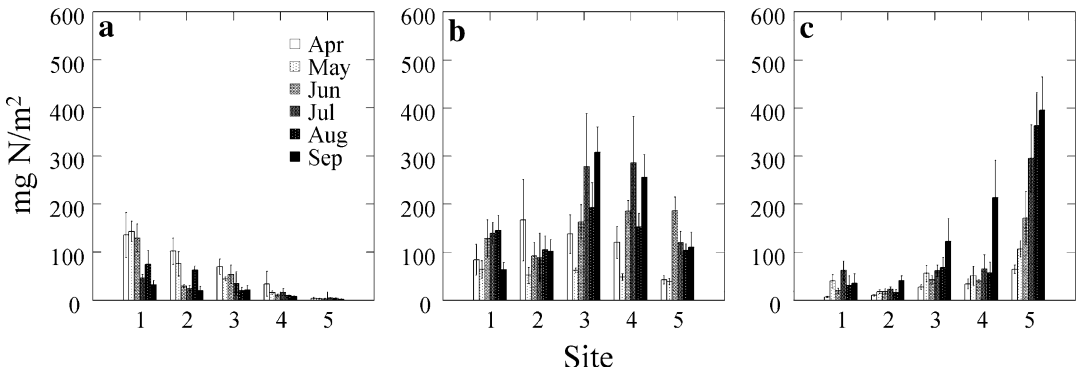

Fig. 2 Standing pools of $CaCl_2$-extractable N forms sampled monthly across the landscape fertility gradient in 2005. Free amino acid N is presented in panel **a**, NH_4^+–N in panel **b**, and NO_3^-–N in panel **c**. Individual bars represent the mean of 5 replicates ±1 SE

season although the linear trend was not statistically significant ($P = 0.054$).

There were significant main effects of site ($P = 0.017$) and sampling date ($P = 0.006$) on soluble peptides; however, there were no significant overall trends across sites ($P = 0.171$; linear) or across dates ($P = 0.610$; linear). The significant main effect of site appeared to be driven largely by lower pools of soluble peptides at the most fertile site (Site 5), with very little difference among the other four sites (Fig. 3). Because soluble peptides are the substrate from which free amino acids are produced through the action of proteolytic enzymes, I also investigated the relationship between soluble peptides and free amino acids in 4 mM $CaCl_2$ extracts. Pooled across all sites and sampling dates free amino acid concentrations increased with increasing soluble peptide concentration, although soluble peptide pool size explained little of the variation in free amino acid pools ($r^2 = 0.249$; $P < 0.001$; Fig. 4a). However, when examined on a site by site basis, it is clear that the nature of the relationship changed markedly, and systematically, among sites along the gradient (Fig. 4b–f). At the lowest fertility site, free amino acid pools were the most strongly correlated with soluble peptide pools ($r^2 = 0.469$). Moving up along the fertility gradient the relationship between free amino acids and soluble peptides weakened to Sites 4 and 5 where free amino acid pools were universally low, despite wide variation in soluble peptide pools.

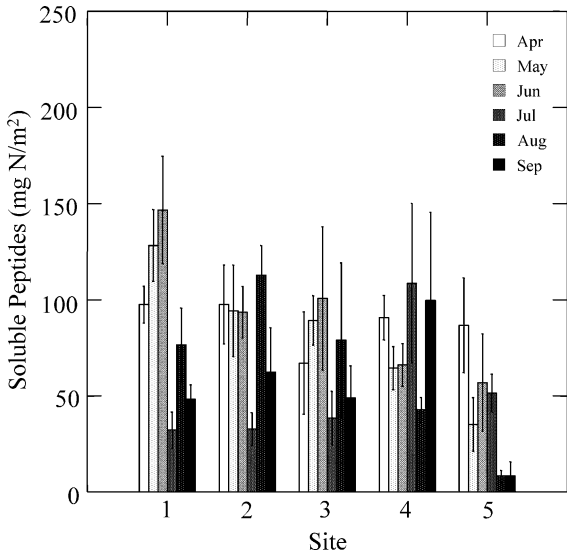

Fig. 3 Standing pools of soluble peptides sampled monthly across the landscape fertility gradient in 2005. Individual bars represent the mean of 5 replicates ±1 SE

On the July sample date. About 16 individual amino acids were present in quantities sufficient for comparison among sites: alanine (Ala), arginine (Arg), asparagine (Asn), aspartate (Asp), glutamine (Gln), glutamate (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), serine (Ser), tyrosine (Tyr) and valine (Val) (Fig. 5). Ala, Asn, Gln, Glu, Gly and Ser were the most abundant individual amino acids, comprising 76% (mole basis) of total free amino acids averaged across all five sites. At this one sample date, I observed systematic variation in amino acid composition across sites, with some notable trends across the fertility gradient. Arg, Asp, Glu, Gly and Lys all showed significant declines in relative abundance from low to high fertility, whereas Ile and Met both increased in relative abundance (Fig. 5). Some of the trends in relative abundance of amino acids across the gradient appeared to be associated with their side chain properties. In general, amino acids with acidic or basic side chains (Arg, Asp, Glu, His, Lys) declined in relative abundance with increasing fertility, whereas amino acids with neutral and non-polar side chains tended to increase in relative abundance. A notable exception to this generalization was the non-polar amino acid glycine which declined markedly in relative abundance from low to high fertility (Fig. 5).

Amino acid fluxes

In contrast to standing pools of free amino acids which declined markedly along the gradient, I could find no apparent pattern of amino acid production among sites as measured by laboratory assays of native proteolysis activity (Fig. 6). In 2005 there was no significant effect of site on proteolysis activity ($P = 0.338$); however, I did observe a significant main effect of sample date ($P < 0.001$) with rates in July and September being consistently lower than rates in May. Note that this pattern cannot be explained by differences in incubation length among dates since the May and July samples were both conducted with 12-h incubations. At a single sample date in 2006, native proteolysis activity appeared to be slightly higher in Sites 1 and 2 (Fig. 6); however, the main effect of site was not statistically significant ($P = 0.106$). Potential

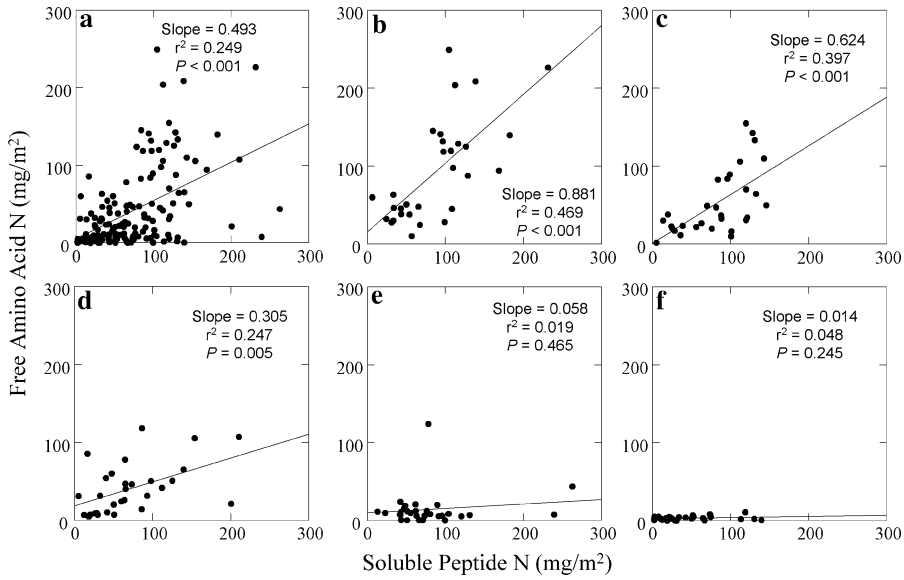

Fig. 4 Pools of soluble free amino acids regressed against those of soluble peptides for all sites together (**a**), Site 1 (**b**), Site 2 (**c**), Site 3 (**d**), Site 4 (**e**) and Site 5 (**f**). Data points are for individual samples and lines and statistics are for best-fit linear regressions ($n = 150$ for panel **a**; $n = 30$ for panels **b**–**f**)

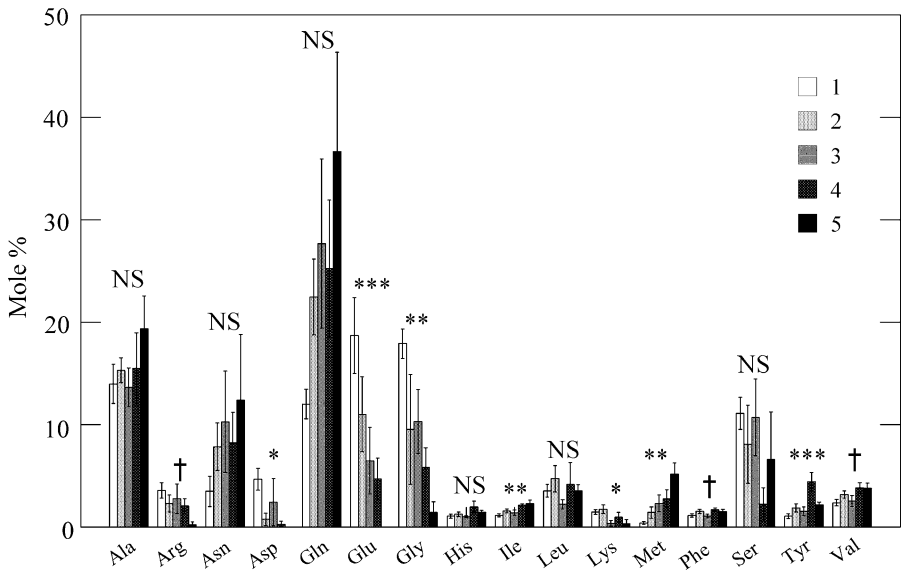

Fig. 5 Free amino acid composition across the landscape fertility gradient at the July 2005 sample date. Values represent the mean percentage contribution of each amino acid to the total for that sample on a molar basis (±1 SE). Individual bars represent the mean of 5 replicates ±1 SE. Significance levels for the main effect of site on each amino acids mole % are noted as follows: *NS* $P > 0.1$, $^{\dagger}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. For Site 5, Asp and Glu levels were below the detection limit (50 pM)

proteolysis activities measured in protein-amended soil slurries was remarkably uniform across sites (site effect $P = 0.549$).

The ^{15}N leucine-labeling experiment demonstrated differences among sites along the gradient in the fate of added leucine (Fig. 7). Total recovery of leucine-^{15}N tracer in all pools combined (NH_4^+, NO_3^-, DON, MN and SON) averaged 97% with no significant differences among sites ($P = 0.689$). Recovery of tracer ^{15}N in the DON pool, presumably dominated by untransformed label, was high (grand mean = 37%) with no apparent effect of site ($P = 0.687$). There was a marginally significant main effect of site on tracer recovery in the DIN pool ($P = 0.090$) and a significant

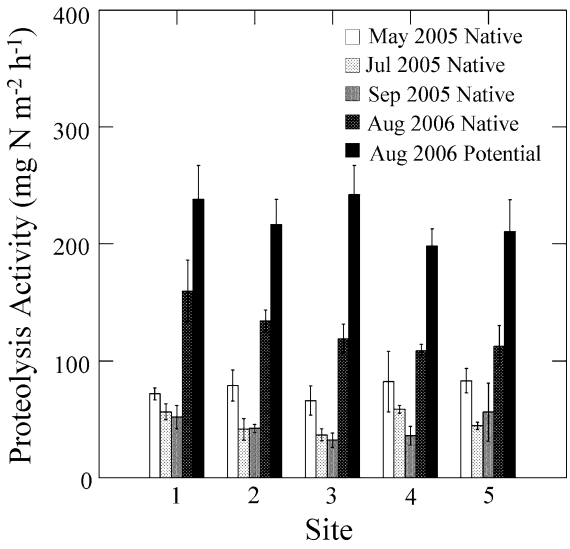

Fig. 6 Rates of free amino acid production as measured by laboratory assays of proteolysis activity across the gradient. Native proteolysis activities were sampled at three times during 2005, whereas native and potential proteolysis activities were sampled once in August, 2006. Individual bars represent the mean of 5 replicates ±1 SE

linear trend of increasing recovery with increasing site fertility ($P = 0.017$). Chloroform-labile MN had the second highest recovery (grand mean 30%), with site means suggestive of a decline across the gradient; however, the main effect of site was not statistically significant ($P = 0.211$). Recovery of ^{15}N-tracer in the SON pool exhibited the most obvious change across the gradient with a significant main effect of site ($P = 0.001$) and a marked linear increase with increasing site fertility ($P < 0.001$).Total microbial biodegradation of ^{15}N leucine (DIN Recovery + MN Recovery) was relatively constant across sites ($P = 0.661$; data not shown). Finally, I calculated total removal of leucine label as the sum of recoveries in DIN, MN and SON, which should represent all of the leucine that was removed from the free amino acid pools through microbial and sorptive processes. Total removal also had site means suggestive of an increase across the gradient; however, the effect of site was not statistically significant ($P = 0.184$; data not shown).

Discussion

As ecologists have sought to incorporate plant-uptake of free amino acids into models of terrestrial ecosystem N cycling, the working hypothesis has been that free amino acid contributions to plant N nutrition should be greatest at low fertility sites (Aber and Melillo 2001; Schimel and Bennett 2004). However, empirical data on soil amino acid availability has been strongly weighted towards arctic, boreal and alpine systems where organic N stocks are high and N mineralization is constrained by low temperatures (e.g. Chapin et al. 1993; Kielland 1994, 1995; Näsholm et al. 1998; Lipson et al. 2001; Jones and Kielland 2002; McFarland et al. 2002; Nordin et al. 2001; Weintraub and Schimel 2005). Relatively little work has been done investigating free amino acid

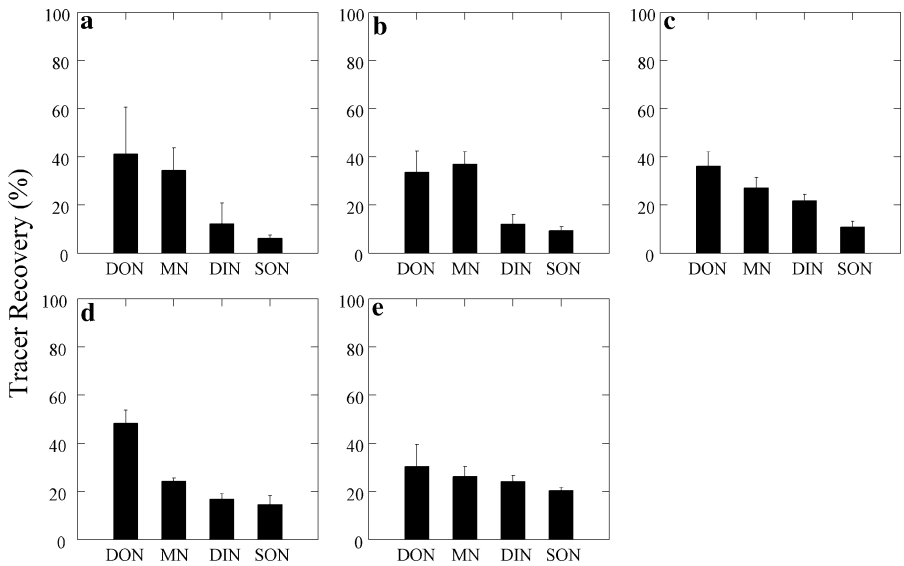

Fig. 7 Percentage recovery of ^{15}N label following a 4-h laboratory incubation of soils amended with 98 atom.% ^{15}N leucine. Values represent percentage of initial label recovered in extractable DON, chloroform-labile microbial N (MN), extractable NH_4^+ and NO_3^- (DIN) and residual soil organic N (SON). Sites 1, 2, 3, 4 and 5 are presented in panels **a**, **b**, **c**, **d**, and **e**, respectively. Individual bars represent the mean of 5 replicates ±1 SE

contributions to plant-available N pools across fertility gradients in temperate regions where feedbacks between soil properties and plant litter quality are thought to drive variability in N mineralization (but see Finzi and Berthrong 2005; Berthrong and Finzi 2006). Results from this study provide strong empirical evidence that free amino acids make diminishing contributions to pools of potentially plant-available N across a temperate forest fertility gradient. In fact, patterns of amino acid-, NH_4^+- and NO_3^-–N availability almost perfectly matched the theoretical predictions of Schimel and Bennett (2004) and Aber and Melillo (2001), with free amino acid pools highest at low fertility, NH_4^+ pools highest at intermediate fertility and NO_3^- pools highest at high fertility (Figs. 1, 2). These results clearly show that that there is a pool of free amino acids available for plant uptake in these low fertility temperate forests, and that standing pools diminish rapidly as site fertility increases.

Interestingly, although Schimel and Bennett (2004; Fig. 2 therein) argued only for shifting *relative* dominance of N forms from amino acids to NH_4^+ to NO_3^-, I found that free amino acids pools declined in absolute terms as well (Fig. 2a). In a similar comparison of soluble N pools along a boreal forest productivity gradient, Nordin et al. (2001) found a slight decrease in soluble amino acids, but large increases in NH_4^+ and NO_3^-. These results contrast to some degree with those of Finzi and Berthrong (2005) who found no change in absolute amino acid pools among three temperate deciduous forests in Connecticut, but a decline in the relative contribution of free amino acids from ca. 44% in low fertility sites to 23% in a high fertility site. These differences may be explained by the fact that Finzi and Berthrong extracted soils in 2 M KCl which likely liberates pools of free amino acids not extracted by water (Nordin et al. 2001) or 4 mM $CaCl_2$ (this study).

In addition to spatial variation along the landscape fertility gradient, I also observed seasonal variability in free amino acid pools within sites, in particular a large decrease from early spring to summer in the low fertility sites. Other researchers have noted a similar decline in free amino acid concentrations from spring to summer in both arctic tundra (Weintraub and Schimel 2005) and a young sub-boreal forest in Wisconsin (Johnson and Pregitzer 2007). In both studies the authors attributed the decline in free amino acid pools to increased N demand by plants and microorganisms in the heart of the growing season, which is consistent with my results where the transition from high to low pool sizes generally coincides with timing of canopy leaf out in these deciduous forests.

While there were some differences in the composition of free amino acids in soils across the gradient, six amino acids tended to dominate across a wide range of sites: alanine, asparagine, glutamine, glutamate, glycine and serine (Fig. 5). These amino acids are all frequently found at high concentrations in soil extracts across a wide variety of ecosystems (reviewed by Lipson and Näsholm 2001). In examining the compositional changes of the free amino acid pool across the gradient, the greater relative contribution of glycine at low fertility is of particular note. Glycine is the most common amino acid used in plant uptake studies (e.g. Raab et al. 1996; Schimel and Chapin 1996; Näsholm et al. 1998; Finzi and Berthrong 2005) and is thought to be particularly important as a N source for plants because of its low molecular weight, low C:N ratio, relatively rapid diffusion rates in soil and the fact that it is not well utilized by soil microorganisms (Lipson et al. 1999; Raab et al. 1999). Thus the high molar fraction of glycine at low fertility sites, suggests that the potential contribution of amino acids to plant nutrition may be even greater than that indicated by total amino acid pools alone. However, compositional differences among sites need to be viewed with a healthy dose of skepticism because these data reflect sampling at a single point in time—I cannot say if these compositional patterns would persist or change over a full growing season.

Whereas it is clear that there is a strong pattern in free amino acid pools across this gradient, it is less clear what underlying mechanisms drive this pattern. The depolymerization of proteins and peptides by extracellular proteolytic enzymes is thought to be the most important process by which free amino acids are produced in soil (Lipson and Näsholm 2001). Rates of proteolysis in soil depend on the availability of substrate (soluble peptides) and on the abundance and activity of proteolytic enzymes. Overall, I found little or no effect of site on either component of proteolysis. There was a significant main effect of site on pools of soluble peptides (Fig. 3); however, this was

driven almost entirely by lower values at Site 5, with no apparent pattern among Sites 1–4. Furthermore, although values were low at Site 5, the scale of change in soluble peptides was slight compared to the huge change in free amino acids (e.g. there was a 25-fold difference in free amino acids between Site 1 and Site 5, but only a 2-fold difference in soluble peptides). This lack of a trend in soluble peptides was mirrored in native proteolysis rates which also did not vary among sites, at any sample date (Fig. 6). Similarly, when I amended soil slurries with a protein substrate, I found remarkable uniformity among sites in potential proteolysis rates indicating no difference in the abundance/activity of extracellular proteolytic enzymes. Thus all available evidence, including reasonably high peptide substrate and high native and potential proteolytic activity, indicates that free amino acids are being produced in the fertile sites at rates comparable to those in infertile sites.

The ^{15}N-leucine labeling experiment was designed to experimentally test the hypothesis that potential microbial degradation of amino acids increases along the gradient from low to high fertility. Supporting this hypothesis, I observed a significant increase in the proportion of leucine-^{15}N that was mineralized to NH_4^+ and NO_3^- across the gradient (Fig. 7). However, this was balanced by a non-significant pattern of decreasing assimilation into microbial biomass with increasing fertility (Fig. 7) such that total microbial consumption of leucine–N did not vary among sites ($P = 0.661$). Contrasting trends for mineralization versus immobilization of tracer ^{15}N are consistent with the observations of Finzi and Berthrong (2005), who found greater mineralization of a ^{15}N-glycine tracer at their high-fertility sites and greater immobilization at their low-fertility site. One notable difference is that they observed much lower recovery of ^{15}N in the DON pool (~5% after 15 min), perhaps due to the fact that they added tracer at much lower concentrations than did I (0.25 μg N/g vs. 2.4 μg N/g). In contrast, my results are consistent with those of several other studies using ^{14}C-labeled amino acids which have also observed significant tracer recovery in the soluble organic fraction after 1–6 h (Jones 1999; Jones and Kielland 2002; Gonod et al. 2006). In particular, my observation of ~60% removal of ^{15}N tracer from the DON pool after 4 h is quite consistent with results of Gonod et al. (2006) who estimated that it took 3 h for 50% of their added leucine (at 0.8 μg N/g) to be removed from the soluble organic fraction.

Surprisingly, the one place I observed a consistent increase in ^{15}N recovery across the gradient was in the residual soil pool, with more than a 3-fold increase from Site 1 to 5. It is possible that increasing recovery of ^{15}N in the residual soil pool across the gradient reflects increasing potential for sorption of amino acids onto the soil solid phase. However, we know that some of this ^{15}N, perhaps the great majority, must be associated with microbial cells because typically only about half of the N contained in microbial biomass is rendered extractable by $CHCl_3$ fumigation (Brookes et al. 1985; Jenkinson 1988). Furthermore, it has been shown that leucine is not as readily sorbed to soil solids as positively-charged amino acids and that sorption does not compete strongly with microorganisms for this amino acid in particular (Gonod et al. 2006). Given these uncertainties, it is impossible to partition the strong increase I observed in residual soil ^{15}N recoveries into microbial versus sorptive components. Regardless of the particular mechanisms at work, total consumptive potential of these soils (DIN + MN + -SON) had site means suggestive of an increase across the gradient, increasing from ~55% at Site 1 to ~70% at Site 5; however, the effect of site was not statistically significant due to high within-site variability. Thus assays of amino acid fluxes conducted in the laboratory were consistent in showing little change in the potential for production or consumption of free amino acids across the gradient.

How then to reconcile the marked changes in pool sizes across this gradient with the relatively consistent flux rates? One possibility is simply that laboratory assays conducted on disturbed soils do not accurately reflect in situ dynamics of amino acid production and consumption. Certainly, sieving and wetting of soils and the concomitant exposure of organic matter may have elevated microbial activity (e.g. Franzluebbers 1999) in both proteolysis and ^{15}N-leucine experiments, potentially masking any in situ differences in these processes. Sieving and homogenizing soils would also have masked any differences in microsite processes that Schimel and Bennett (2004) argue are a key driver of N dynamics in soils. In this case, higher standing pools at low fertility sites may not result from greater overall rates of production or lower overall consumption, but,

rather from greater uncoupling of sites of production from sites of consumption. A final possibility is that I may have underestimated true rates of amino acid production at low fertility sites by focusing solely on depolymerization of peptides, and excluding leaching of amino acids from organic residues. Because soil organic matter at the low fertility sites is composed of a large portion of particulate organic matter, fine roots and fungal hyphae (personal observation), leaching of this material following wetting of the soil may make a greater contribution to free amino acid pools than at high fertility sites where highly decomposed, humic material dominates. Free amino acids from these sources would have been captured by the 4 mM $CaCl_2$ extracts, but not by the native proteolysis assays.

Implications for N cycling in temperate forests

Results from this study clearly demonstrate that free amino acids can make an important contribution to plant-available N pools in temperate forest soils, with amino-acid N pools even exceeding inorganic N pools early in the growing season at low fertility sites (Fig. 2). This finding, together with recent results from the eastern US (Finzi and Berthrong 2005), suggests that we need to expand our thinking on the nature of N cycling in temperate forest ecosystems. Previous work on this and other landscape fertility gradients in the Upper Lake States has focused solely on inorganic N availability (Pastor et al. 1984; Zak et al. 1986, 1989; Reich et al. 1997), demonstrating increasing availability of NH_4^+ and NO_3^- from low to high fertility sites. My results suggest that focusing solely on inorganic N may underestimate actual plant N availability and N cycling rates in low-fertility sites. When free amino acids are included as a component of plant-available N, then the apparent increase in N availability across the gradient is moderated relative to consideration of inorganic N alone (Fig. 2).

It is also important to consider the changes in plant species composition that go along with changes in amino acid availability across this gradient. In particular, note that low-fertility sites with high amino acid availability are dominated by tree species forming ectomycorrhizal (EM) associations (e.g. *Quercus* spp.; Table 1), whereas, arbuscular-mycorrhizal (AM) tree species (*Acer saccharum*, *Tilia americana*) dominate the richer sites with low amino acid availability. This difference in plant species composition also occurs in the study of Finzi and Berthrong (2005) where systems dominated by EM tree taxa (*Pinus*, *Tsuga*, *Quercus* and *Fagus*) exhibited both greater amino acid availability and greater fine-root amino acid uptake relative to mineral forms of N. Similarly, Högberg et al. (2007) showed that EM fungal biomarker fatty acids declined markedly along a boreal forest gradient where amino acids pools decline and mineral N pools increase from low to high fertility (Nordin et al. 2001). Ectomycorrhizal fungi are thought to be particularly important in facilitating plant acquisition of N from organic N sources through their production of proteolytic enzymes (Abuzinadah and Read 1986; Finlay et al. 1992; Buee et al. 2007), suggesting that the importance of organic N uptake across this gradient may also depend on tree species composition and/or mycorrhizal association.

Acknowledgments I gratefully acknowledge the USDA Forest Service for providing access to field sites. I thank A. Holcomb, S. LeDuc, N. Longbucco, M. McDermott, J. Mooney, A. Mueller, E. Scott, G. Smith and S. Spaulding for assistance in the field and laboratory. I thank D. Zak and two anonymous reviewers for helpful comments on earlier drafts. This project was funded by NSF grant 0448058 to D. E. Rothstein and by the Michigan Agricultural Experiment Station.

References

Aber JD, Melillo JM (2001) Terrestrial ecosystems, 2nd edn. Harcourt Academic Press, San Diego

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 Phytol 103:481–493. doi:10.1111/j.1469-8137.1986.tb02886.x

Aerts R, Chapin FS III (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67. doi:10.1016/S0065-2504(08)60016-1

Berthrong ST, Finzi AC (2006) Amino acid cycling in three cold-temperate forests of the northeastern USA. Soil Biol Biochem 38(5):861–869. doi:10.1016/j.soilbio.2005.08.002

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 Biol Biochem 17(6):837–842. doi:10.1016/0038-0717(85)90144-0

Buee M, Courty PE, Mignot D, Garbaye J (2007) Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. Soil Biol Biochem 39:1947–1955. doi:10.1016/j.soilbio.2007.02.016

Cabrera ML, Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci Soc Am J 57(4):1007–1012

Chapin FSIII, Moilanen L, Kielland K (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal sedge. Nature 361(6408):150–153. doi:10.1038/361150a0

Finlay RD, Frostegård Å, Sonnerfeldt 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 Phytol 120:105–115. doi:10.1111/j.1469-8137.1992.tb01063.x

Finzi AC, Berthrong ST (2005) The uptake of amino acids by microbes and trees in three cold-temperate forests. Ecology 86(12):3345–3353. doi:10.1890/04-1460

Franzluebbers AJ (1999) Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biol Biochem 31(8):1083–1090. doi:10.1016/S0038-0717(99)00022-X

Gonod LV, Jones DL, Chenu C (2006) Sorption regulates the fate of the amino acids lysine and leucine in soil aggregates. Eur J Soil Sci 57(3):320–329. doi:10.1111/j.1365-2389.2005.00744.x

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization and nitrification. In: Methods of soil analysis, part 2: microbiological and biochemical properties. Soil Science Society of America, Wisconsin

Hobbie SE (1992) Effects of plant species on nutrient cycling. Trends Ecol Evol 7(10):336–339. doi:10.1016/0169-5347(92)90126-V

Högberg MN, Högberg P, Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590–601. doi:10.1007/s00442-006-0562-5

Holmes WE, Zak DR (1994) Soil microbial biomass dynamics and nitrogen mineralization in northern hardwood ecosystems. Soil Sci Soc Am J 58(1):238–243

Holmes WE, Zak DR (1999) Soil microbial control of nitrogen loss following clearcut harvest in northern hardwood ecosystems. Ecol Appl 9(1):202–215. doi:10.1890/1051-0761(1999)009[0202:SMCONL]2.0.CO;2

Holmes WE, Zak DR, Pregitzer KS, King JS (2003) Soil nitrogen transformations under *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* following 3 years exposure to elevated CO_2 and O_3. Glob Chang Biol 9(12):1743–1750. doi:10.1046/j.1365-2486.2003.00705.x

Host GE, Pregitzer KS, Ramm CW, Lusch DP, Cleland DT (1988) Variation in overstory biomass and ecological land units in northwestern Lower Michigan. Can J For Res 18(6):659–668. doi:10.1139/x88-101

Jenkinson DS (1988) Determination of microbial biomass carbon and nitrogen in soil. In: Wilson JR (ed) Advances in nitrogen cycling in agricultural eco-systems. CAB, International, Wallingford

Johnson RM, Pregitzer KS (2007) Concentration of sugars, phenolic acids, and amino acids in forest soils exposed to elevated atmospheric CO_2 and O_3. Soil Biol Biochem 39(12):3159–3166. doi:10.1016/j.soilbio.2007.07.010

Jones DL (1999) Amino acid degradation and its potential effects on organic nitrogen capture by plants. Soil Biol Biochem 31:613–622

Jones DL, Hodge A (1999) Biodegradation kinetics and sorption reactions of three differently charged amino acids in soil and their effects on plant organic nitrogen availability. Soil Biol Biochem 31(9):1331–1342. doi:10.1016/S0038-0717(99)00056-5

Jones DL, Kielland K (2002) Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. Soil Biol Biochem 34(2):209–219. doi:10.1016/S0038-0717(01)00175-4

Jones DL, Owen AG, Farrar JF (2002) Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biol Biochem 34(12):1893–1902. doi:10.1016/S0038-0717(02)00203-1

Jones DL, Kemmitt SJ, Wright D, Cuttle SP, Bol R, Edwards AC (2005) Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy. Soil Biol Biochem 37(7):1267–1275. doi:10.1016/j.soilbio.2004.11.023

Kielland K (1994) Amino-acid absorption by arctic plants—implications for plant nutrition and nitrogen cycling. Ecology 75(8):2373–2383. doi:10.2307/1940891

Kielland K (1995) Landscape patterns of free amino acids in arctic tundra soils. Biogeochemistry 31(2):85–98. doi:10.1007/BF00000940

Kielland K, McFarland JW, Ruess RW, Olson K (2007) Rapid cycling of organic nitrogen in taiga forest ecosystems. Ecosystems (N, Y Print) 10(3):360–368. doi:10.1007/s10021-007-9037-8

Lipson D, Näsholm T (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128(3):305–316. doi:10.1007/s004420100693

Lipson DA, Raab TK, Schmidt SK, Monson RK (1999) Variation in competitive abilities of plants and microbes for specific amino acids. Biol Fertil Soils 29(3):257–261. doi:10.1007/s003740050550

Lipson DA, Raab TK, Schmidt SK, Monson RK (2001) An empirical model of amino acid transformations in an alpine soil. Soil Biol Biochem 33(2):189–198. doi:10.1016/S0038-0717(00)00128-0

McFarland JW, Ruess RW, Kielland K, Doyle AP (2002) Cycling dynamics of NH4+ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. Ecosystems (N Y, Print) 5(8):775–788

Morris SJ, Boerner REJ (1998) Landscape patterns of nitrogen mineralization and nitrification in southern Ohio hardwood forests. Landscape Ecol 13(4):215–224. doi:10.1023/A:1007967630020

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392:914–916. doi:10.1038/31921

Nordin A, Högberg P, Näsholm T (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. Oecologia 129:125–132. doi:10.1007/s004420100698

Pastor J, Aber JD, McClaugherty CA, Melillo JM (1984) Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecology 65(1):256–268. doi:10.2307/1939478

Paul JP, Williams BL (2005) Contribution of alpha-amino N to extractable organic nitrogen (DON) in three soil types from the Scottish uplands. Soil Biol Biochem 37(4):801–803. doi:10.1016/j.soilbio.2004.09.011

Raab TK, Lipson DA, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. Oecologia 108(3):488–494. doi:10.1007/BF00333725

Raab TK, Lipson DA, Monson RK (1999) Soil amino acid utilization among the Cyperaceae: plant and soil processes. Ecology 80(7):2408–2419

Reich PB, Grigal DF, Aber JD, Gower ST (1997) Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. Ecology 78(2):335–347

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602. doi:10.1890/03-8002

Schimel JP, Chapin FSIII (1996) Tundra plant uptake of amino acid and NH_4^+ nitrogen in situ: plants compete well for amino acid N. Ecology 77(7):2142–2147. doi:10.2307/2265708

Watanabe K, Hayano K (1995) Seasonal variation of soil protease activities and their relation to proteolytic bacteria and *Bacillus* spp in paddy field soil. Soil Biol Biochem 27(2):197–203. doi:10.1016/0038-0717(94)00153-R

Weintraub MN, Schimel JP (2005) Seasonal protein dynamics in Alaskan arctic tundra soils. Soil Biol Biochem 37(8):1469–1475. doi:10.1016/j.soilbio.2005.01.005

Zak DR, Pregitzer KS, Host GE (1986) Landscape variation in nitrogen mineralization and nitrification. Can J For Res 16(6):1258–1263. doi:10.1139/x86-223

Zak DR, Host GE, Pregitzer KS (1989) Regional variability in nitrogen mineralization, nitrification, and overstory biomass in northern Lower Michigan. Can J For Res 19(12):1521–1526. doi:10.1139/x89-231

Zsolnay A (2003) Dissolved organic matter: artefacts, definitions, and functions. Geoderma 113(3–4):187–209. doi:10.1016/S0016-7061(02)00361-0