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Glycine mineralization in situ closely correlates with soil carbon availability across six North American forest ecosystems

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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, comparatively little attention has been given to their role as labile carbon (C) substrates that might influence the metabolic status of resident microbial populations. 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 (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 in situ glycine-C turnover and C_{min} measured in the laboratory. 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 consumption of these low-molecular-weight substrates by soil microorganisms may be governed as much by the overall decomposability of soil C as by N limitation to microbial growth.

Keywords Soil free amino acid · Glycine · ^{13}C · Soil C and N · Mineralizable C · Forest soils

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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. 2001a, b; 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. 2001a, b) or transferred to plants via fungal symbionts.

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 has been given less attention. There is increasing evidence that heterotrophic growth in many soil environments is C- rather than N-limited (Morita 1988; Zak et al. 1994; De Nobli et al. 2001; Mondini et al. 2006; Treseder 2008). 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 (ectomycorrhizal versus arbuscular mycorrhizal), 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). However, these studies focused within a particular ecosystem or landscape in which many of the state factors influencing soil development (climate, parent material, vegetation, or time) were held constant. 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 where C availability was expected to vary widely.

Our study was designed to test the idea that turnover dynamics of FAA in forest soils are 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*). Relevant features for forest types are discussed below while specific stand characteristics are presented in Table 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. These stands are predominantly ectomycorrhizal (EM). 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 well-developed organic horizons extending to 10 cm or more in depth. The climate is strongly continental, and forests are exposed to sub-freezing conditions for much of

Table 1 Select stand characteristics for each of the six forest ecosystems

Stand parameter	Site					
	Southern temperate		Northern temperate		Boreal	
	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	AM	EM	AM	EM	EM	EM
Stand age (year)	40	>60	95–100 overstory	50	80–100	150–250
Mean annual temperature (°C)[a]	12.7	16.5	3.8	3.8	−3.3	−3.3
Mean annual precipitation (mm)[a]	1816	1263	841	883	287	287
Percent overstory[a]	85	68	92	100	70	98
Stem density (trees ha^{-1})[b]	2396	391	707	522	922	400
Basal area (m^2 ha^{-1})[a]	34	26	33	34	37	30
Total litterfall (g m^{-2} $year^{-1}$)[c]	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

AM arbuscular mycorrhizae, *EM* ectomycorrhizae

[a] MAT, MAP, percent overstory (as % basal area), and basal area from Pregitzer et al. (2002)

[b] Stem density measured 1998 for balsam poplar and white spruce. Data for tulip poplar, white oak, red pine, and sugar maple from R. Hendrick and K. Pregitzer (*unpublished*)

[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

the year. Though 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 plant community composition changes through succession. 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 were about 95–100 years old. A second harvest occurred about 25 years prior to our study, at which time approximately 2/3 of the basal area was left intact. Overstory taxa in this stand are predominantly arbuscular mycorrhizal (AM). 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 (EM) is located at the William Payne La Croix plantation established in 1950 near Houghton, Michigan. This stand consists of evenly spaced (1.8 m × 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 (AM) 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.

The oak site (EM) 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 range 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 3 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. We define the

core divisions in white spruce and balsam poplar as approximate because compression within the organic horizons during sample collection likely resulted in core depths that were slightly deeper than 20 cm. Moreover, spatial heterogeneity in depth of the forest floor along with buried organic layers among white spruce and balsam poplar stands contributed differing amounts of organic matter to the mineral cores. We chose these divisions to coordinate our laboratory incubation experiment with temperature-dependent models of soil respiration developed from field measurements taken throughout the growing season at each site (*unpublished data*).

Subsequent to partitioning, 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) 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 3 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

Table 2 Select soil properties for each of the six forest ecosystems in our study

Soil parameter	Site					
	Southern temperate		Northern temperate		Boreal	
	Tulip poplar	White oak	Sugar maple	Red pine	Balsam poplar	White spruce
Soil organic carbon (g C kg^{-1})						
Upper horizons[a]	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}
Lower horizons[b]	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}
$C:N_{UPPER}$	13.9 ± 0.1^{a}	16.6 ± 0.8^{b}	13.3 ± 0.5^{a}	21.7 ± 1.1 ^{cd}	18.8 ± 0.4^{bc}	23.3 ± 1.1^{d}
Total soil nitrogen (g N kg^{-1})						
Upper horizons[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 ± 0.3^{a}	16.9 ± 0.7^{a}	12.8 ± 0.2^{b}	20.0 ± 0.6^{c}	16.7 ± 0.3^{a}	19.4 ± 0.6^{c}
Soil pH (0–20 cm)[c]	5.7	5.2	4.4	4.5	6.0	5.5
TFAA (μg N 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 (μg N g^{-1})[d]	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

For soil C and N ($n = 6$), TFAA ($n = 15$), and DIN ($n = 18$), values are mean ± S.E. Letters denote significant differences ($P \leq 0.05$) between forest types

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

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

[c] Values from Pregitzer et al. (2002)

[d] Values from McFarland et al. (in press)

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 h prior to each measurement and then aerated before being returned to the incubation chamber. Between sampling periods, each jar was covered with 0.8 mil 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). Due to differences in C content among soil types we normalized $C_{\min}$ by total soil organic C (C_{total}), so that instantaneous rate constants (K_C) reflect site to site variation in C_{total}. Additionally, we tested a double exponential model which considers two soil organic matter pools with differing susceptibility to decomposition (Alavarez and Alavarez 2000). However, due to the relatively short duration of our soil incubation experiment, we found that a single pool C mineralization model adequately described our results.

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 (see McFarland et al. 2002 for visual depiction of grid design). Within each injection grid, we administered either U-[$^{13}C_2$]-glycine (glycine treatment) or distilled water (control) to a depth of 10 cm. As part of a companion experiment examining plant-microbial competition for N, we added $(^{15}NH_4^+)_2SO_4$ to the ^{13}C-glycine treatment and established a second set of injection cores in which glycine was labeled with ^{15}N. Following gas measurements for glycine-derived $^{13}CO_2$, we harvested a 5.5 × 12 cm ($w \times d$) core to track the fate of our N additions from the $(^{15}NH_4^+)_2SO_4$ and ^{15}N-glycine treatments. These cores were harvested in the manner of McFarland et al. (2002) which permitted us to reasonably estimate total recovery of NH_4^+- and glycine-derived N in dissolved inorganic N (DIN), dissolved organic N, (DON), microbial N, (MBN) and fine root N pools. Results from the ^{15}N tracer study are briefly discussed in the context of microbial C:N balance below and in greater detail elsewhere (McFarland et al. in press). Total injection volume was 37 ml ($\sim$1 ml cm^{-2}), which delivered 0.39 g ^{13}C m^{-2} and ensured that cross-site differences in soil moisture were minimized.

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 pressed against the soil surface for 3 min at which time a 15 ml sample was collected. We exercised caution in sealing the chamber to the soil so as to avoid depressing the surface and releasing excess CO_2. Gas in the sampling 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., UK) pre-evacuated 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 h)

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., UK). 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 and DIN extraction and quantification

We randomly collected 15 soil cores to a depth of 12 cm along our transect using a 5.5 cm (I.D.) steel corer combusted at 450°C for 6 h prior to use. Sampling depth was chosen to coincide with cores harvested to trace the fate of our ^{15}N additions. All cores were handled with nitrile gloves and stored in clean polyethylene bags on ice during transport to the laboratory. Within 4 h, 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, USA.). 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, VT, 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, VT, 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 equations 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. Simple linear regression analyses were used to relate $k_{glycine}$ and $1/k_{glycine}$ with soil temperature, soil C:N, 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 $\alpha = 0.05$. Results for $k_{glycine}$, $^{13}C_{APEcum}$, and residence time for glycine ($1/k_{glycine}$), did not meet these criteria for statistical significance. However, since these parameters were derived from measurements made in an open system as opposed to a highly controlled laboratory microcosm, we discuss these results as a non-significant trend where $P < 0.10$.

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 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 kg^{-1} (Table 2). Carbon content in the lower horizon was more similar among all forest types, ranging from 45 g C kg^{-1} in tulip poplar to 16 g C 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 kg^{-1} in white oak to 12.4 g N 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 kg^{-1}) and the lowest in red pine (0.8 g N 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 (Fig. 1a). Carbon

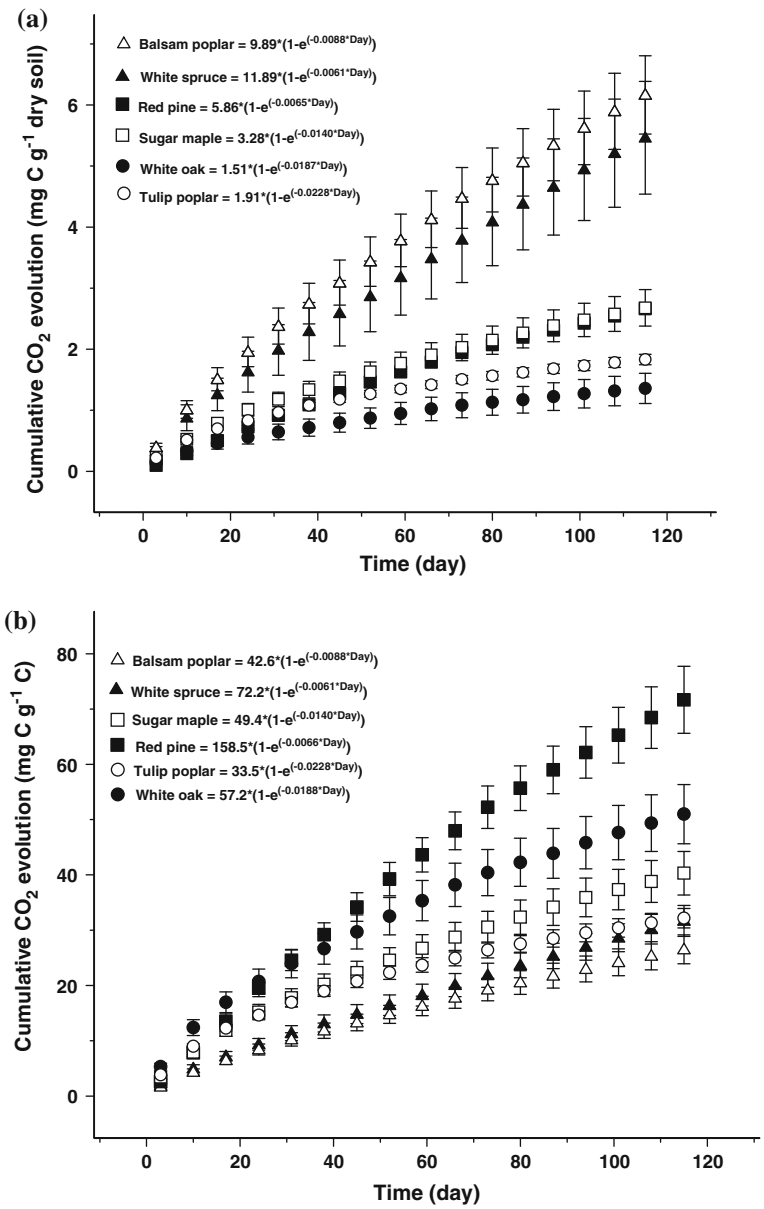

Fig. 1 Cumulative CO_2 evolution from upper soil horizons (0–7 cm depth; see methods) for six North American forest ecosystems. Values, expressed as C g^{-1} dry mass in panel (**a**) and C g^{-1} C in panel (**b**), were fitted to a nonlinear single exponential model

dioxide accumulation curves constructed for each site reveal that net mineralizable C (C_{min}) ranged from 1.51 g C kg soil^{-1} in white oak to 11.89 g C kg soil^{-1} in white spruce, and was positively correlated with C_{total} ($r^2 = 0.65$, $P < 0.001$; $n = 36$). Though C_{min} increased significantly with increasing latitude ($P < 0.001$), this trend was no longer apparent when respired C was adjusted for C_{total} (Fig. 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} across all samples ($r^2 = 0.41$, $P < 0.001$; $n = 36$; Fig. 2a). Soils 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), but similar values for C_{min}. When adjusted for C_{total} (Fig. 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 at different temperatures. Therefore, net C mineralization rates reflect differences in both temperature and microbial C utilization. The highest C turnover rates were observed in tulip poplar soils (0.0228 ± 0.0009 days^{-1}) and the lowest in white spruce (0.0061 ± 0.0012 days^{-1}; Table 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 3). Mean residence time for C_{min} in red pine was 92.3 days in the lower soil horizon versus 184.0 days in the upper soil horizon. Similarly, average C_{min}/C_{total} was almost 2-fold 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 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

Table 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

Stand type	Upper soil horizon			Lower soil horizon		
	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	0.0188 ± 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 ± 35.9^{bc}	15.67 ± 1.13^{b}	0.0119 ± 0.0016^{b}	92.3 ± 13.4^{b}	7.98 ± 0.74^{a}
Balsam poplar	0.0089 ± 0.0011^{c}	124.8 ± 21.2^{bc}	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}

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

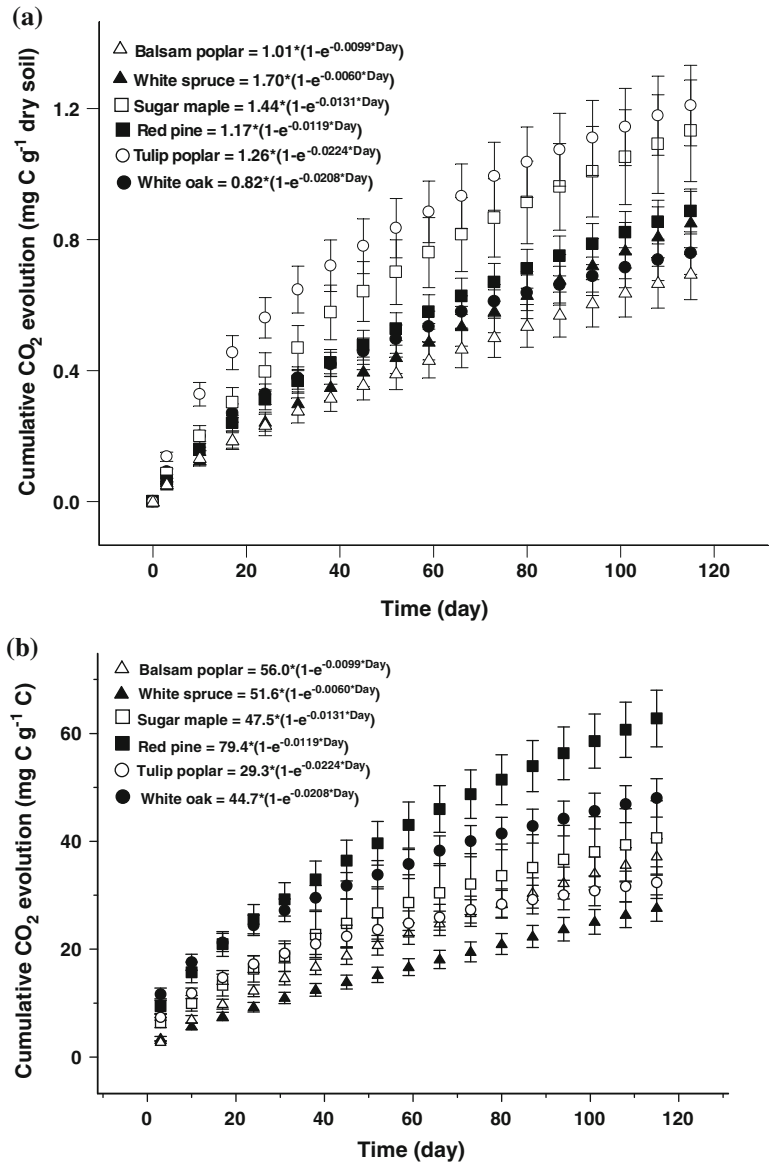

Fig. 2 Cumulative CO_2 evolution from lower soil horizons (7–20 cm depth; see methods) for six North American forest ecosystems. Values, expressed as C g^{-1} dry mass in panel (**a**) and C g^{-1} C in panel **b**, were fitted to a nonlinear single exponential model

observed variation was attributed to soils from Alaskan stands, which in some instances had over 10-fold higher concentrations of FAA than soils from the southern deciduous stands (Fig. 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). 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 4); nevertheless, rate

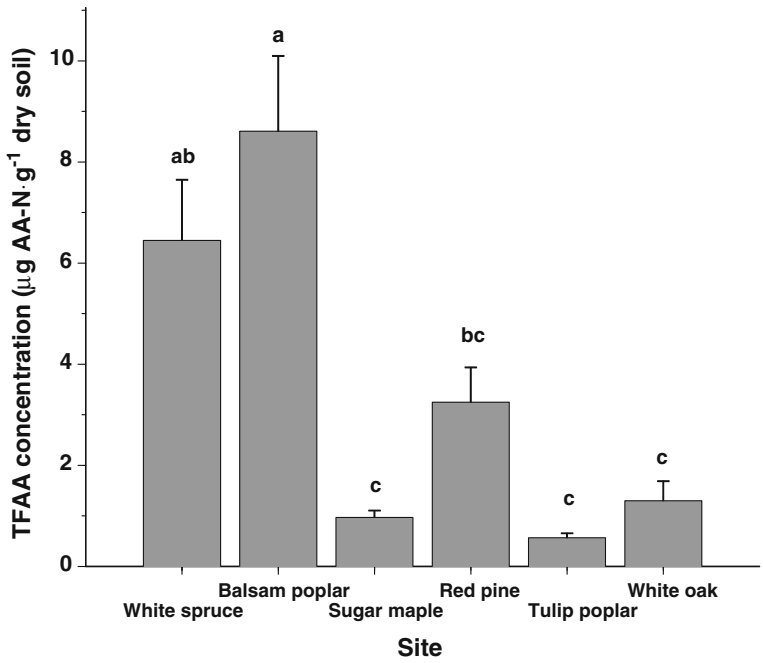

Fig. 3 Concentration of soil-free amino acid-N across a latitudinal gradient of six forest ecosystems. Data are means ($n = 15$) ± S.E

Table 4 Soil temperature and turnover parameters derived from nonlinear single exponential models fitted to cumulative production of $^{13}CO_2$ from cores treated with U-^{13}C-glycine

Stand type	Soil temperature (°C)	k (h^{-1})	Residence time (h)	APE $^{13}C_{cum}$ (24 h)
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^{a}	1.44 ± 0.08^{a}	1.00 ± 0.11^{a}
Sugar maple	13.8	0.88 ± 0.15^{a}	1.27 ± 0.23^{a}	0.87 ± 0.07^{ab}
Red pine	15.7	0.64 ± 0.10^{a}	1.71 ± 0.20^{a}	0.53 ± 0.05^{c}
Balsam poplar	12.8	0.69 ± 0.06^{a}	1.50 ± 0.12^{a}	0.55 ± 0.07^{bc}
White spruce	9.3	0.53 ± 0.04^{a}	1.96 ± 0.19^{a}	0.43 ± 0.04^{c}

Values are mean ± S.E. ($n = 6$). Letters denote significant differences ($P \leq 0.05$) between forest types. Soil temperatures represent a temporal ($n = 24$) and spatial ($n = 6$) average of hourly values for each site collected at 7 cm depth for the duration of the glycine mineralization assay

constants for glycine turnover (k_{gly}) determined from our single exponential model were statistically different across all forest types ($F_{5,30} = 3.34$, $P = 0.02$; Table 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 (Fig. 4). Contrary to predictions, mean in situ turnover rates for glycine were significantly faster in tulip poplar soils than in white spruce soils ($P \leq 0.05$) 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 and 0.69 h^{-1}, respectively), whereas northern sugar maple demonstrated non-significantly higher turnover rates for glycine (0.88 h^{-1}; Table 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 (Fig. 5; $r^2 = 0.82$, $P = 0.01$). The highest values for k_{gly} were observed in soils with

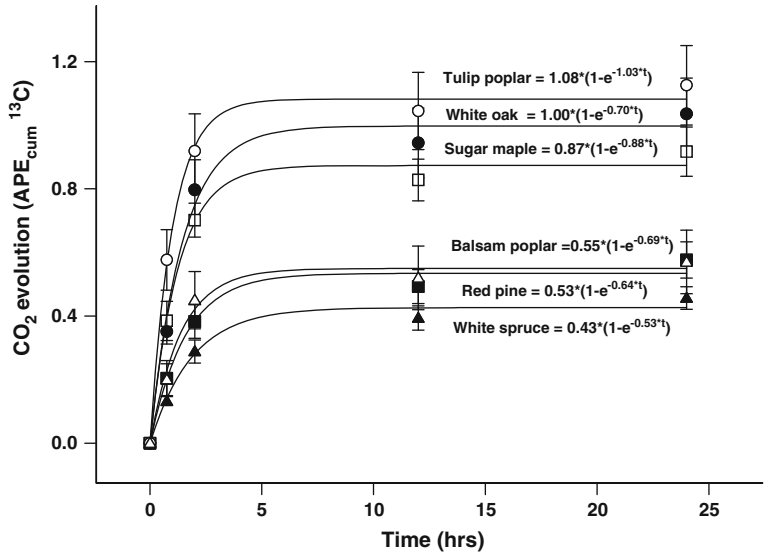

Fig. 4 The time dependent mineralization of ^{13}C-labelled glycine in situ across six North American forest ecosystems. Values are expressed as atom% enrichment of ^{13}C-CO_2. CO_2 efflux above each injection area was sampled at 45 min, 2, 12, and 24 h. Data are means ($n = 6$) ± S.E

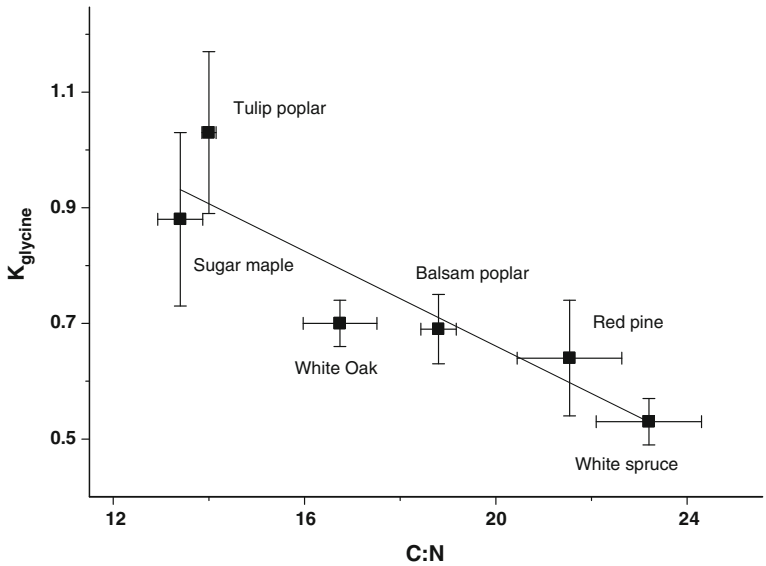

Fig. 5 Relationship between the rate constants for in situ glycine mineralization ($k_{glycine}$) and the soil C:N (*upper horizon*; see "Materials and Methods"). Mineralization constants are means ± S.E. ($n = 6$) for each constant calculated from nonlinear single exponential models fitted to cumulative product curves. The line is a linear regression fitted to the data [$r^2 = 0.82$]

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"). 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 (Fig. 6; $r^2 = 0.67$, $P < 0.05$). 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

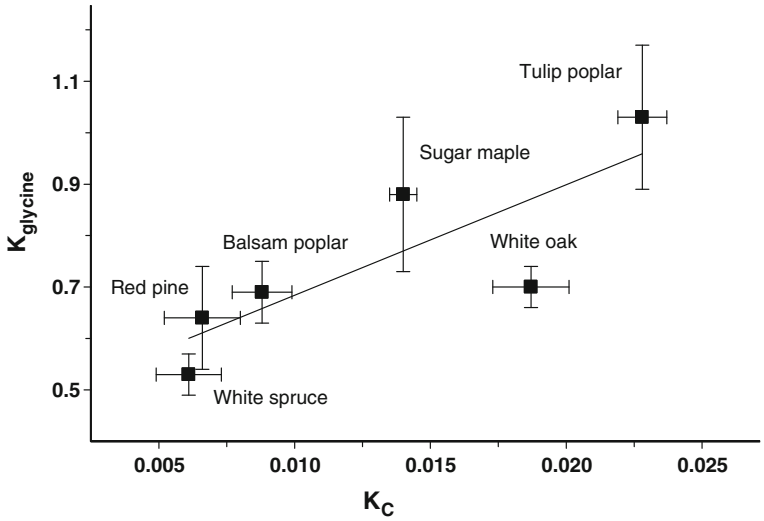

Fig. 6 Relationship between the rate constants for in situ glycine mineralization ($k_{glycine}$) and the decomposition potential for soil C (upper horizon; see methods) expressed per unit C (k_C) among all forest types. Values are means ± S.E. ($n = 6$) calculated for each constant from nonlinear single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

to the turnover of soil C pools. In general, mineralization of our glycine additions increased with decreasing pools of labile C, suggesting a microbial response to C limitation. Although soil microorganisms in all forest types rapidly mineralized 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 Nobli 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 with decreasing latitude—were not as straightforward as predicted.

Results from our laboratory incubations show that boreal forest soils yielded substantially larger pools of respired C than mid-latitude soils (Fig. 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 (MAT), 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 MAT or litter quality (Fig. 1b) particularly in the temperate regions. This suggests that neither MAT nor litter quality alone provided an adequate explanation for FAA turnover and $C_{min.}$

Forests dominated by species producing high quality (low lignin:N; Table 5) aboveground (AG) litter, e.g. tulip polar and sugar maple, would be expected to have higher rates of litter decomposition

Table 5 Generalized lignin: N ratios of overstory taxa for the stand types used in our investigation

Stand type	Tulip poplar	White oak	Sugar maple	Red pine	Balsam poplar	White spruce
Lignin:N	15.1[a]	24.0[b]	14.6[b]	64.4[b]	23.4[c]	25.2[c]

[a] Values reported in White et al. 1987 for stands in the Coweeta LTER research site

[b] Values from a study on Blackhawk Island, WI (McClaugherty et al. 1985) as reported by Aber et al. (1990)

[c] Values reported in Taylor et al. (1989) for stands in Alberta, Canada

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). Contrary, C_{min} accounted for a larger proportion of total C in stands producing lower quality AG litter, e.g. red pine, despite that turnover rates (k_C) for soil C were slower for red pine than for sugar maple or tulip poplar. Organic matter from both soil horizons contained 2–4 times more labile C under red pine versus sugar maple or tulip poplar (Figs. 1b, 2b). This apparent discrepancy might reflect differences in early-stage versus 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 more recalcitrant mass remains during the late stages of decomposition (Berg and Ekbohm 1991; Berg and Meentemeyer 2002; but see Hobbie 2000).

In our study we observed a strong relationship between soil C:N and k_{gly} whereby soils with lower C:N trended towards higher turnover rates for glycine (Fig. 5). Assuming a C-limited soil environment and a high metabolic demand in soils with lower C:N could explain why microbes in tulip poplar and sugar maple responded to our glycine additions with faster turnover rates than observed in oak or red pine where C_{min}/C_{total} is higher. 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, e.g. rhizo-deposition (Mondini et al. 2006). Still, glycine represents a source of labile N as well as C, and to link the turnover of glycine to C-limitation in stands with low C:N, we must first reject the argument that the residence time for glycine was not linked as well to N-limitation in those stands.

In our companion experiment (McFarland et al. in press) we observed several interesting trends in the fate of our NH_4^+- and glycine-derived ^{15}N tracers. First, microbial immobilization represented the largest short-term biotic sink for both N forms regardless of forest type. Second, long-term measurements of MBN turnover across our latitudinal gradient indicated that the majority of ^{15}N for both N treatments was ultimately transferred to stable soil N pools. These results suggest that microbes do indeed target both substrates for N and thus may be N-limited. However, there were deviations in the cycling patterns for glycine and NH_4^+ for some forest types that warrant further consideration. First, 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. Second, the mineralization 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. Third, the incorporation of NH_4^+ into MBN was significantly lower than the incorporation of glycine-N under sugar maple and relatively low for both N forms under tulip poplar. Together, these results indicate that soil microorganisms in the two AM-dominated stands were likely utilizing glycine primarily as a C source. Unfortunately, since the cycling dynamics of NH_4^+ and glycine-N varied more or less in concert for the other forest types, we cannot rule out the possibility that the turnover of glycine is linked to microbial N-limitation in those soils.

Processes affecting the biodegradation of glycine reflect a complex of interacting factors that we did not study including, among others, microbial community composition. Community structure of soil microorganisms can strongly influence the incorporation of plant litter into SOM and thus the availability of labile C (Elliot et al. 1993). The size of the microbial biomass may be less important than the activity of the community in predicting decomposition rates, particularly if metabolic activities of microbes are adapted to available substrates (Elliot et al. 1993; Waldrop and Firestone 2004; but see McClaugherty et al. 1985). Data from our companion study, (McFarland et al. in press), indicate no correlation between MBN and glycine turnover rates. Using MBN as a surrogate for microbial biomass, this suggests that patterns in glycine turnover are likely driven by differences in heterotrophic consumption rather than the size of the microbial biomass per se. Recent evidence from low fertility black oak/white oak and high fertility sugar maple/basswood

ecosystems indicates that standing pools of free amino acids are higher when mineral N availability is low despite that amino acid production does not vary significantly between these two forest types (Rothstein 2009). We propose that differences in amino acid consumption, related to microbial C rather than N balance, may explain this discrepancy between production and pool size.

In our study, forest types are characterized as predominantly arbuscular (AM) or ectomycorrhizal (EM) with respect to the overstory taxa (Pregitzer et al. 2002; Lansing, "unpublished data") and we suspect that these fungal associations represent a significant portion of the soil microbial biomass. Mycorrhizal fungi can influence SOM quality by regulating both availability and turnover of soil C, but the effects of AM and EM on rhizosphere processes appear to differ (Langley and Hungate 2003). Ectomycorrhizal fungi 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 some AM roots have a capacity to promote the activity of rhizobacteria through root or fungal exudation into mycorrhizospheric soil (Olsson et al. 1996b; Andrade et al. 1997). During the degradation of organic matter, calorimetric values can be significantly higher for bacteria than fungi (Critter et al. 2004), though the relative importance of bacteria and fungi to total metabolism may be soil and substrate dependent. Elliot et al. (1993) found that fungal contributions to microbial metabolism within the forest floor increased significantly with substrate recalcitrance across a range of forest types. We did not assess the ratio of fungi:bacteria in soils from any of our sites in relation to mycorrhizal association; however, it is worth noting in our study that in situ turnover rates for glycine were highest in the stands dominated by AM species (sugar maple and tulip poplar), and lowest in the EM-dominated white spruce and red pine stands. If AM associations tend to enrich bacterial flora while EM associations render the rhizopshere and surrounding soil less hospitable to bacterial growth, the predominance of one mycorrhizal type over the other among our experimental forests could partially explain differences in glycine turnover (given its lability) among stand types.

There are several caveats to our interpretations regarding FAA turnover in the field given the limitations of our experimental design. First, microbial preference for a particular substrate as well as differential partitioning of that substrate into anabolic versus catabolic pathways can influence the turnover of that substrate in microbial pools. We concede that the magnitude of the mineralization response we measured might be amino acid specific. In our study, comparing turnover dynamics of a common substrate (glycine) versus a site-specific amino acid cocktail as determined from pool constituency most certainly would have been more informative. However, we feel the overall pattern of response among our sites using glycine alone is still useful in evaluating microbial response to these substrates in intact systems with varying soil properties. Second, we added the same quantity of glycine to soils for each stand type rather than a fixed percent of background pools. Prior to our experiment, values for soil concentrations of FAA across our latitudinal gradient were not available and a priori characterization of soil FAA pool size for each stand type was not logistically feasible. Glycine amendments represented a 2–5-fold increase in FAA-N concentrations for our deciduous stands, but less than a 2-fold increase in FAA-N for the coniferous stands plus balsam poplar. Our experimental design reflects a trade-off between detecting the ^{13}C-tracer against background pools in an open system, and potentially initiating a priming effect. Therefore, it is worth noting that the response (e.g., k_{gly}) we measured in the field could be driven in part by an imbalance in nutrient to C ratios particularly among stands with narrow soil C:N. Finally, other factors, some of which we measured (soil temperature) and some of which we didn't (microbial C) could also have influenced FAA turnover among forest types. However, regardless of their contribution to the cycling dynamics of FAA, these effects were not strong enough to disrupt the robust relationship between the cycling rates of FAA and labile fractions of SOM across disparate forest types.

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 much this research effort 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 turnover rates for low-molecular-weight organic substrates such as amino acids.

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