

Rapid Cycling of Organic Nitrogen in Taiga Forest Ecosystems

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ABSTRACT

We examined the dynamics of organic nitrogen (N) turnover in situ across a primary successional sequence in interior Alaska, USA, in an attempt to understand the magnitude of these fluxes in cold, seasonally frozen soils. Through a combination of soil extraction procedures and measurements of ¹³C-enriched CO₂ efflux from soils amended in the field with ¹³C-labeled amino acids, we were able to trace the fate of this N form. Amino acid turnover in situ at soil temperatures of 10°C or below show that amino acids represent a highly dynamic soil N pool with turnover times of approximately 3–6 h. The rapid turnover of free amino acids is associated with high soil proteolytic activity, which in turn is tightly correlated with soil protein concentration. Moreover, these estimates of soil amino acid turnover in the field correspond well with measure-

ments of amino acid turnover under equivalent temperatures in the laboratory. The gross flux of amino acid-N over the growing season greatly exceeded the annual vegetation N requirement, suggesting that microbial biomass represent a significant sink for this organic N. Depending on the strength of this sink, N flow via free soil amino acids can potentially account for the entire N demand of vegetation in the absence of net N mineralization. These relationships underscore the important biogeochemical role of labile DON fractions in high-latitude forest ecosystems.

Key words: amino acid; boreal forests; DON; nitrogen cycling; protease activity; soil processes; taiga ecosystems.

INTRODUCTION

The growing momentum for the idea that plants can acquire organic nitrogen (N) directly, by short-circuiting the mineralization step, has contributed to a reevaluation of plant–nitrogen relationships in northern ecosystems (Kielland 2001; Lipson and Näsholm 2001 and references therein), as well as the conceptual framework for the N cycle in terrestrial ecosystems in general (Chapin 1995; Atkin 1996; Schimel and Bennett 2004). Organic N serves both as an important mineralization substrate (Jones 1999; Jones and Hodge

1999) as well as a direct source of N to a variety of plants in arctic (Kielland 1994; Schimel and Chapin 1996; Henry and Jefferies 2002; Nordin and others 2004), boreal (Näsholm and others 1998; McFarland and others 2002; Falkengren-Grerup and others 2004), and alpine ecosystems (Raab and others 1999; Lipson and others 1999a). Moreover, many agricultural species also readily absorb organic N (Yamagata and Ae 1996; Näsholm and others 2000; Okamoto and others 2003). The primary focus of many recent ecological studies of organic N has been the uptake of amino acid by plants, whereas the dynamics of amino acids in soil has received modest attention.

Forests underlain by permafrost are ubiquitous landscape features of the boreal regions of North America. In Alaska and Canada these forests are

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Table 1. Selected Physical and Chemical Parameters of Soils across the Primary Successional Sequence on the Tanana River Floodplain

Soil parameter	Willow	Alder	Balsam poplar	White spruce	Black spruce
Carbon (%)	1.5 (0.1)	2.6 (0.4)	6.9 (1.3)	10.5 (1.2)	25.5 (2.4)
Nitrogen (%)	0.07 (0.0)	0.14 (0.0)	0.39 (0.1)	0.43 (0.1)	0.85 (0.1)
TFAA: DIN	1.09	1.85	2.04	2.62	5.10
Moisture (%)	32 (0.9)	32 (0.2)	29 (0.3)	35 (0.1)	56 (0.3)
pH	8.1 (0.0)	8.2 (0.1)	6.7 (0.1)	6.1 (0.2)	5.1 (0.1)
$\Sigma^{\circ}\text{C}_{\text{soil}}$	1,206	841	633	664	427

TFAA and DIN refer to total free amino acids, and ammonium and nitrate, respectively. The temperature sum ($\Sigma^{\circ}\text{C}$) refers to soil temperatures at 10 cm depth during the growing season (June–September). (Mean \pm SE, $n = 15$).

typically dominated by black spruce (*Picea mariana*), and interspersed with tamarack (*Larix laricina*) and white spruce (*P. glauca*) depending on slope, aspect, and stand age (Viereck and others 1983). Low soil temperatures coupled with recalcitrant litter inputs from the coniferous canopy are attributed to slowing both decomposition (Fox and Van Cleve 1983), net N mineralization (Van Cleve and others 1993b), and productivity (Van Cleve and others 1981). These soils are typically high in organic matter, total N, and dissolved organic nitrogen (DON), but exhibit small pools of inorganic N and low rates of net N mineralization and nitrification (Walker 1989; Klingensmith and Van Cleve 1993; Kielland and others 2006b, in press). On the floodplains of interior Alaska, forest ecosystems are represented by a diversity of vegetation types, including deciduous stands of willow and alder in early succession to permafrost-dominated black spruce forests in late succession. Across these forest ecosystems net primary productivity and net N mineralization vary approximately five-fold (Van Cleve and others 1983; Klingensmith and van Cleve 1993; Ruess and others 2006) with concomitant large differences in other soil characteristics, including soil temperature, soil pH, and soil N composition.

Here we examine the dynamics of free amino acid turnover in situ across several taiga forest ecosystems that span a primary successional sequence encompassing young, alluvial soils dominated by deciduous vegetation through late successional coniferous forest with deep peat horizons and stands up to 300 years old. We hypothesized that turnover of soil amino acids would be most rapid in the relatively warm, early successional soils compared to the permafrost-dominated soils of late succession. To our knowledge this is the first study of amino acid turnover in the field in natural ecosystems.

METHODS

The study was conducted at the Bonanza Creek Taiga LTER sites approximately 20 km SW of Fairbanks, Alaska (65°45'N, 148°15'W), across a primary successional sequence along the Tanana River. These successional stages are named after the dominant species as follows: stage 1—willow (*Salix* sp.), 2—alder (*Alnus tenuifolia*), 3—balsam poplar (*Populus balsamifera*), 4—white spruce (*P. glauca*), and 5—black spruce (*P. mariana*). The soils in early floodplain succession are sandy, with a thin silt loam layer on the surface. Because of shallow water tables, the soil often remains very moist throughout most of the growing season. On older terraces (in later succession), the soils are predominantly silt textured. The soils are classed as Typic Cryofluvents (Orthic Regosols) (Viereck and others 1993). In the oldest stages of succession, dominated by coniferous forests (white and black spruce), the silt loam soils are cold and wet, and in the case of black spruce stands, often underlain by shallow permafrost (Van Cleve and others 1993a). Soils in these stages of succession are classed as Histic Pergelic Cryaquepts (Gleysolic Static Cryosols) (Viereck and others 1993). Soil carbon and N content increase with succession, whereas soil pH decreases. Similarly, soil heat sums decrease across succession, reflecting the insulative effects of organic matter accumulation, a continuous moss cover, and an eventual dominance of permafrost. Selected soil physical and chemical properties in each stand type are presented in Table 1. The climate is strongly continental with temperature extremes ranging from -50°C in winter to more than $+30^{\circ}\text{C}$ during the summer. Average annual precipitation is 269 mm, 37% of which falls as snow. Snow covers the ground 6–7 months of the year. Complete site descriptions regarding climate, vegetation, and

soils can be found through the web address for the Bonanza Creek LTER Program: <http://www.lter.uaf.edu/>.

We conducted tracer experiments in August 2002 in all the five forest types representative of the primary successional sequence on the Tanana River floodplain as described above. We injected 90 µg amino acid-N in 9 ml DI water of equimolar amino acid solutions containing U-¹³C-labeled alanine, aspartic acid, and glycine into the top 10 cm of the soil. These amino acids are common in Alaskan taiga soils across succession (Werdin 2006). The solutions were injected in a 9-point hexagonal pattern using a double side-port spinal syringe (McFarland and others 2002; Kielland and others 2006a). The templates measured 81 × 15 cm and consisted of three 8 cm diameter injection grids with nine holes each. The amount of substrate was equal to 11–87% of the background concentrations of free amino acids. Syringes were raised during injections to evenly distribute the label throughout the 10-cm soil core. Injections were replicated three times in each successional stand. Prior to the injections we collected background ¹³C values of CO₂ released from the soil. We recovered the label as respired ¹³C–CO₂ using a 10 cm diameter (800 cm³) PVC chamber, fitted with gas sampling ports. The chamber was firmly placed over the injection grid for 3 min at which time a gas sample was collected after thoroughly mixing the chamber by slowly pumping the sample syringe ten times. Gas samples of 15 ml were withdrawn from the chamber and injected overpressurized into 10 ml exetainers (Labco. Ltd., UK) evacuated to 50 µm Hg at 5 time periods after injection: 0.5, 2, 4, 6, and 24 h. Because of the logistics involved in river boat travel between sites, the actual time of sampling varied somewhat from this schedule as indicated in data presentation. Soil temperatures were measured at a depth of 10 cm during each sampling period. Gas samples were analyzed for ¹³C–CO₂ using a Europa Scientific continuous flow mass spectrometer (SPEC-PDZ Europa, Inc.). The data are expressed as atom percent enrichment (APE), which was determined by subtracting the atom% ¹³C of control cores from the atom% ¹³C of treated cores. Control values were averaged within a site prior the experiment for estimating isotope enrichment. To estimate the turnover of amino acids in the field we fitted rate equations to the ¹³C–CO₂ efflux data, using non-linear regression in the curve-fitting program Axum[®] 7.0 (MathSoft, Cambridge, MA, USA 2001). These equations take the following general form:

$$A_t = A_0^*(1 - e^{-k*t}), \quad (1)$$

where A_t is the ¹³C APE of the CO₂, A_0 is maximum isotopic enrichment over the experimental period, k is the rate constant, and t is time. We estimated gross flux of amino acid in each successional soil as the product of the soil amino acid concentration and the individual rate constants derived from each successional soil.

Soils were sampled monthly from prior to green-up in May to freeze-up in early October. Samples were taken to a depth of 10 cm (excluding live moss and previous-year's litter) with a 5.5 cm diameter stainless steel corer at six random intervals along randomly oriented 50 m transects in each successional stage. Each of the five successional stages was replicated three times to avoid pseudoreplication (Hurlburt 1984). The samples were kept cool during transport to laboratory, where individual cores were homogenized by hand and woody debris larger than 1 cm in diameter was removed. Here we report average seasonal values for nitrate, ammonium, and free amino acids based on these sampling times.

Soil protein was extracted with NaCO₃ from fresh field-moist samples (Lipson and others 1999b), and analyzed using the Bradford procedure (Bradford 1976). Total free soil amino acids were measured using a modified ninhydrin method (Moore 1968; Lipson and Monson 1998) with leucine as a standard. Because ninhydrin reacts with amine groups other than free amino acids, notably ammonium, free amino acid concentrations were calculated after subtraction of ammonium (Berthrong and Finzi 2006). Potential soil proteolytic activity was measured in the presence of toluene (200 µl/g soil) using the ninhydrin reaction (Watanabe and Hayano 1995). Soil samples were incubated in a 50 mM sodium citrate buffer (Lipson and others 1999b), where the pH was adjusted to match specific soils. Amino acid production was measured every hour for six hours and the reaction stopped with the addition of TCA (Ladd and Butler 1972; Lipson and others 1999b). Absorbance at 570 nm was measured on a PerkinElmer UV/Vis Lambda 25 spectrometer. Concentrations of soil ammonium and nitrate were determined on soils extracted with 0.5 M K₂SO₄, equilibrated for 12 h (Robertson and others 1999), then analyzed by flow-injection colorimetry using a modified Technicon II autoanalyzer (Whitledge and others 1981). Concentrations of soil N fractions were analyzed using one-way analysis of variance (ANOVA) using Statistix 8.0 (Analytical Software, Inc, Tallahassee, FL, USA)

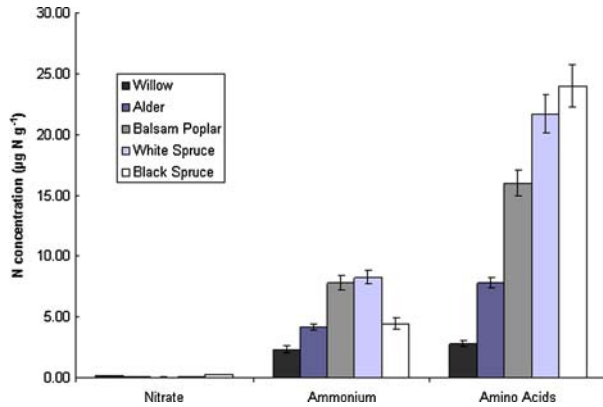


Figure 1. Concentrations of soil nitrate, ammonium and free amino acids across a primary successional sequence on the Tanana River, interior Alaska. Values are seasonal average concentrations from monthly measurements in June–October. Mean \pm SE, $n = 3$.

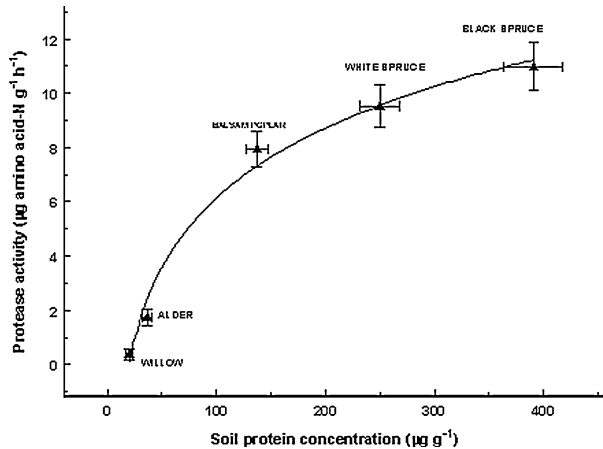


Figure 2. Soil proteolytic activity in relation to soil protein concentrations across a primary successional sequence on the Tanana River floodplain, interior Alaska. Mean \pm SE, $n = 3$.

followed by Tukey's multiple comparisons test when results from the ANOVA were significant. Relationships between soil proteolysis, amino acid turnover and edaphic characteristics were examined through linear regression on untransformed data.

RESULTS

Soil amino acid concentrations differed significantly ($F = 26.5$, $P < 0.0001$) among successional stages, increasing nearly tenfold over the successional sequence, from $2.7 \mu\text{g N g}^{-1}$ in willow stage soil to over $24 \mu\text{g N g}^{-1}$ in black spruce soils

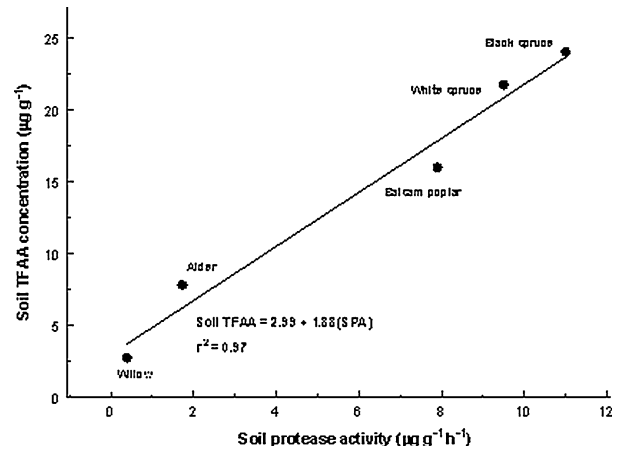


Figure 3. Relationship between soil protease activity and soil total free amino acid (TFAA) concentrations across successional soils on the Tanana River floodplain, interior Alaska. Mean \pm SE, $n = 3-6$.

(Figure 1). Soil ammonium concentrations also showed significant differences among stages ($F = 13.7$, $P < 0.0001$), but varied less and exhibited a different pattern across succession from soil amino acid-N. Concentrations of soil nitrate were very low. The ratio of free amino acid-N to DIN (NH_4^+ plus NO_3^-) increased from near unity in early succession (willow stage) to approximately 5:1 in late succession (black spruce stage) (Table 1).

Soil protease activity exhibited a curvilinear relationship to soil protein concentration across succession in a manner suggestive of Michaelis-Menten kinetics (Figure 2). We found a significant relationship between soil protease activity and soil amino acid concentration across the successional sequence (Figure 3), indicating that the latter is under strong productive control. The estimated rates of gross amino acid production based on these rates of protease activity were much greater than previous estimates of gross N mineralization in these forests (Vance and Chapin 2001), which may in part explain the high concentration of free amino acids in taiga soils.

Amino Acid Turnover in the Field

The pattern of amino acid turnover in situ was very similar across the successional sequence, with all soils exhibiting a rapid efflux of the label over the first 4–6 h of the experiment. However, the rate of $^{13}\text{C-CO}_2$ efflux was inversely related to successional age. That is, the most rapid rates were observed in late-successional black spruce soils and the slowest rates were observed in early-successional willow stands (Figure 4). Turnover

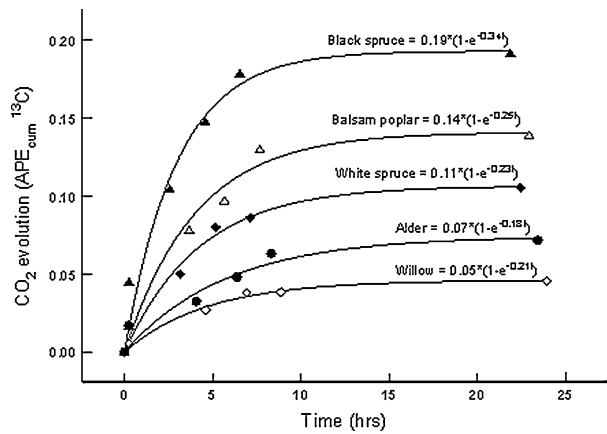


Figure 4. Time course of ^{13}C -carbon dioxide evolution in situ from successional soils amended with aspartic acid, glycine, and alanine. Values expressed as atom% enrichment of ^{13}C - CO_2 . Mean \pm SE, $n = 3$.

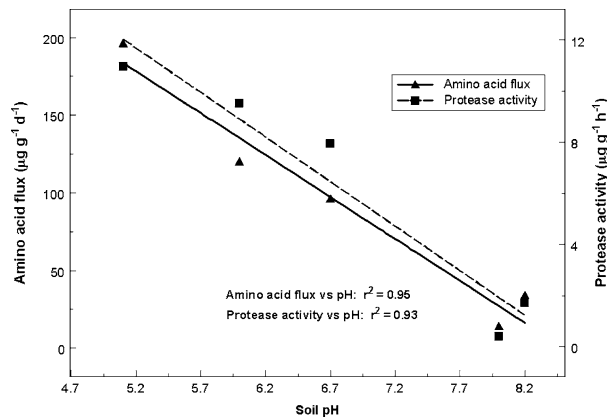


Figure 5. Relationship between soil pH, soil proteolytic activity and soil amino acid flux across the primary successional sequence on the Tanana River floodplain, interior Alaska.

times for soil free amino acids ranged between approximately 3 h (black spruce) and 6 h (willow). Thus, the capacity of the soils to mineralize amino acids in situ was not offset by the large differences in prevailing ambient soil temperature ($\approx \Delta 10^\circ\text{C}$) across successional stages. Estimated rates of potential amino acid flux based on the observed rates of amino acid turnover in the field and the seasonal average amino acid concentration ranged approximately 14-fold, being lowest in willow stands and highest in black spruce soils. Both soil protease activity and amino acid flux were highly correlated with soil pH, which explained over 90% of the variation in these processes (Figure 5).

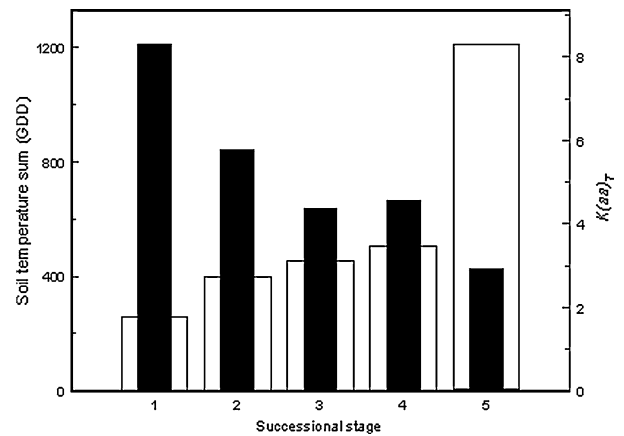


Figure 6. Soil amino acid turnover (*open bars*) expressed as "thermal capacity" ($K(aa)_T$), the rate constant for soil amino acid turnover in the field divided by the average soil temperature during the experiment ($\times 100$), and the respective seasonal soil temperature sums across succession (*filled bars*). The successional stages 1–5 are: 1 willow, 2 alder, 3 balsam poplar, 4 white spruce, 5 black spruce.

DISCUSSION

Proteinaceous N forms dominate the soil organic N pool in most natural ecosystems (Schulten and Schnitzer 1998). Moreover, increases in absolute concentrations of labile soil amino acids and protein across the successional sequence were also associated with greater dominance of these compounds relative to DIN in the soil solution, as the ratios of free amino acids to DIN ranged from approximately unity in early succession to approximately 5:1 in late succession (Table 1). The concentrations of soil protein and protease activities were quite similar to concentrations and activities observed in arctic tundra (Weintraub and Schimel 2005), but higher than those reported from alpine tundra (Lipson and others 1999a). However, we observed no significant increase in proteolytic activity when soils were amended with protein (data not shown), suggesting that protease activity was not substrate limited.

Contrary to our central hypothesis, the rate of amino acid turnover increased across succession, despite the significant reduction in soil temperatures ($12\text{--}3^\circ\text{C}$) as well as a shift in vegetation from deciduous to evergreen-dominated (coniferous) forests, with concomitant increases in soil C:N ratio (Table 1) and decrease in soil organic matter turnover (Flanagan and Van Cleve 1983). Thus, we reject our initial hypothesis that the apparent recalcitrant soil organic matter and low soil temper-

atures in late successional coniferous ecosystems (Van Cleve and others 1983; Klingensmith and Van Cleve 1993) result in low rates of organic N turnover. In particular, the nearly twofold increase in the rate amino acid turnover between early successional shrub communities vs. late-succession black spruce forests, despite a nearly 10°C difference in ambient soil temperatures (during the experiment), suggest that cold, late successional soils exhibit an apparent temperature compensation for amino acid turnover. Normalizing amino acid turnover for in situ differences in soil temperature (by dividing the fractional turnover rate, k , by the average soil temperature during the experiment), magnified the difference between early and late successional soils (Figure 6). Analogous relationships to temperature have been observed for organic matter turnover both in laboratory and field experiments (Kirschbaum 2004), and may be pertinent to soil N turnover as well. The control of amino acid turnover by temperature can vary among specific amino acids (Vinolas and others 2001), but the temperature response of amino acid turnover is minor between 1 and 10°C (Vinolas and others 2001), which may in part explain our results.

Microbial communities in taiga soils are dominated by fungi rather than bacteria (Flanagan and Van Cleve 1983). Though we have very little information regarding precise changes in microbial community composition across primary succession in interior Alaska, we know that fungal biomass (active mycelium) increases as a function of increases in soil organic matter (Flanagan and Van Cleve 1983). This increase in microbial activity is another potential factor explaining the high rate of N turnover in late successional soils.

We recognize that our estimates of soil amino acid turnover and associated N fluxes are in part a reflection of the substrates we chose for our analysis (Lipson and others 1999a; Jones and others 2005). Aspartate, alanine, and glycine are common amino acids in high-latitude ecosystems (Kielland 1995; Henry and Jefferies 2002; Werdin 2006). Amino acids such as aspartate and glutamate are rapidly degraded by soil microbes, whereas glycine turns over at a slower rate (Kielland 1995; Lipson and others 1999a; Vinolas and others 2001; Jones and Kielland 2002). Thus, we surmise that the mixture of amino acids used in the present study is fairly representative of the behavior of the common amino acids in these soils. Although not part of the present study, we know from previous research that amino acids may be differentially partitioned between respiration and microbial biomass pro-

duction (Lipson and others 1999b; Vinolas and others 2001; Persson and others 2003). In mid to late-successional white spruce stands only 8% of the amino-acid carbon applied in situ was released as CO₂ (McFarland and others in prep), whereas in a black spruce ecosystem microbes in vitro consistently respired 25% of the amino-acid carbon while the remainder was incorporated into new cell biomass (Jones and Kielland 2002). These observations suggest that our current estimates of organic N turnover in black spruce are relatively conservative.

We do not know the causative relationship between soil protease activity and soil protein concentration. Evidence has been advanced from a diversity of ecosystems in support of control over protease activity by increased protein concentration (Lipson and others 1999a) or induction of proteolysis by N deficiency (Smith and others 1989; Weintraub and Schimel 2005). In the present study, the high concentrations of organic N in the form of amino acids appear to be sustained through high proteolytic activity. This observation can in part be explained by pronounced increases in soil acidity in late succession which favors proteolysis to a greater extent than net N mineralization (Read and Bajwa 1985; Leake and others 1987; Chapin and others 1988). Decreased soil pH may also accelerate amino acid turnover. For example, in an upland black spruce ecosystem, amino acid turnover increased fourfold with a drop in soil acidity by less than half a pH unit (Jones and Kielland 2002).

Our finding that late successional, coniferous ecosystems, particularly permafrost-dominated black spruce forests appear to exhibit greater pool sizes of labile DON, higher rates of turnover of these pools, and consequently greater fluxes of organic N than the warmer, more productive deciduous forests, suggests that some revision of conventional paradigms of N cycling in high-latitude ecosystems is warranted. Soil organic matter quality (OMQ) is a major control over decomposition in Alaskan taiga forests (Flanagan and Van Cleve 1983; Fox and Van Cleve 1983; Kielland and others 2006b, in press). At first glance black spruce soils would be expected to have comparatively low OMQ, as indicated by Jenny's k (aboveground production divided by forest floor biomass) (Fox and Van Cleve 1983) and ratio of soil lignin to soil N. However, concentration of forest-floor lignin in alder stands is actually nearly twice that of spruce. Furthermore, indices such as soil C:N ratios or Jenny's k (Jenny 1980) represent an average of total soil carbon (and nitrogen pools), which yield

little information about small, labile pools with very rapid turnover. We hypothesize that the fast turnover of soil amino acids in black spruce, and possibly other soils, is in part a consequence of the uncoupling of protein/amino acid dynamics from soil organic matter decomposition. That is, soil amino acids are a small, but rapidly cycling N pool (Kuz'yakov 1996; Schimel and Bennett 2004), with production and consumption dynamics very different from that of the bulk soil C pool. Rapid decay and decomposition of fine roots may be the source of this labile N pool in interior Alaska black spruce stands, where fine root production accounts for nearly 60% of total stand production (Ruess and others 2003). Moreover, concentrations of proteinaceous N in the plant cell are high relative to the surrounding bulk soil and lysis of fine-root epidermal cells could contribute substantially to the rapidly cycling DON pool (Jones and Darrah 1994).

CONCLUSION

The cold, seasonally frozen soils typical of taiga forests in interior Alaska are strongly dominated by organic N forms, the labile fractions of which are principally proteins and amino acids. Nitrate pools are largely undetectable in floodplain ecosystems irrespective of successional stage, leaving these soils ammonium-dominated. Free amino acid concentration increases nearly tenfold across succession, and the ratio of free amino acid-N to inorganic N increases approximately fivefold.

In the present study gross amino acid production (protease activity) could account for the turnover of the entire pool of soil free amino acids. Amino acid turnover in situ at soil temperatures below 10°C show that amino acids represent a highly dynamic soil N pool with turnover times of approximately 3–6 h. The rapid turnover of free amino acids is associated with high soil proteolytic activity, which in turn is tightly correlated with soil protein concentration and pH. Both amino acid production and consumption are better explained by changes in soil pH, than site variation in soil temperature and moisture, suggesting that the controls over the dynamics of organic N involve additional controls than those typically invoked for inorganic N fluxes. We hypothesize that the rapid turnover of amino acids in black spruce soils is more closely associated with high rates of fine root production, mortality and decomposition, than with the decomposition of bulk soil organic matter.

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