



Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils

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Abstract

Black spruce (*Picea mariana*) forests represent the dominant vegetation type throughout the North American and Siberian taiga and are generally considered to be pristine, N-limiting environments. The aim of this study was to investigate the fundamental underlying mechanisms which control N availability in these soils with particular reference to the dynamics of dissolved organic nitrogen (DON). Our results showed that in these highly organic and low pH soils, soluble N is dominated by organic forms with correspondingly low concentrations of ammonium and nitrate. Amino acids, which are known to be directly taken up by plants growing in these soils, were calculated to constitute 10–20% of the total DON pool. The microbial mineralization of amino acids was rapid in all surface horizons (L, O and Ah; $t_{1/2} = 5$ h), conformed well to Michaelis–Menten kinetics and appeared to have a common mineralization pathway across all horizons and soils tested. The results indicated that the amino acid pool of soil solution is extremely transient, turning over approximately 20 times per day. From these results, we suggest that the transformation of protein to amino acids and not amino acids to NH_4^+ is the major factor limiting N availability in these soils. As DON turnover constitutes a large proportion of the annual N flux in these forests, it warrants inclusion in models of climate change in high-latitude ecosystems. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Amino acids; Dissolved organic nitrogen; Microbial kinetics; N mineralization; N flux; Permafrost; Taiga ecosystems

1. Introduction

Black spruce (*Picea mariana*) ecosystems are the dominant vegetation type throughout much of the northern forested areas in Siberia and North America, including Alaska. The distribution of this forest type corresponds closely with the distribution of discontinuous permafrost, attesting to the cold temperature regimes that characterize these soils. Low soil temperatures, coupled with high moisture content, high organic matter content, and low pH, renders these soils ammonium-dominated with relatively low rates of nitrification in situ (Van Cleve and Alexander, 1981; Walker, 1989; Klingensmith and Van Cleve, 1993). The low rate of annual net nitrogen mineralization is thought to represent a major bottleneck in the flux of N in both taiga and tundra systems (Van Cleve and Alexander, 1981; Chapin, 1983). However, recent calculations of the nitrogen budget in taiga ecosystems suggest that the fluxes of inorganic N (dry and wet deposition, N fixation and

mineralization) cannot account for plant N uptake (Ruess et al., 1996). In black spruce forests, these conventional measures of N flux represent less than 50% of the annual N requirement of the vegetation (Kielland, 2001). The reason for this discrepancy between N supply and N demand might be that taiga trees absorb part of their nitrogen in an organic form (Näsholm et al., 1998). Soluble organic N concentrations in soils are typically in an order of magnitude greater than the combined pools of ammonium and nitrate, and the organic N fraction contains a large proportion of free amino acids. The recent appreciation that Arctic and subArctic vegetation can acquire amino acids directly, i.e. in the absence of N mineralization, has focused renewed attention on the role of soluble organic soil N in plant nutrition and nutrient cycling (Chapin, 1995; Atkin, 1996; Raab et al., 1996; Lipson and Monson, 1998). Taiga ecosystems have very high soil organic N stocks (Van Cleve and Alexander, 1981) and a large proportion of the forest biomass exists in the form of fine roots and mycorrhizas (Ruess et al., 1996). Further, the potentially rapid turnover of amino acids in a variety of soils, ranging from temperate grasslands (Jones, 1999) to Arctic tundra (Kielland, 1995), suggest that this soil N fraction accounts for a major proportion of

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Table 1
Correlation matrix for soil parameters from 22 individual soil samples collected from under black spruce forests in Alaska. Asterisks represent significant differences where * is $P < 0.05$, ** is $P < 0.01$, and *** is $P < 0.001$

	Soil pH	Moisture content	Total C	Total N	DIN ^a	DON	Basal respiration ^b	Glucose SIR ^c	Arginine SIR ^d	Amino acid half-life ^e
Moisture content	0.29									
Total C	0.51*	0.74***								
Total N	0.44*	0.52*	0.69***							
DIN	0.02	0.09	0.02	0.08						
DON	0.33	0.09	0.25	0.40	0.16					
Basal respiration	0.43	0.04	0.29	0.17	0.18	0.18				
Glucose SIR	0.47	0.06	0.47	0.22	0.14	0.22	0.92***			
Arginine SIR	0.62**	0.35	0.71***	0.51	0.01	0.34	0.41	0.57**		
Amino acid half-life	0.63**	0.30	0.54**	0.57**	0.01	0.17	0.58**	0.61**	0.62**	
Soil C/N	0.23	0.36	0.51	0.06	0.01	0.01	0.01	0.20	0.25	0.19

^a Dissolved inorganic N (i.e. $\text{NH}_4^+ + \text{NO}_3^-$).

^b Basal respiration = non-substrate induced.

^c SIR indicates substrate induced respiration with glucose.

^d Substrate induced respiration with arginine (Fig. 7(A)).

^e Amino acid half-life in soil when a mixture of amino acids are added (Fig. 7(B)).

the annual total soil N flux. Here we examine the rates of amino acid turnover in permafrost-dominated black spruce forest in interior Alaska, and discuss the controls relative to the flux of inorganic N, in the context of present climatic change that has been documented in this environment.

2. Materials and methods

2.1. Soil sites and sampling regime

The study was conducted near Fairbanks, Alaska (64°51'N, 147°43'W, elevation 305 m) in two upland black spruce (*P. mariana*) taiga forests. One site (Site 1) was located just west of Fairbanks in the Cripple Creek catchment, and one (Site 2) was located approximately 20 km north of Fairbanks in the Caribou Creek catchment. The latter site is part of the Bonanza Creek Long Term Ecological Research program boreal forest site network. The mean annual air temperature is -3.3°C (minimum in December–January of -51°C , maximum in July–August of 32°C), and mean annual precipitation 269 mm yr^{-1} , of which approximately 37% falls as snow (Viereck et al., 1993). Soil degree days (above 0°C) average 640 and annual above-ground tree productivity is approximately $110\text{ g m}^{-2}\text{ yr}^{-1}$ (Viereck et al., 1983). The mean soil temperature in July–August ranges from 0 to 10°C with a mean temperature of 6°C . The soils are classified as Histic Pergelic Cryaquepts, derived from a loess parent material (Viereck et al., 1983). Both sites are characterized by poor drainage and are high in organic matter content, relatively acidic, and underlain by permafrost (Van Cleve et al., 1983). The forest floor chemistry is characterized by high lignin content and high C/N ratios. These forests exhibit very low rates of decomposition and nitrogen mineralization (Fox

and Van Cleve, 1983; Van Cleve et al., 1983; Klingensmith and Van Cleve, 1993).

Soil samples were collected with a 6.5 cm i.d. stainless steel corer to a depth of 40 cm during mid-August, 1999. Thirty samples were randomly collected along a 200 m transect in the Cripple Creek catchment (Site 1) and 12 samples were collected from the Caribou Creek catchment (Site 2). Soil cores were subsequently sub-divided based on horizon (L, O and Ah). At each site, soil was sieved to pass 1 cm and stored in sealed, gas permeable plastic bags at $2 \pm 1^\circ\text{C}$ to await analysis. Soils were stored for a maximum of six months over which time basal respiration or $\text{NH}_4^+/\text{NO}_3^-$ contents did not change significantly from those analyzed immediately after collection in agreement with Jones and Shannon (1999). For each analysis, unless otherwise stated, six cores from Site 1 and six cores from Site 2 were randomly selected and used for each analysis.

2.2. Soil characterization

Moisture content was determined by oven-drying for 24 h (80°C for L and O horizons; 105° for Ah horizons), while pH and electrical conductivity were determined in 1:1 (v/v) soil/water extracts (Smith and Doran, 1996). Soil NO_3^- -N, NH_4^+ -N and dissolved organic N (DON) concentrations were estimated by performing 1:10 (w/v) soil/0.5 M K_2SO_4 extractions on a reciprocating shaker (30 min, 10°C) followed by centrifugation at $10\,000\text{g}$ (30 min, 4°C) and storage of supernatant solutions at -20°C . Extract N concentrations were determined using the colorimetric procedures of Downes (1978) for NO_3^- , the hydrazine, *N*-1-naphthylethylenediamine assay; Keeney and Nelson (1982) for NH_4^+ , the salicylic acid assay; and Williams et al. (1994) for DON the persulfate-autoclave oxidation assay. Total organic C and total N were determined on oven-dried soil samples using a LECO

CHN2000 analyzer (Leco Corporation, St Joseph, MI). Basal, non-substrate induced, soil respiration was determined by placing 10 g of field-moist soil in a 50 ml respirometer cuvette and measuring CO₂ production continuously at 10 ± 1 °C over a 6-h period using an automated CIRAS-IRGA soil respirometer (PP Systems Ltd, Hitchin, UK). Soil respiration values were taken as the mean CO₂ output after steady state had been achieved (typically 0.5–1 h after removal from storage).

2.3. Mineralization rate of amino acid mixtures

Amino acid mineralization was determined by adding a mixture of 15 uniformly ¹⁴C labeled amino acids to soil and their subsequent evolution as ¹⁴CO₂ measured over a 24-h period. The standard amino acid mixture (pH 5.60) was obtained by mixing together the following L-isomeric amino acids in equimolar proportions to give an individual amino acid concentration of 333 μM and specific activity of 0.4 kBq ml⁻¹: alanine, arginine, aspartate acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine. The concentration was chosen based upon the total amount of amino acid in plant cells (5–20 mM; Jones and Darrah, 1994) and the likely concentration present in the soil after lysis of a root epidermal cell. Briefly, 500 μl of the standard amino acid mixture was mixed with 5 g of soil in a 50 ml polypropylene tube. A 1 ml, 1 M NaOH trap was added to each sample to capture evolved ¹⁴CO₂ and the tubes sealed with gas-tight stoppers. Samples were incubated at 10 ± 1 °C with changes of the NaOH trap after 1, 3, 6 and 24 h. This temperature reflects those experienced by soil microorganisms on a summer day (July–August). After 24 h, the amino acid remaining in the soil (free plus exchangeable; ¹⁴C_{K₂SO₄}) was removed by performing 1:10 (w/v) soil/0.5 M K₂SO₄ extracts as described earlier and in Jones and Hodge (1999). Amino acid mineralization in replicate cores from each site were performed in duplicate and all radioactivity was determined by liquid scintillation counting using a Wallac 1404 liquid scintillation counter and Wallac Optiphase 3 scintillation fluid (Wallac EG and G Ltd, Milton Keynes, UK). The amount of ¹⁴C present in the microbial biomass (¹⁴C_{biomass}) after 24 h was determined as follows

$$^{14}\text{C}_{\text{biomass}} = ^{14}\text{C}_{\text{total}} - (^{14}\text{CO}_2 + ^{14}\text{C}_{\text{K}_2\text{SO}_4}) \quad (1)$$

where ¹⁴C_{total} is defined as the total amount of ¹⁴C amino acid added at the start of the experiment. The amount of amino acid C partitioned into microbial biomass versus respiration (i.e. microbial yield; Y_{mic}) was calculated as follows:

$$Y_{\text{mic}} = ^{14}\text{C}_{\text{biomass}} / (^{14}\text{C}_{\text{biomass}} + ^{14}\text{CO}_2) \quad (2)$$

2.4. Amino acid uptake kinetics

The microbial uptake of glycine, glutamate and lysine, which represent the neutral, acidic and basic groups of amino acids, respectively, were determined by measuring the depletion of uniformly ¹⁴C labeled amino acids from the soil solution and subsequent production of ¹⁴CO₂. Three contrasting soils were used; a high biological activity topsoil (L2 horizon) and a low biological activity subsoil (Ah horizon) from Site 1 and a topsoil (L/O horizon) from Site 2. Briefly, 100 μl of an individual amino acid (4 kBq ml⁻¹) ranging from 50 to 1000 μM mixture was mixed with 1 g of soil in a 5 ml polypropylene tube. A 1 ml, 1 M NaOH trap was added to each sample to capture evolved ¹⁴CO₂ and the tubes sealed with gas-tight stoppers. Samples were incubated at 10 ± 1 °C for 1 h, after which the ¹⁴CO₂ production and amino acid remaining in solution (¹⁴C_{K₂SO₄}) were determined as described earlier. Duplicate samples were used for each individual concentration.

2.5. Substrate induced respiration

Substrate induced respiration (SIR) was measured using a CIRAS-IRGA as described earlier but after the addition of 1 ml of 100 mM glucose to the soil, 2 h after steady state, basal respiration at 10 ± 1 °C had been achieved. SIR CO₂ outputs were calculated after a new quasi steady-state level had been achieved (typically 1 h after glucose addition).

To determine the time-dependent response of the soil microbial biomass to additions of N, inorganic N (NO₃⁻ or NH₄⁺) or DON (urea, glycine, glutamate, aspartate, lysine, phenylalanine, methionine, serine, leucine and arginine, bovine serum albumin, casein) were added to the soil as described earlier for glucose with a solution concentration of 25 mM (inorganic N, urea, amino acids) or 3 mg g⁻¹ (protein; corresponds to 25 mM amino acids assuming an average amino acid M_w of 120). SIR rates were compared against controls in which a distilled water solution was added to the soil at an identical rate. Two independent replicates of each treatment were undertaken.

2.6. N mineralization assays

To compare the rates and stoichiometry of amino acid C mineralization and inorganic N release at both sites, 1 g of soil was incubated with 100 μl of ¹⁴C labeled arginine (25 mM) for 24 h at 10 °C with ¹⁴CO₂ evolution measured as described earlier. After 24 h, the soil was extracted with 0.5 M K₂SO₄ and NH₄⁺ and NO₃⁻ concentrations measured as described earlier. Control incubations were performed where arginine was replaced with distilled water. Net N mineralization was calculated as the difference between the arginine and the distilled water incubations. Two replicates of each soil horizon was used in this assay.

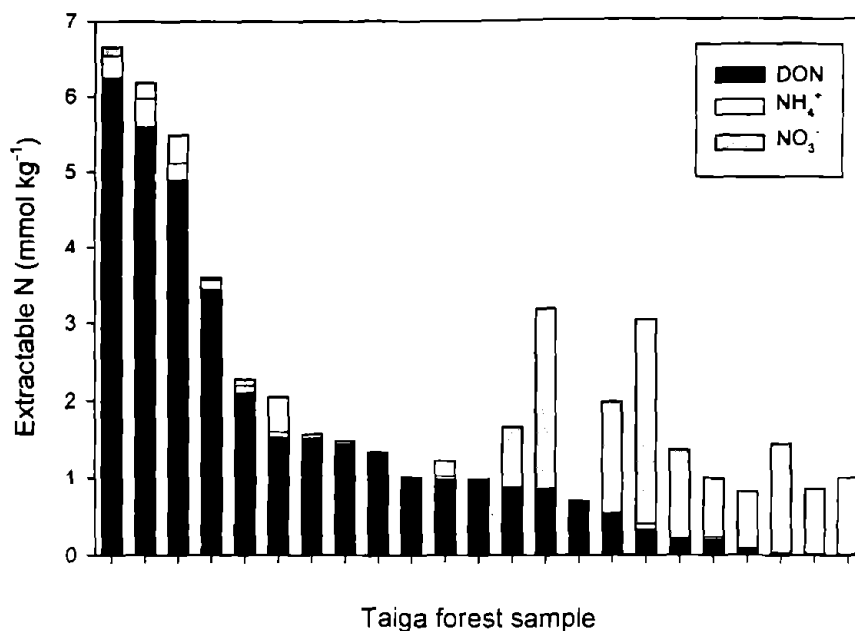


Fig. 1. K_2SO_4 -extractable dissolved organic N (DON), ammonium and nitrate present in 22 taiga forest soil samples. Samples are ranked in order of DON concentration with the same sample order used for NO_3^- and NH_4^+ panels.

2.7. Statistical and data analysis

Amino acid half-lives were estimated by linear interpolation of respiration curves as described in Jones and Hodge (1999) and Jones and Shannon (1999) using the computer package Sigmaplot 4.01 (SPSS Inc, Chicago, ILL). SIR response times were calculated by linear interpolation using Sigmaplot 4.01 with response time defined as the time taken to achieve half-maximal CO_2 evolution (SIR_{50}). To mathematically describe the microbial uptake kinetics of individual amino acids, the utilization rates (V , $\mu mol g^{-1} soil h^{-1}$) were fitted to a single Michaelis–Menten equation using a computerized least squares optimization procedure using Sigmaplot 4.01 where the uptake equation takes the form

$$V = (V_{max}C)/(C + K_m) \quad (3)$$

where V_{max} is the maximum uptake rate ($\mu mol g^{-1} soil h^{-1}$), C is substrate concentration (μM) and K_m is the concentration at which $V_{max}/2$ occurs (affinity constant, μM). All linear regression analyses were performed using Sigmaplot 4.01.

3. Results

The soils collected for this study were generally characterized by highly organic surface horizons which possess a high C/N ratio, low pH, high moisture content and constitute the upper 20–30 cm of the soil profile. These surface horizons are underlain by predominantly mineral Ah soils which have a significantly ($P < 0.001$) lower organic C content,

lower C/N ratio, higher pH and lower moisture content. Across all the samples analyzed at both sites, there were close correlations between soil moisture, pH and organic matter content (Table 1).

The amount of soluble organic and inorganic N recovered in K_2SO_4 extracts of 22 black spruce soils from both sites is shown in Fig. 1. The mean concentration of DON across all samples was $1.59 \pm 0.36 mmol N kg^{-1}$, approximately twofold greater than the total inorganic N present in the samples ($NH_4^+ + NO_3^-$; $0.77 \pm 0.17 mmol N kg^{-1}$). A close linear correlation was observed between the amount of DON and NH_4^+ present in the samples ($r^2 = 0.87$) although the amount of DON was on average 45 ± 8 fold greater than the amount of NH_4^+ present. This result is similar to previous studies in Arctic tundra soils (Kielland, 1997). We found an inverse relationship between the concentrations of soil DON and NO_3^- (Fig. 1). Typically, the levels of DON were much greater in the organic surface horizons ($2.2 \pm 0.5 mmol N kg^{-1}$) in comparison to subsurface Ah mineral horizons ($0.5 \pm 0.3 mmol N kg^{-1}$). In contrast, the levels of NO_3^- were higher in mineral horizons ($0.8 \pm 0.3 mmol N kg^{-1}$) in comparison to organic surface horizons ($0.3 \pm 0.2 mmol N kg^{-1}$). Comparison with total N values indicated that $99.75 \pm 0.04\%$ of the N in the soil was present in an insoluble form whilst 0.24% could be recovered as DON. The findings are similar to other studies of high-latitude soils (Kielland, 2001).

The rate of amino acid mineralization is shown in Fig. 2. Only the results for one soil core from Site 1 are presented; however, the results are representative of all the soil cores tested. Generally, mineralization of the amino acids to CO_2 was most rapid in the surface L and O1 horizons in

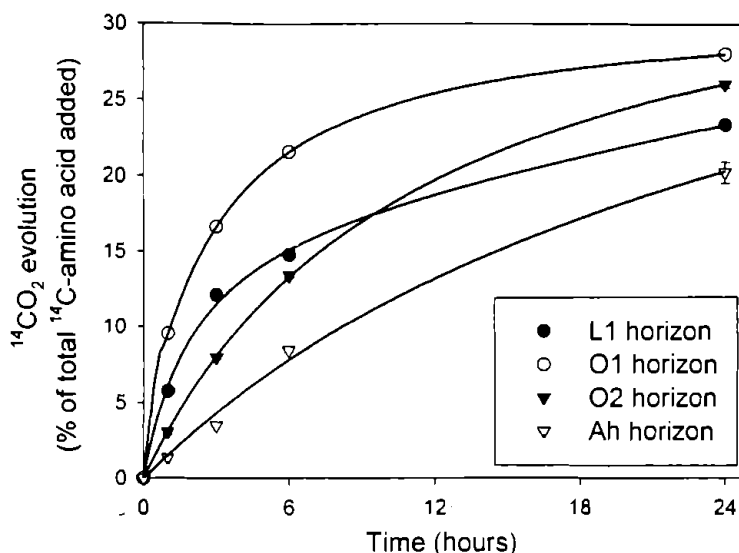


Fig. 2. Impact of soil horizon on the time-dependent mineralization of ^{14}C labeled amino acids to $^{14}\text{CO}_2$. Values represent means \pm SEM ($n = 2$).

comparison to Ah horizons. The mean half-life ($t_{1/2}$) of the amino acids in soil was estimated based upon the amount of amino acid mineralized after 7 days (data not presented). At this time-point, >98% of the amino acids had been removed from the soil. For the L horizon, the mean $t_{1/2}$ was estimated to be 4.0 ± 0.7 h while for the O horizon, it was 6.4 ± 1.0 h and for the Ah horizon, 13.9 ± 0.7 h.

After accounting for the amino acid still remaining in the soil after 24 h, the C partitioning within the microbial biomass was determined (Fig. 3). Across all soils and depths, the microbial biomass consistently utilized 25% of the amino acid C for respiration while the remainder was incorporated into new cell biomass. The pattern of C partitioning was independent of the rate of substrate utilization (Fig. 3). The rate of amino acid turnover was proportional to the total soil N concentration, but inversely related to soil pH (Fig. 4). However, other soil properties including C/N ratio, moisture content, DON and dissolved inorganic N (DIN) concentrations, were insignificantly related to this process (Table 1).

The concentration-dependent uptake of three contrastingly charged amino acids (glutamate $^{-1}$, glycine 0 and lysine $^{+1}$) by the soil microbial community in three soil horizons is shown in Fig. 5. In agreement with the amino acid mineralization results presented in Fig. 2, uptake followed the horizon series L > O > Ah. The uptake of the amino acids by the soil microbial pool was amino acid-dependent following the series glutamate > lysine > glycine in all three soil horizons. The uptake of the amino acids appeared to show saturating tendencies especially in the O and Ah horizons over the concentration range tested (0–1 mM). Generally, the experimental data conformed well to a single Michaelis–Menten equation yielding a mean r^2 value of 0.98 ± 0.01 . No relationship was apparent between the parameter values obtained for K_m and V_{max} across all horizons and amino acids ($r^2 = 0.01$; Table 2).

Short-term incubation of soils with a range of C and N substrates indicated that in both L and Ah horizons the microbial community responded very rapidly to the addition of labile C substrates in the form of monosaccharides, amino acids and urea (Fig. 6). Typically a half-maximal response to these substrates was observed within 10 min of addition to the soil. In contrast, the microbial biomass generally responded more slowly to inputs of polymeric sugars and amino acids to the soil in the form of starch and protein. The largest respiration response was to urea for both the L and Ah horizon, whereas no increase in soil respiration was observed upon the addition of either NO_3^- or NH_4^+ .

Generally, a close correlation ($r^2 = 0.70$) was observed between the rate of NH_4^+ production and CO_2 evolution across all samples when amino acids were added to the soil (Fig. 7(A)). As observed in previous amino acid turnover experiments in these soils, the rate of arginine mineralization and ammonification followed the horizon sequence L > O > Ah. Close correlations were observed between the gross rate of ammonification and CO_2 production with that of the amino acid half-life measured in Fig. 2 ($r^2 = 0.65$; Fig. 7(B)). Significant correlations of ammonification with intrinsic soil properties such as pH, total C and substrate-induced respiration were apparent as were significant correlations with general soil microbial activity (Fig. 7(C), Table 1).

4. Discussion

The results presented here confirm previous findings, which have indicated that DON constitutes a major pool of nitrogen in Arctic and taiga soils. The amount of DON appeared to be inversely correlated with levels of NO_3^- giving credence to the hypothesis that a nitrification block may exist in these soils (Walker, 1989). Although the

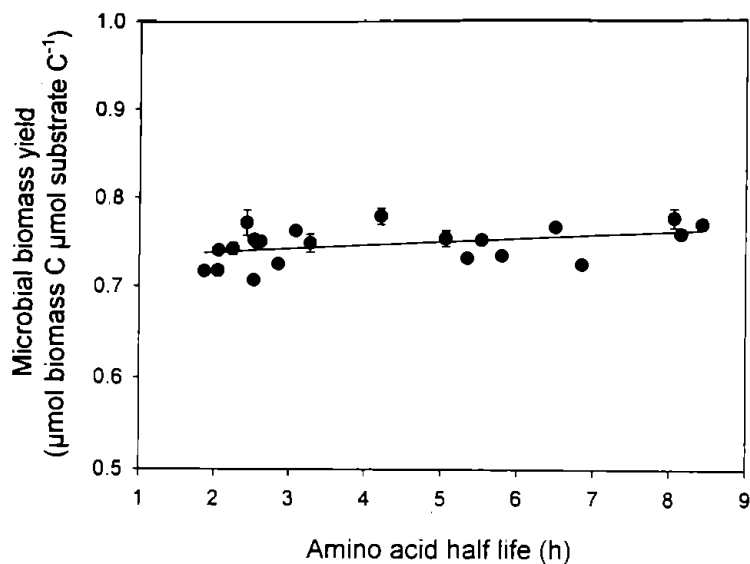


Fig. 3. Rate of amino acid-C incorporation into new cell biomass (microbial yield) in 22 taiga forest soil samples plotted as a function of amino acid half-life. Values represent means \pm SEM ($n = 2$). The linear regression line can be described by $y = 0.00393x + 0.729$.

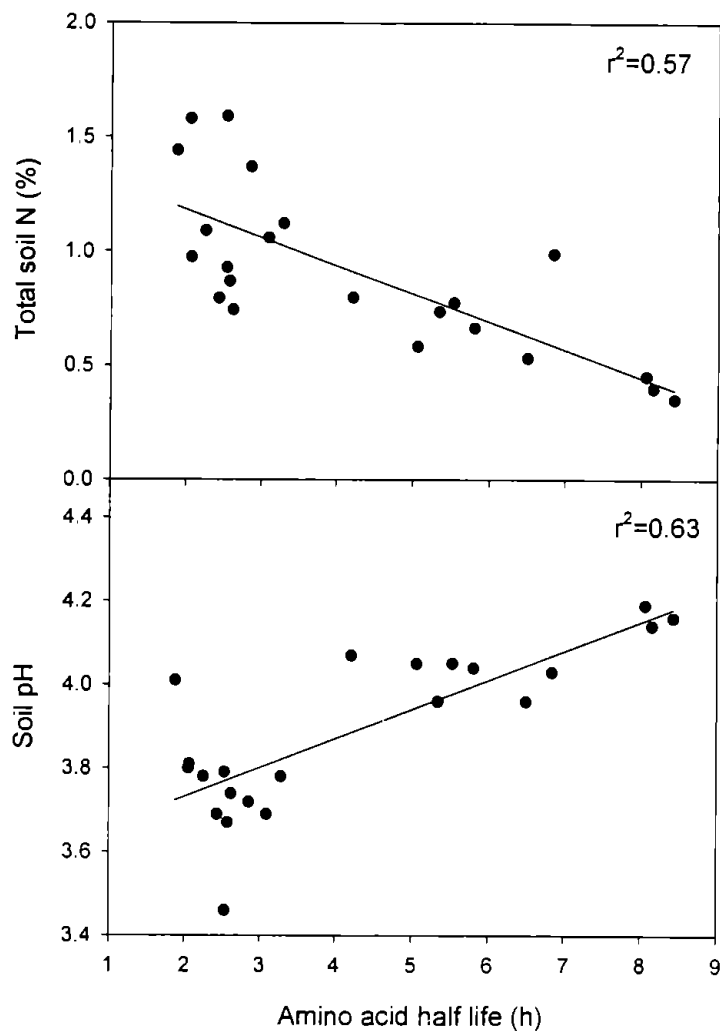


Fig. 4. Linear regression correlations between amino acid half-life and soil N and pH in taiga black spruce soils.

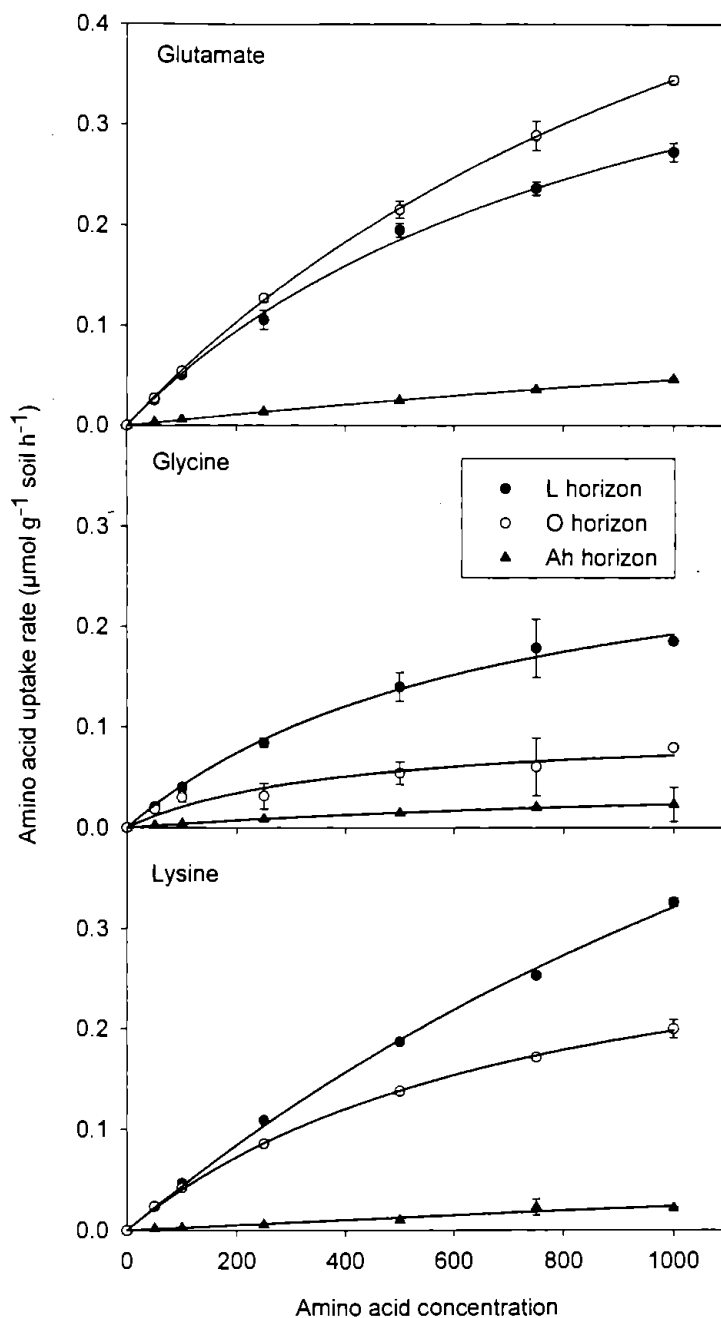


Fig. 5. Uptake kinetics of three amino acids (glycine, glutamate and lysine) measured after a 1 h substrate incubation time in three taiga forest soil horizons (L, O and Ah). Symbols represent experimentally determined data points (mean \pm SEM, $n = 2$), while lines represent curves fitted to a single Michaelis–Menten equation using a least squares optimization procedure.

individual N species within the extracted DON was not ascertained in this study, it can be expected based upon other studies at the same location that a proportion will be in the form of amino acids. If it is assumed that the total amino acid concentration in soil solution is $100 \mu\text{M}$, the water content is 30%, and their sorption characteristics are typical for organic soils (Jones et al., 1994), the amount of extractable amino acids can be estimated between 0.05 and $0.25 \text{ mmol N kg}^{-1}$. This compares to a mean extractable DON in the soils tested here of $1.36 \pm 0.33 \text{ mmol N kg}^{-1}$,

suggesting that free amino acids comprise 4–20% of the total DON in soil. This would also support other evidence, which suggests that a major proportion of the DON and DOC in soil solution is of high molecular weight due to slow microbial turnover of this component (Jones et al., submitted; Jones and Hodge, 1999; Hongve et al., 2000).

Within taiga ecosystems, above-ground litter and, especially root turnover, constitutes the dominant N input into soil (Ruess et al., 1996) with the majority of N contained within these residues associated with proteins. While the

Table 2
Michaelis–Menten kinetic parameters describing the microbial uptake of three contrastingly charged amino acids (glycine, glutamate and glycine) after incubation for 1 h in different horizons from a black spruce forest soil. Parameters V_{\max} and K_m were derived by a least square optimization procedure. Values describe the curves illustrated in Fig. 5 and represent mean \pm SEM, $n = 2$

	V_{\max} ($\mu\text{mol g}^{-1} \text{h}^{-1}$)	K_m (μM)	r^2
<i>Glutamate</i>			
L horizon	0.825 ± 0.018	1397 ± 46	0.999
O horizon	0.241 ± 0.032	4269 ± 685	0.997
Ah horizon	0.531 ± 0.039	927 ± 121	0.969
<i>Glycine</i>			
L horizon	0.321 ± 0.025	666 ± 105	0.995
O horizon	0.099 ± 0.018	377 ± 178	0.934
Ah horizon	0.050 ± 0.003	1178 ± 129	0.998
<i>Lysine</i>			
L horizon	1.067 ± 0.130	2325 ± 399	0.999
O horizon	0.348 ± 0.008	756 ± 36	0.999
Ah horizon	0.168 ± 0.036	6056 ± 148	0.944

direct uptake and assimilation of amino acids by soil microorganisms has been well documented (Kay and Gronlund, 1971; Glover et al., 1975), the direct use of amino acids as a N source by plant roots and algae in N-limiting terrestrial and freshwater environments has drawn renewed interest (Atkin, 1996). On the basis of the results presented here, it appears that the rate of amino acid mineralization is much

greater than the rate of protein turnover. This is presumably due to the fact that amino acids can be taken up directly into microbial cells by specific transporters while proteins must be broken down into peptides, preferably amino acids before transport into the cell (Anraku, 1980). The rate of amino acid turnover in these soils was rapid ($t_{1/2} = 3$ h) and is comparable to results presented for soils from temperate environments (Jones, 1999; Owen and Jones, 2001; Vinolas et al., 2001).

Our results also indicated that amino acid uptake into the soil microbial community was very rapid even at large exogenous concentrations (e.g. >1 mM), possibly indicating that the biomass is capable of responding to large inputs of amino acids (e.g. when a root cell bursts and its contents are released into the soil; root cell = 10 mM amino acids). Individual free amino acid soil solution concentrations reported for these soils are typically in the region of 1–20 μM (Chapin et al., 1993; Kielland, 1995). In comparison, the K_m values presented here for individual amino acid uptake indicate that half-maximal uptake occurs at an external concentration of ca. 2 mM (Table 2). While an obvious disparity exists between these concentrations, it can be partially explained by the knowledge that the values presented here represent the summation of the kinetics from many soil species and that microorganisms can possess multiple uptake systems of varying affinity for amino acids (Tang and Howard, 1973; Glover et al., 1975). Further, it is not uncommon for these transport systems to be operating

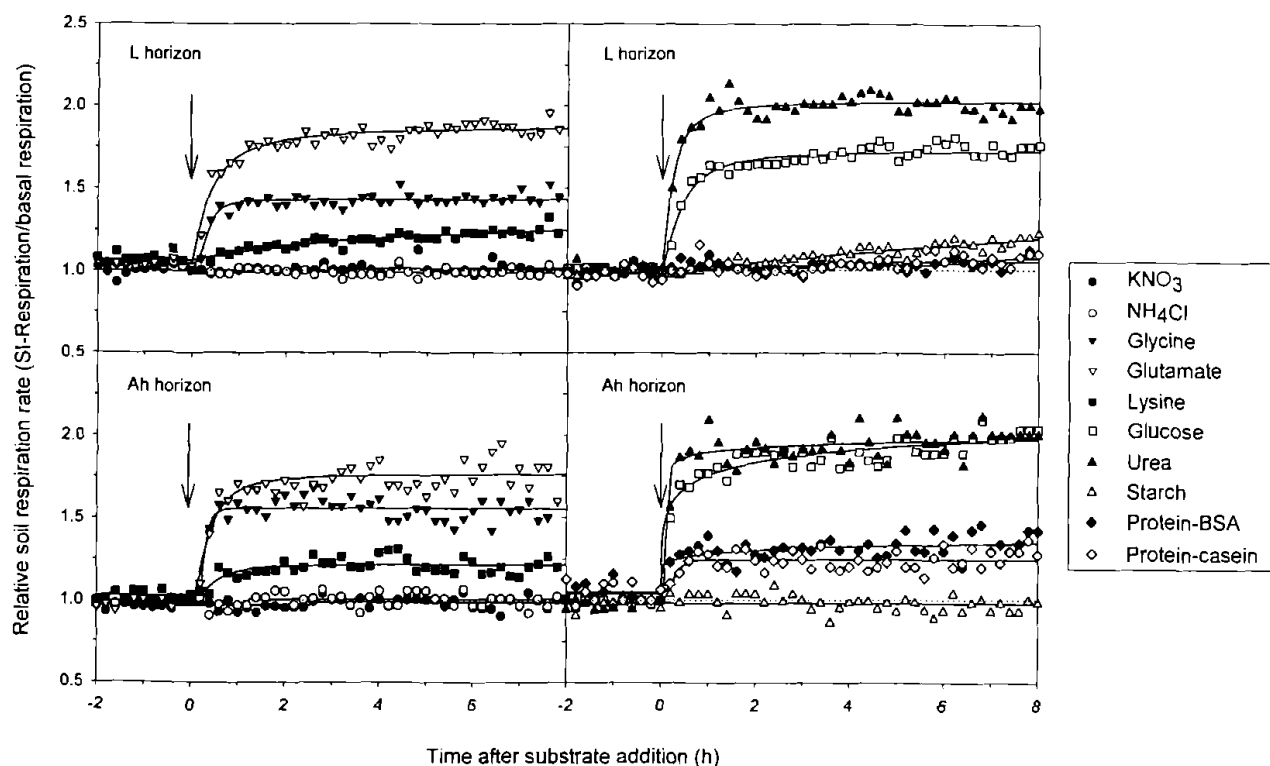


Fig. 6. Relative respiration rate of two taiga forest soil horizons in response to the addition of high and low molecular weight C substrates and inorganic N. Substrates were added at $t = 0$ as indicated by the arrow. The basal respiration in the L horizon was $44 \text{ pmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ while in the Ah horizon it was $8 \text{ pmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$. Respiration in the control soil (water only added) is indicated by a dashed line. Values represent means ($n = 2$).

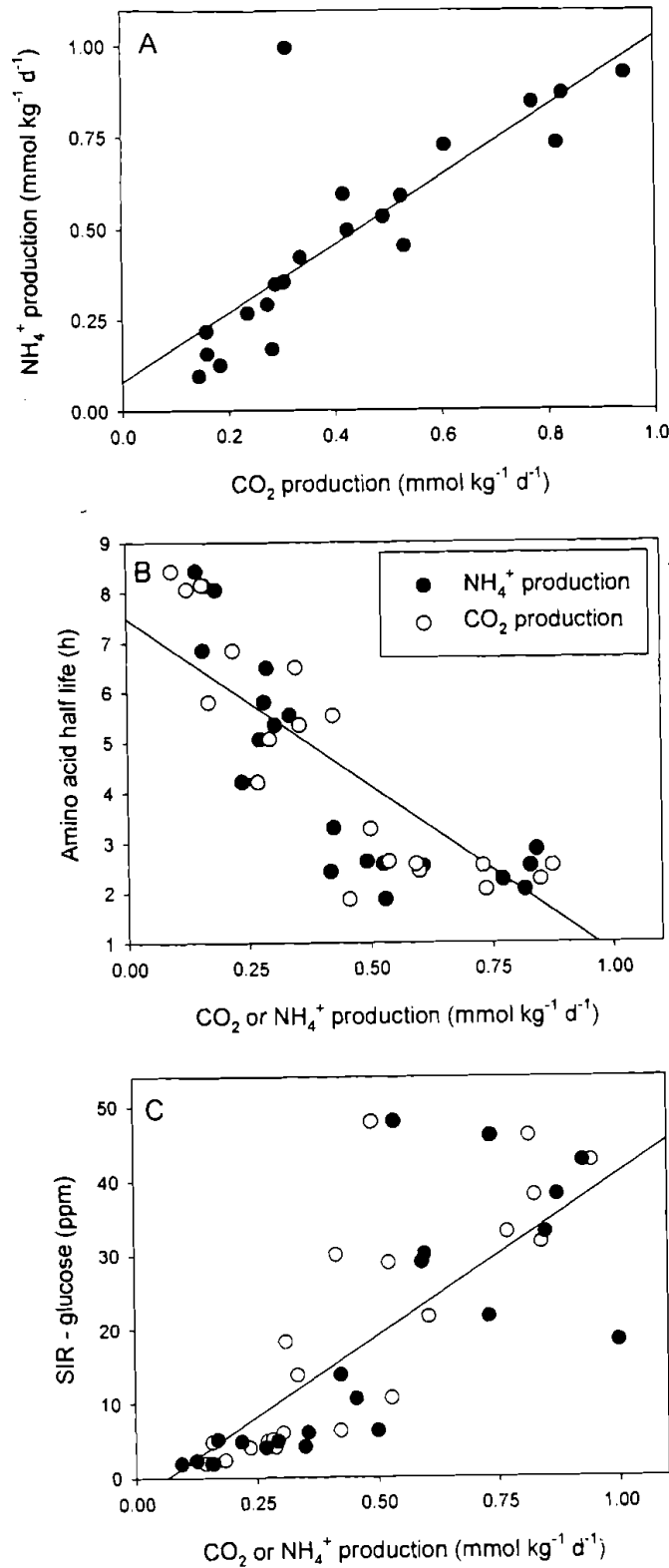


Fig. 7. (A) Relationship between NH_4^+ production and $^{14}\text{CO}_2$ production as a result of the mineralization of ^{14}C labeled arginine in 22 taiga forest soil samples ($y = 0.079x + 0.95$; $r^2 = 0.71$). (B) Relationship between NH_4^+ or $^{14}\text{CO}_2$ production and amino acid half-life for 22 taiga forest soil samples. Half-life data is from Fig. 4 ($y = 7.48x - 6.70$; $r^2 = 0.61$). (C) Relationship between NH_4^+ or $^{14}\text{CO}_2$ production and substrate (glucose) induced respiration for 22 taiga forest soil samples ($y = -2.82x + 43.7$; $r^2 = 0.62$). The legend for panels B and C is identical.

simultaneously within the same organism (Anraku, 1980). It can be speculated, therefore, that the high uptake rates observed at high external concentrations could represent uptake by the zymogenous proportion of the soil biota (Fig. 1). Although the kinetic parameters K_m and V_{max} were measured in this study, no attempt was made to determine C_{min} , the point at which microbial uptake (influx) is balanced by efflux (Barber, 1995). As the molecular and biochemical basis of the transport systems in plants and microorganisms are similar, some parallels can be drawn (Zhou et al., 1998; Buttner and Sauer, 2000). For plant roots, the C_{min} value has been estimated at around $1 \mu\text{M}$ indicating that below this concentration plant roots are net losers of amino acids to the soil while above this concentration, net uptake occurs giving rise to a positive N balance (Barber, 1995; Jones and Darrah, 1994). If soil microorganisms have a similar C_{min} value to plants, this might partially explain why soil solution amino acid concentrations are rarely depleted below $1 \mu\text{M}$.

A main feature of the results presented in this study is that although the soils possessed dramatically different chemical characteristics and microbial activities they appeared to process amino acid C and N in a very similar way (Fig. 7). This appears to be promising for modeling where universal soil reactions and constants are often applied (e.g. ratio of N excreted and C use).

Assuming a steady state free amino acid concentration in the soil solution of $100 \mu\text{M}$ ($0.045 \mu\text{mol amino acid cm}^{-3}$ soil; Chapin et al., 1993; Kielland, 1995; Atkin, 1996; Kielland, unpublished results), we can estimate from our results that the microbial amino acid uptake rate is $0.94 \mu\text{mol amino acid cm}^{-3} \text{ soil d}^{-1}$ (L/O horizon). We therefore conclude that the free amino acid pool in soil solution is extremely transitory and turned over approximately 20 times per day. This can be compared to the total amount of amino acids held in the surface horizon organic matter of $230 \mu\text{mol amino acid cm}^{-3}$ soil, assuming that 30% of the organic N is present as amino acids (Stevenson, 1982). However, it must be noted that a large proportion of the amino acids held in this soil organic matter may be present in a non-available form (e.g. protein–humic complexes; Stevenson, 1982). Based on published data for root turnover in these soils ($24 \text{ kg ha}^{-1} \text{ d}^{-1}$; Ruess et al., 1996) and free amino acid concentrations inside root cells (ca. 10 mM), we can estimate that the direct input of free amino acids as a result of root turnover is $0.002 \mu\text{mol cm}^{-3} \text{ soil d}^{-1}$ with a further $0.10 \mu\text{mol amino acid cm}^{-3} \text{ d}^{-1}$ added in the form of root protein. The amount of amino acids added to the soil in the form of exudates from living roots can be estimated at $0.05 \mu\text{mol amino acid cm}^{-3} \text{ soil d}^{-1}$ based upon a fine root density of $0.9 \text{ cm root cm}^{-3}$ soil and published root exudation data (Cakmak and Marschner, 1988; Jones and Darrah, 1993). For comparison, the standing live root biomass in these soils ($2.2 \text{ mg DW cm}^{-3}$ soil; Ruess et al., 1996) contains $8 \mu\text{mol protein-amino acids cm}^{-3}$ soil and $0.2 \mu\text{mol free amino acids cm}^{-3}$ soil. While these calcula-

tions show a discrepancy between total amino acid inputs to the soil solution and total outputs, we have not included mycorrhizal inputs, native soil organic matter turnover, addition from shoots or rainfall/throughfall inputs. On the basis of the total soil respiration figures obtained here and elsewhere ($6\text{--}12 \mu\text{mol CO}_2 \text{ cm}^{-3} \text{ soil d}^{-1}$; Vance and Chapin, 2001), and the proportion of amino acid C mineralized to CO_2 (35%), we further calculate that between 8 and 17% of the total soil respiration is directly attributable to turnover of amino acids. It must be noted that the calculations performed here are based partially on laboratory results and therefore further work is required to validate these findings in the field.

5. Conclusions

In conclusion, we have presented experimental evidence showing that black spruce forest soils contain large concentrations of DON relative to inorganic N. Further, we have shown that inorganic N formation in these soils is probably not limited by the transformation of amino acids to NH_4^+ but moreover by the transformation of proteins to amino acids. In this forest type, the microbial turnover of the free amino acid pool is extremely rapid with the flux being over two orders-of-magnitude greater than the rate of net N mineralization (Vance and Chapin, 2001). These data coupled to the observation that many taiga species (trees, mosses, etc.) can absorb organic N in situ, lend support to the hypothesis that DON may constitute an important and direct supply of N for taiga forest trees. Finally, the amino acids appear to be processed by a common pathway by the microbial biota that should enable greater confidence of N models in Arctic and taiga environments.

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