



Soil amino acid composition across a boreal forest successional sequence

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ABSTRACT

Soil amino acids are important sources of organic nitrogen for plant nutrition, yet few studies have examined which amino acids are most prevalent in the soil. In this study, we examined the composition, concentration, and seasonal patterns of soil amino acids across a primary successional sequence encompassing a natural gradient of plant productivity and soil physicochemical characteristics. Soil was collected from five stages (willow, alder, balsam poplar, white spruce, and black spruce) of the floodplain successional sequence on the Tanana River in interior Alaska. Water-extractable amino acid composition and concentration were determined by HPLC. Irrespective of successional stage, the amino acid pool was dominated by glutamic acid, glutamine, aspartic acid, asparagine, alanine, and histidine. These six amino acids accounted for approximately 80% of the total amino acid pool. Amino acid concentrations were an order of magnitude higher in coniferous-dominated late successional stages than in early deciduous-dominated stages. The composition and concentration of amino acids were generally constant throughout the growing season. The similar amino acid composition across the successional sequence suggests that amino acids originate from a common source or through similar biochemical processes. These results demonstrate that amino acids are important components of the biogeochemical diversity of nitrogen forms in boreal forests.

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1. Introduction

The role of dissolved organic nitrogen (N) in meeting the nutritional requirements of forest and agricultural plants recently has received much attention. There is a worldwide geographic distribution of plant species and growth forms that use organic N in the form of amino acids, peptides, and proteins directly through roots without mycorrhizae or via ericoid, ecto-, or other mycorrhizal associations (Kielland, 2001; Lipson and Näsholm, 2001). Numerous laboratory, *in situ*, and field studies have shown that plants can directly absorb organic forms of N, namely free amino acids, thereby circumventing the traditional mineralization bottleneck (Chapin et al., 1993; Kielland, 1994; Raab et al., 1996; Schimel and Chapin, 1996; Näsholm et al., 1998; Nordin et al., 2001). Inferences regarding the importance of amino acids as an organic N source have been largely based on physiological studies of plant uptake; few studies have examined the free amino acid composition of the soil.

Dissolved organic N may be especially significant in boreal regions where N mineralization and bulk organic matter decomposition predominantly are slow due to a cold, dry climate (Van Cleve and Alexander, 1981; Flanagan and Van Cleve, 1983; Van

Cleve et al., 1991). Organic forms of N dominate over inorganic N forms in the Tanana River floodplain soils in interior Alaska (Kielland et al., 2007). In addition, the boreal forests of interior Alaska receive minimal N in the form of atmospheric deposition ($0.58 \text{ kg N ha}^{-1} \text{ y}^{-1}$ in 2001; NADP, 2002). The Tanana River floodplain successional sequence accordingly provided an ideal setting to study the soil amino acid composition of a relatively pristine, organic N-dominated ecosystem.

Primary succession on the Tanana River occurs over a timescale of more than 300 years (Viereck et al., 1993a) and is well documented by over three decades of research (Van Cleve et al., 1993b). The successional sequence represents a natural gradient of plant productivity and soil physicochemical characteristics, including soil temperature, organic matter content, pH, carbon and N concentration, and decomposition rates (Van Cleve et al., 1993a,c; Viereck et al., 1993a; Yarie et al., 1993). Along the successional sequence, there are several critical “turning points” that result in major functional changes to the ecosystem: 1) canopy closure and increased litter deposition promotes forest floor development, 2) addition of the N-fixing species thinleaf alder (*Alnus incana* subsp. *tenuifolia*) increases soil N, 3) forest functional type shifts from shrub to tree dominance, 4) overstory composition changes from deciduous to coniferous species, and 5) development of an insulating moss layer reduces soil temperature and promotes the formation and maintenance of permafrost (Viereck, 1989). These

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vegetation, soil, and ecosystem changes with succession may affect the composition and concentration of soil amino acids across this successional sequence.

Soil amino acid concentrations have been shown to vary over the growing season (Abuarghub and Read, 1988; Kielland, 1995; Lipson et al., 1999; Weintraub and Schimel, 2005) and likely reflect seasonal changes in soil and plant biology. Increased uptake of available soil amino acids during plant and microorganism growth cycles may deplete the amino acid concentrations in the soil (Jones et al., 2005b; Weintraub and Schimel, 2005). Seasonal freeze–thaw and dry–rewet events may cause flushes of amino acids from lysis of microbial, mycorrhizal, or root tissue (Lipson and Monson, 1998), as well as the physical disintegration of soil organic matter structure (Ivarson and Sowden, 1966). Seasonal changes in microbial activity may alter microbial production of exoenzymes that break down proteins into soluble peptides and amino acid constituents (Abuarghub and Read, 1988; Lipson and Näsholm, 2001; Read and Perez-Moreno, 2003). Other relevant seasonal amino acid sources may include turnover of roots and mycorrhizae (Ruess et al., 2006), root exudation (Jones, 1999; Jones et al., 2005b), and litter inputs (Chapin and Kedrowski, 1983; Chapin et al., 1986). Although the body of work on soil amino acids, including their temporal changes and the causes for those changes, has grown rapidly in the past decade, fundamental information on the composition and seasonal dynamics of amino acids is still very limited.

Our research quantified the composition, temporal, and landscape patterns of free amino acids in the soils of the Tanana River successional sequence in interior Alaska. We hypothesized that the composition of the soil amino acid pool would vary across the successional sequence, with amino acids with simple molecular structures (e.g., glycine, aspartic and glutamic acid) prevalent in the early deciduous-dominated successional stages with younger and less chemically complex organic matter, and amino acids with a more complex molecular structure (e.g., arginine, histidine, phenylalanine) common in the later coniferous-dominated successional stages with an older and larger reservoir of chemically complex and recalcitrant organic matter. Second, we hypothesized that the concentration of soil amino acids would increase across the successional sequence, as plant-derived organic matter accumulated and N mineralization and microbial immobilization of amino acids slowed with reduced soil temperatures and more recalcitrant soil organic matter. Finally, we hypothesized that soil amino acid concentration would be highest in the spring because of root and microbial lysis over winter. To test these hypotheses, we investigated the composition of the soil amino acid pool, the concentration of soil amino acids, and the seasonal dynamics of soil amino acids across a primary successional sequence on the Tanana River floodplain in interior Alaska. To our knowledge, this is one of the first studies to examine the full suite of possible amino acids across a primary forest successional sequence.

2. Materials and methods

2.1. Study sites

The study area was located on the Tanana River floodplain within the Bonanza Creek Long Term Ecological Research (LTER) site, 20 km southwest of Fairbanks, in interior Alaska (lat. 64°44'N, long. 148°15'W; elevation 120 m). The climate is strongly continental and semiarid with cold winters and warm, dry summers (Vioreck et al., 1993b). Mean annual temperature is -3.3°C , with temperature extremes ranging from -50°C in winter to 35°C in summer. Mean annual precipitation is 269 mm y^{-1} , with approximately 37% falling as snow. The growing season is 100 days or less. The Tanana River, nearly 1000 km in length (Collins, 1990), is the

largest tributary of the Yukon River and carries a heavy silt load, with 85% of its discharge from glacier-fed tributaries originating in the Alaska Range (Anderson, 1970). The Tanana River floods frequently due to snowmelt in spring and increased rainfall and glacial meltwater in late summer, thereby producing alluvium terraces of progressively greater age with increasing distance from the river's edge (Van Cleve et al., 1993a; Yarie et al., 1998).

The study sites, comprising a primary successional sequence that encompasses a natural gradient of terrace age, plant productivity, and soil physicochemical characteristics (Table 1), included five major successional stages: willow, alder, balsam poplar, white spruce, and black spruce. We established three replicate 900 m² plots (each 30 × 30 m or 20 × 45 m) in each of the five successional stages, for a total of 15 plots located along an 11 km stretch of the Tanana River. Replicated plots of each successional stage had a similar vegetative community, stand structure, and age.

2.2. Vegetation

We conducted a vegetation inventory at each plot to characterize the general vegetation composition of the five successional stages. Willow plots were characterized by the dominance of *Salix* L. species (*Salix alaxensis* (Anderss.) Coville, *Salix niphoclada* Rydb., *Salix interior* Rowlee, *Salix lucida* subsp. *lasiantra* (Benth.) E. Murr., and *Salix pseudomyrsinites* Anderss.), *Populus balsamifera* L. seedlings, and scattered clumps of *A. incana* subsp. *tenuifolia* (Nutt.) Breitung; the understory was dominated by *Equisetum variegatum* Schleich. ex F. Weber & D. Mohr and *Equisetum arvense* L. Alder plots were characterized by a dense canopy of *A. incana* subsp. *tenuifolia*, scattered *S. pseudomyrsinites* shrubs, and *P. balsamifera* saplings and trees; the understory was dominated by *E. arvense* and *Chamerion angustifolium* (L.) Holub. Balsam poplar plots were characterized by a tall, mature *P. balsamifera* overstory and a dense shrub layer of *A. incana* subsp. *tenuifolia*; the understory was fairly sparse due to the high litter cover. White spruce plots were characterized by a large, mature *Picea glauca* (Moench) Voss overstory and a dense tall shrub layer of *Rosa acicularis* Lindl., *Alnus viridis* subsp. *fruticosa* (Rupr.) Nyman, *A. incana* subsp. *tenuifolia*, and *Viburnum edule* (Michx.) Raf.; the understory was dominated by a thick feather moss layer of *Hylocomium splendens* (Hedw.) Schimp. Black spruce plots were characterized by an open canopy of mature *Picea mariana* (Mill.) B.S.P. trees, a diverse tall shrub layer of *R. acicularis*, *A. viridis* subsp. *fruticosa*, *A. incana* subsp. *tenuifolia*, *Salix arbusculoides* Anderss., *S. alaxensis*, *Betula glandulosa* Michx., and *Betula nana* L.; the understory was dominated by a dense low shrub layer of *Ledum groenlandicum* Oeder, *Vaccinium vitis-idaea* L., *Vaccinium uliginosum* L., and *Empetrum nigrum* L.; and a thick, nearly continuous moss layer of *H. splendens*, *Aulacomnium palustre* (Hedw.) Schwaegr., *Pleurozium schreberi* (Brid.) Mitt., and *Sphagnum* L. species.

2.3. Soils

Soils of the five successional stages are alluvial and have coarse-loamy texture, but each successional stage differs in soil classification (Ping and Johnson, 2000). Willow soils are classified as Typic Cryofluvents, alder soils as Typic Cryaquents, balsam poplar soils as Fluvaquentic Cryaquepts, white spruce soils as Aquic Eutrocrypts, and black spruce soils as Typic Historthels. All soils from the Tanana River floodplain are generally cold, wet, and poorly developed. Roots tend to be concentrated close to the soil surface, with nearly 90% of annual fine root production located in the top 30 cm of mid-successional soils (Ruess et al., 2006). Organic matter accumulates over succession, with forest floor thickness ranging from 0 cm in willow to 30 cm in black spruce soils (Table 1; Vioreck et al., 1993a). Soil temperature generally decreases over succession, and

Table 1
Characteristics of the five successional stages on the Tanana River floodplain, interior Alaska.

Successional stage	Typical terrace age (y) ^a	Typical terrace height (m) ^a	Forest floor thickness (cm) ^a	Annual litterfall (g m ⁻² y ⁻¹) ^a	Aboveground biomass (kg m ⁻²) ^b	Aboveground annual production (g m ⁻²) ^b	Soil temperature over growing season (°C) ^c	Permafrost ^a	Maximum active layer thaw depth (cm) ^d
Willow	2–10	1.8–2.2	0	8–10	0.2–0.6	200–300	9.7 ± 0.9	None	ND ^e
Alder	10–40	2.0–2.4	5–10	150–300	1.0–6.4	290–350	6.4 ± 0.3	None	ND ^e
Balsam poplar	80–150	2.5–2.8	8–12	250–650	4.1–18.0	264–952	3.9 ± 0.3	None	ND ^e
White spruce	200–300	3.2–4.0	6–20	50–250	14.6–22.7	331–540	4.8 ± 0.3	Intermittent	191 ± 12
Black spruce	500+	4–5	18–30	30–100	1.6–10.9	57–148	3.6 ± 0.2	Present	112 ± 23

^a Adapted from Viereck et al. (1993a). Terrace heights were measured as height (m) above winter low water level.

^b Van Cleve et al. (1991).

^c 5-Year mean (June–September 1997–2001) of soil temperature at 10 cm depth, LTER sites FP1A, FP2A, FP3A, FP4A, and FP5A (Bonanza Creek LTER database, 2002b). Values are means ± 1 SEM, *n* = 5.

^d 5-Year mean (1997–2001) of maximum active layer thaw depths at LTER white spruce sites FP4A, FP4B, and FP4C; and black spruce sites FP5A, FP5C, and FP5D (Bonanza Creek LTER database, 2002a). Values are means ± 1 SEM, *n* = 13 and *n* = 14 for white and black spruce respectively.

^e Not determined because the first three successional stages do not have permafrost.

permafrost occurs in the late successional stages of white and black spruce (Table 1; Viereck et al., 1993a).

2.4. Soil sampling and extraction

We sampled soils three times during 2001: June, just after soil thaw, early August, in the middle of the growing season, and late September, just prior to freeze-up. For each sampling event, we used a stainless steel corer to obtain eight soil cores (5.8 cm diameter × 20 cm depth) starting at the top of the Oe horizon (after clearing off the fresh Oi litter horizon), along a randomized coordinate grid system in each 900 m² replicate plot of the five successional stages. Generally, two plots were sampled per day, for a total sampling duration of eight days. The three replicate plots for each successional stage were sampled successively.

Numerous precautions were taken to ensure minimal effects on amino acids before soil samples were chemically extracted. After field coring, soil cores were transported in an ice-filled cooler to a temporary field laboratory, where all samples were extracted and extracts frozen within 4 h of collection. In addition, to minimize potential amino acid contamination, all field and laboratory equipment, glassware and polyethylene plasticware were washed in soap and water, acid-washed (10% HCl), and thoroughly rinsed in nanopure water before use. All glass fiber filters, foil, and glassware were heated in a muffle furnace for 8 h at 450 °C. Personnel wore PVC gloves at all times to minimize contamination during processing.

We separated each soil core into organic and mineral horizons, with buried organic horizons (BOHs) greater than 0.5 cm thickness pooled with the forest floor (Oe/Oa) horizon, and successive mineral horizons combined. BOHs that were less than 0.5 cm thick were combined with the mineral soil. Woody debris and roots >1 mm in diameter were removed, and soils were homogenized by mixing them inside a sterile polyethylene bag. We extracted amino acids from the soil by gently shaking 10 g field-moist soil in 75 ml nanopure water for 10 min in a polyethylene cup. Soil extracts were vacuum-filtered through a Whatman GF/A glass fiber filter, and an aliquot of supernatant was filtered by syringe through a Whatman 0.2 µm GD/X polyvinylidene fluoride (PVDF) membrane filter into sterile 2.0 ml cryovials. We used PVDF membrane filters because they are hydrophilic and do not bind proteins, and the 0.2 µm pore size eliminated the majority of microbes that could alter the amino acid pool by utilization or lysis. Immediately after filtration, soil extracts were frozen and stored in a freezer until analysis. Three procedural blanks of nanopure water were extracted for each replicate plot.

We determined soil moisture content by oven drying a subsample of each soil (60 °C for organic soils and 105 °C for mineral soils) until

constant mass (48 h). We measured soil pH of air-dried soil samples collected in September on a Denver Instrument Company Model 220 pH/conductivity meter, using soil to water ratios of 1:5 for organic soils and 1:2 for mineral soils (Hendershot et al., 1993). Oven-dried September soils were ground with mortar and pestle, and total soil carbon (C) and N concentrations were analyzed on a LECO CNS-2000 (LECO Corporation, St. Joseph, MI, USA).

2.5. Chemical analysis by HPLC

Concentrations of individual amino acids were determined by high performance liquid chromatography (HPLC; Gilson Inc., Middleton, WI, USA) with an automated pre-column *o*-phthalaldehyde (OPA)/2-mercaptoethanol derivatization procedure modified from Jarret et al. (1986). The automated protocol ensured sample reproducibility, consistency of OPA derivatization reaction times, and enabled 24-h HPLC operation for over one thousand samples. Good peak separation was obtained using an Adsorbosphere OPA-HS 5 µm column (100 × 4.6 mm) with accompanying 7.5 × 4.6 mm guard column (Alltech Associates, Inc., Deerfield, IL, USA). A FD-100 filter fluorescence detector (Groton Technology Inc./SpectroVision, Acton, MA, USA) with a 326 nm excitation filter, 470 nm emission filter, and pulsed xenon lamp was used to detect amino acid fluorescence after column separation.

We identified eighteen of the twenty primary L-amino acids; the exceptions were proline (no reaction with OPA derivative; Roth, 1971) and cysteine (yields very low fluorescence; Roth, 1971; Jarret et al., 1986). All amino acid peaks eluted within 35 min. The merged peaks of glutamic acid/asparagine and histidine/glutamine were separated, and all eighteen individual amino acid peak areas were integrated using PeakFit software (Version 4.11, SYSTAT Software Inc., Point Richmond, CA, USA), with manual baseline subtraction, Savitsky–Golay smoothing, and an Exponentially Modified Gaussian peak fitting model (SYSTAT Software Inc., 2002). Amino acid concentrations were calculated from peak areas using a calibration curve of known amino acid standards (A9656 food hydrolysate amino acid standard, with addition of L-glutamine and L-asparagine standards, Sigma-Aldrich, St. Louis, MO, USA) run on the HPLC the same day. The series of amino acid standards covered the range of measured sample values. Any amino acid peaks detected in the procedural blanks were subtracted from the samples.

2.6. Calculations

Although we measured soil properties and amino acid concentration on both organic and mineral horizons within a soil core, we

present results of soil properties and amino acid contents with the organic and mineral horizons combined (i.e., the complete 0–20 cm depth soil core). Combining the organic and mineral horizons allowed for effective comparisons across the successional sequence, where organic horizons made a progressively greater fraction of the total core volume over succession, ranging from 0% for willow to 79% for black spruce. To calculate the soil amino acid concentration of the combined organic and mineral horizons, we multiplied the individual horizon concentration by the horizon percent mass fraction of the entire 20 cm soil, then added the two horizon components together. The final concentration unit is ng N g^{-1} dry soil, where the g^{-1} dry soil represents 1 g of the entire 20 cm core.

Total free amino acid (TFAA) concentration was calculated from the sum of all eighteen amino acids. The composition of the amino acid pool is expressed as a proportion of the total, by dividing each individual amino acid concentration by the TFAA concentration. Proportions thereby standardized a wide range of concentrations and allowed for effective comparisons of amino acid composition among the different successional stages. We also calculated soil amino acid concentration on an areal basis, by multiplying the concentration (ng N g^{-1} dry soil) by the soil bulk density and depth of soil core. The areal unit is useful because bulk density of the 0–20 cm soil depth declines substantially across the successional sequence.

2.7. Statistical analysis

Successional and seasonal differences in amino acid composition and concentration were tested with the UNIVARIATE, CORR, and MIXED procedures of SAS version 8.2 (SAS Institute Inc., Cary, NC, USA). We examined differences across the successional sequence using a nested, repeated measures, mixed analysis, with all months combined. We examined differences among the months over the growing season using a nested, repeated measures, mixed analysis, with each successional stage analyzed separately. Nearly all of the soil amino acid data were transformed (square root, log, or arcsine square root) to meet the assumptions of normality and homoscedasticity. We used the autoregressive covariance structure for repeated measures analyses (Littell et al., 2002) and Tukey or Tukey–Kramer adjustments to post hoc significance tests. Comparisons were deemed statistically significant when P values were ≤ 0.05 . The eight soil cores collected from each plot were used in all statistical analyses; however, the three replicated plots of each successional stage represent the true experimental units. Accordingly, plot was treated as a nested and random factor in each analysis, and plot was the repeated subject in the repeated measures design. The number of stand level replicates was three when each successional stage or each month was analyzed separately, and nine when all months were combined over the growing season. Values presented in tables and figures are the means ± 1 SEM of the untransformed data. Relationships between soil parameters and amino acid concentration were tested with Spearman's correlations. Absolute values of correlation coefficients ≥ 0.7000 with P values < 0.0001 were deemed as strongly correlated (Hatcher and Stepanski, 1994).

3. Results

3.1. Successional patterns

3.1.1. Soil properties

The five successional stages showed marked differences in soil properties (Table 2). Gravimetric moisture content was lowest in balsam poplar and highest in black spruce. Bulk density and soil pH generally decreased across the successional sequence. Soil C and N

concentrations increased across the successional sequence. Consequently, the C:N ratios of soils from the coniferous stages of white and black spruce were higher than the C:N ratio of the soils from the first three deciduous stages. Soil C pools increased progressively over the successional sequence, whereas soil N pools were lowest in willow, peaked in balsam poplar, and remained essentially unchanged from balsam poplar to black spruce.

3.1.2. Amino acid composition

Soil amino acid composition was diverse, with eighteen of the twenty primary amino acids detected in most plots (Table 3). Despite the diversity of amino acids found, the composition of the amino acid pool was very similar with six amino acids dominant in all successional stages: alanine, asparagine, aspartic acid, glutamic acid, glutamine, and histidine. These six amino acids accounted for 71–86% of the TFAA concentration. Across the successional sequence, proportions of the six dominant amino acids ranged from 17–24% for glutamic acid, 11–26% for glutamine, 9–15% for alanine, 9–14% for aspartic acid, 7–13% for asparagine, and 3–11% for histidine. In all successional stages, there were low proportions (0–3%) of isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, and tyrosine.

The six amino acids prevalent across all successional stages (alanine, asparagine, aspartic acid, glutamic acid, glutamine, and histidine) include a variety of molecular sizes, C:N ratios, charges, and solubilities. Five of the six dominant amino acids are polar, with alanine (non-polar) as the exception. Polar amino acids averaged 77% of the total amino acid pool for all successional stages. Non-polar amino acids and those containing aromatic rings (phenylalanine, tryptophan, and tyrosine) comprised a low proportion of the amino acid pool.

Despite the similar overall composition of the amino acid pool across the successional sequence, there were several notable differences (Fig. 1a). Proportions of glutamine were significantly higher in the willow and black spruce stages than in the balsam poplar and white spruce stages. Histidine increased in proportion across the successional sequence, and the deciduous (willow, alder, and balsam poplar) stages had significantly lower histidine proportions than the coniferous (white and black spruce) successional stages ($P < 0.001$). Finally, alder had lower proportions of alanine and aspartic acid than the other successional stages, but alder had a higher proportion (7%) of arginine.

3.1.3. Amino acid concentration

Whereas the overall composition of the amino acid pool was relatively similar among successional stages, concentrations of soil amino acids were extremely different across the successional sequence (Fig. 1b; Table 3). Individual amino acid concentrations generally increased by an order of magnitude across the successional sequence, and TFAA concentration ranged from 438 ng N g^{-1} dry soil for willow to 4867 ng N g^{-1} dry soil for black spruce. There were highly significant differences ($P < 0.001$) among the successional stages for each of the six dominant amino acids and TFAA concentrations. Moreover, the deciduous stages had significantly lower ($P < 0.001$) amino acid concentrations than the coniferous successional stages. Each of the six dominant amino acids and TFAA concentrations was negatively correlated with soil pH and bulk density, but positively correlated with soil C and N concentrations ($P < 0.001$ for all), which shows the influence of increasing organic matter across succession.

Patterns of soil amino acid concentration across the successional sequence changed dramatically when calculated on a soil areal basis (Fig. 1c). The high amino acid concentrations in black spruce were diminished by the lower bulk density in black spruce soils. Nevertheless, black spruce still had the highest glutamine, glutamic

Table 2Soil properties (0–20 cm depth) of the five successional stages. Values are means \pm 1 SEM, $n = 9^a$ and $n = 3^b$.

Successional stage	Gravimetric moisture content (%) ^a	Bulk density (g cm ⁻³) ^a	pH ^b	%C ^b	%N ^b	C:N ^b	C pool (g C m ⁻²) ^b	N pool (g N m ⁻²) ^b
Willow	32 \pm 1	0.81 \pm 0.02	8.00 \pm 0.03	1.2 \pm 0.1	0.06 \pm 0.01	21.4 \pm 0.8	1948 \pm 76	92 \pm 7
Alder	29 \pm 1	0.85 \pm 0.01	7.98 \pm 0.06	1.8 \pm 0.1	0.10 \pm 0.00	18.3 \pm 0.4	3044 \pm 23	168 \pm 5
Balsam poplar	21 \pm 2	0.75 \pm 0.02	6.96 \pm 0.23	4.0 \pm 0.1	0.21 \pm 0.01	19.9 \pm 0.5	5808 \pm 588	295 \pm 38
White spruce	36 \pm 3	0.42 \pm 0.04	5.78 \pm 0.03	11.1 \pm 1.2	0.41 \pm 0.04	26.8 \pm 1.0	6931 \pm 349	262 \pm 21
Black spruce	55 \pm 3	0.22 \pm 0.02	5.27 \pm 0.27	21.3 \pm 2.8	0.73 \pm 0.12	29.7 \pm 2.4	7106 \pm 1198	256 \pm 60

^a Mean of June, August, and September soil sampling.^b Mean of September soil sampling.

acid, histidine, and TFAA concentrations on an areal basis across the successional sequence. TFAA concentration increased across succession, ranging from 70 mg N m⁻² for willow to 104 mg N m⁻² for black spruce, and TFAA concentration was 1.5 times greater in black spruce than in willow. Glutamine concentrations were significantly greater in black spruce than in alder, balsam poplar, and white spruce. Glutamic acid concentrations were significantly lower in the shrub successional stages than in the tree stages ($P < 0.001$). Histidine concentrations increased across succession when calculated on a soil areal basis, similar to the pattern of increasing histidine concentration across succession that was calculated on a soil mass basis (Fig. 1b).

3.2. Seasonal dynamics

3.2.1. Amino acid composition

Composition of the soil amino acid pool was fairly constant across the growing season for each successional stage (Fig. 2). The only trend was that proportions of glutamine were highest in September for all successional stages. Pairwise comparisons showed that glutamine proportions were significantly higher ($P < 0.05$) in September than in August for the alder, balsam poplar, and white spruce stages.

3.2.2. Amino acid concentration

Concentrations of soil amino acids in each successional stage showed few clear and consistent patterns during the growing season (Fig. 3). Glutamic acid concentrations tended to be highest in June for the four earliest successional stages, but the differences were not statistically significant. The balsam poplar stage exhibited

Table 3Soil amino acid concentration on a soil mass basis (ng N g⁻¹ dry soil) for each successional stage over the growing season. Values are means \pm 1 SEM, $n = 9$.

Amino acid	Willow	Alder	Balsam poplar	White spruce	Black spruce
Alanine	58 \pm 7	48 \pm 4	112 \pm 13	327 \pm 32	612 \pm 67
Arginine	18 \pm 6	38 \pm 8	19 \pm 5	71 \pm 8	121 \pm 14
Asparagine	40 \pm 5	67 \pm 10	71 \pm 9	196 \pm 27	302 \pm 44
Aspartic acid	58 \pm 6	48 \pm 5	98 \pm 6	282 \pm 24	471 \pm 63
Glutamic acid	81 \pm 9	89 \pm 8	194 \pm 30	417 \pm 46	967 \pm 100
Glutamine	87 \pm 4	81 \pm 6	90 \pm 16	299 \pm 58	1402 \pm 231
Glycine	12 \pm 2	17 \pm 2	22 \pm 1	48 \pm 4	63 \pm 6
Histidine	15 \pm 3	30 \pm 3	50 \pm 8	221 \pm 30	510 \pm 60
Isoleucine	6 \pm 1	8 \pm 1	13 \pm 1	30 \pm 3	36 \pm 3
Leucine	8 \pm 1	9 \pm 1	8 \pm 1	14 \pm 2	36 \pm 2
Lysine	1 \pm 1	8 \pm 3	8 \pm 3	6 \pm 2	7 \pm 4
Methionine	3 \pm 1	3 \pm 1	3 \pm 0	4 \pm 1	6 \pm 1
Phenylalanine	5 \pm 1	5 \pm 1	7 \pm 1	15 \pm 2	29 \pm 3
Serine	13 \pm 2	19 \pm 1	36 \pm 4	92 \pm 9	146 \pm 13
Threonine	11 \pm 2	23 \pm 7	24 \pm 3	47 \pm 5	71 \pm 10
Tryptophan	10 \pm 3	3 \pm 1	3 \pm 1	11 \pm 3	16 \pm 4
Tyrosine	3 \pm 0	4 \pm 1	4 \pm 1	9 \pm 2	16 \pm 3
Valine	10 \pm 2	12 \pm 1	28 \pm 3	59 \pm 7	56 \pm 7
TFAA ^a	438 \pm 28	514 \pm 37	792 \pm 80	2148 \pm 206	4867 \pm 563

^a Total free amino acid concentration (sum of the 18 amino acids).

the greatest variation in seasonal patterns of amino acid concentrations. In this successional stage, twelve of the eighteen amino acids (including the six dominant) had the highest concentrations in June, although these concentrations were not always statistically significant. Despite predictable variations in soil temperature and moisture, concentrations of TFAA showed no clear seasonal patterns in any successional stage (Fig. 4).

4. Discussion

4.1. Soil amino acid composition across succession

Despite major differences in terrace age, vegetation inputs, productivity, and soil properties, the composition of the amino acid pool was remarkably similar in each of the five successional stages. Amino acid production and consumption in these soils entail a variety of sources (e.g., overstory and understory plant litter, root exudates, animals, and microbial cells) and processes (e.g., plant uptake, microbial uptake, and abiotic sorption). In addition, the production and utilization of amino acids within plant or microbial cells, as well as the ecosystem processes linking them, likely share the same biochemistry and amino acid constituents regardless of vegetation type, soil environment, or climate. Although there are these commonalities, the capacity of plants and microbes to utilize amino acids has been shown to vary among different plant species and functional types (Chapin et al., 1993; Kielland, 1994; Weigelt et al., 2005), with amino acid molecular size (Kielland, 1994), as well as being correlated with the concentration of the amino acid pool (Kielland, 1994; Jones et al., 2005a).

Elucidating the potential origin of amino acids in the soil is complicated due to the intertwined nature and speed of rhizosphere dynamics. Analysis of amino acid composition of overstory plant litter in these successional stages shows high proportions of alanine, glutamine, and histidine, similar to soil (unpublished observations). Other amino acids commonly found in arctic plants include alanine, arginine, asparagine, glutamine, and glutamic acid (Chapin et al., 1986); the amino acid pool in boreal forest vegetation (shrub, herb, and grass foliage) is dominated by asparagine, glutamine, and arginine (Ohlson et al., 1995). In addition to the amino acids found in plant litter, asparagine and glutamine are common constituents of plant xylem and phloem (Pate, 1973; Kielland, 1994; Rochat, 2001), whereas asparagine, glutamine, and arginine are common storage amino acids in above and belowground plant tissue (Chapin et al., 1986; Näsholm et al., 1994; Ohlson et al., 1995; Nordin and Näsholm, 1997). Microorganisms, whose cell walls have high proportions of alanine, aspartic acid, and glutamic acid (Friedel and Scheller, 2002; Yu et al., 2002), are also a likely source of amino acids in the soil.

A review of other studies demonstrates that there are common soil amino acids across a variety of natural and managed ecosystems from different climates, further supporting the view that soil amino acids originate from similar ecosystem components or through similar biochemical processes (Table 4). Alanine and

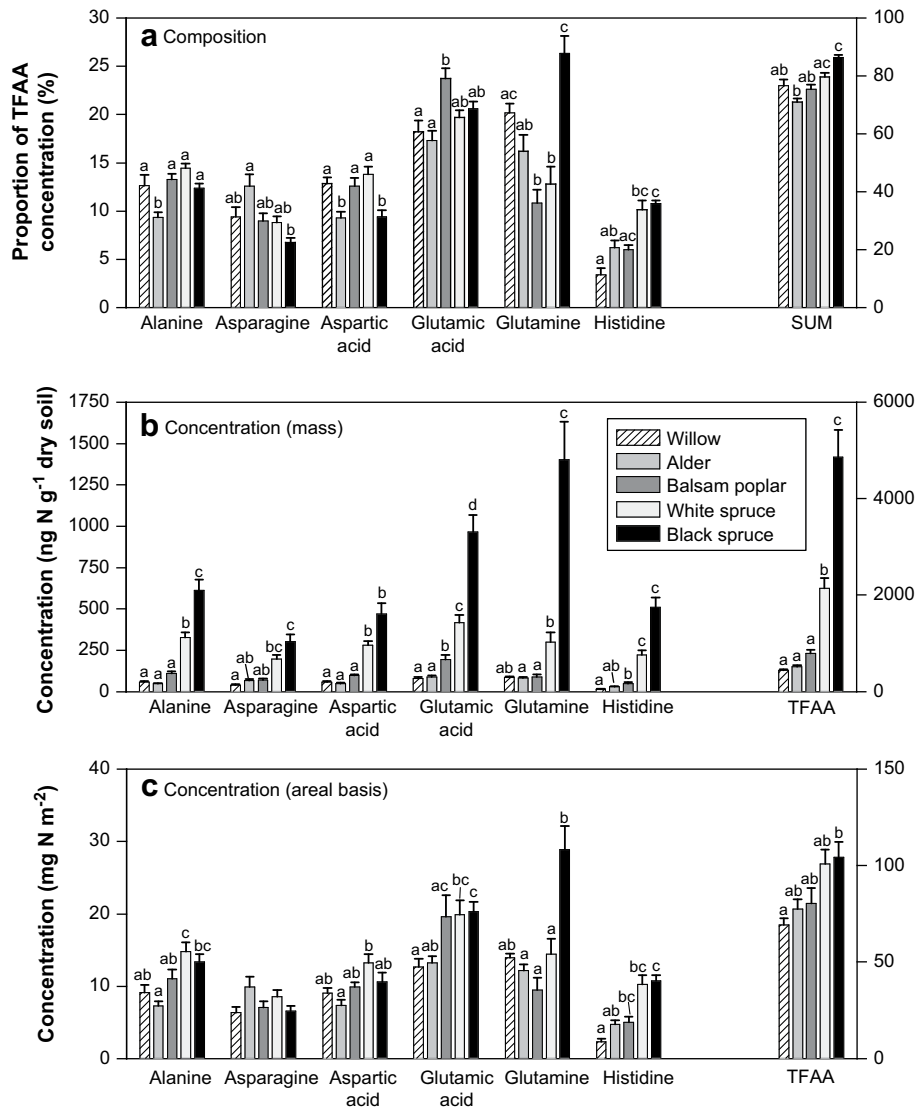


Fig. 1. Amino acid composition (a) and concentration on mass (b) and areal (c) basis across the successional sequence for the six dominant amino acids. Scale on right side applies to the sum of the six dominant amino acid proportions (SUM in (a)) and total free amino acid concentration (TFAA in (b) and (c)). Different lower case letters indicate statistical significance among the successional stages for a given amino acid. Values are means (± 1 SEM), $n = 9$.

glutamic acid are two common important amino acids across a variety of ecosystems. Notably missing from our results were the large proportions of the amino acids arginine, glycine, and serine found in nearly every other study, including those in the boreal forest in Sweden (Nordin et al., 2001) and Alaskan tundra (Kielland, 1995). We did detect a large proportion (7%) of arginine in the alder successional stage, perhaps because alder transports amino acids from their N-fixing root nodules in the form of citrulline (Pate, 1973; Gaudillère, 2001), a precursor to arginine. In addition, arginine is an efficient N storage compound (Chapin et al., 1986; Nordin and Näsholm, 1997), facilitating N storage during rapid N-fixation (Uliassi and Ruess, 2002). Only a few studies report data for asparagine, glutamine, and histidine, but these amino acids have been found to be important components of the amino acid pool (Ivarson and Sowden, 1969; Abuarghub and Read, 1988; Nordin et al., 2001; Yu et al., 2002). The amides asparagine and glutamine combined made up 27% of the soil amino acid pool in both the boreal forest in Sweden (Nordin et al., 2001) and the Alaskan boreal forest of this study. Only three other studies found similarly large proportions of aspartic acid (Ivarson and Sowden, 1969; Read and

Bajwa, 1985; Kielland, 1995). Several amino acids were found in low proportions across all studies: isoleucine, methionine, phenylalanine, tryptophan, and tyrosine. The similar amino acid composition across a variety of different soils is consistent with reviews by Abuarghub and Read (1988) and Schulten and Schnitzer (1998).

There are only a handful of studies describing the amino acid composition in soil, but the majority of laboratory and field studies of amino acid uptake have focused on glycine. Results of this study indicate that glycine accounted for only 3% of the TFAA pool (Table 4). Thus, we suggest that the influence of specific amino acids to plant nutrition would be strengthened by further examination of the abundance of these individual amino acids in the soil.

4.2. Soil amino acid concentration across succession

Amino acid concentrations generally increased across the successional sequence (Fig. 1b and c), probably due simply to the accumulation of soil organic matter (Tables 1 and 2; Viereck et al., 1993a), which is a source of amino acids. In addition, boreal forest soils high in organic matter also have high fungal biomass

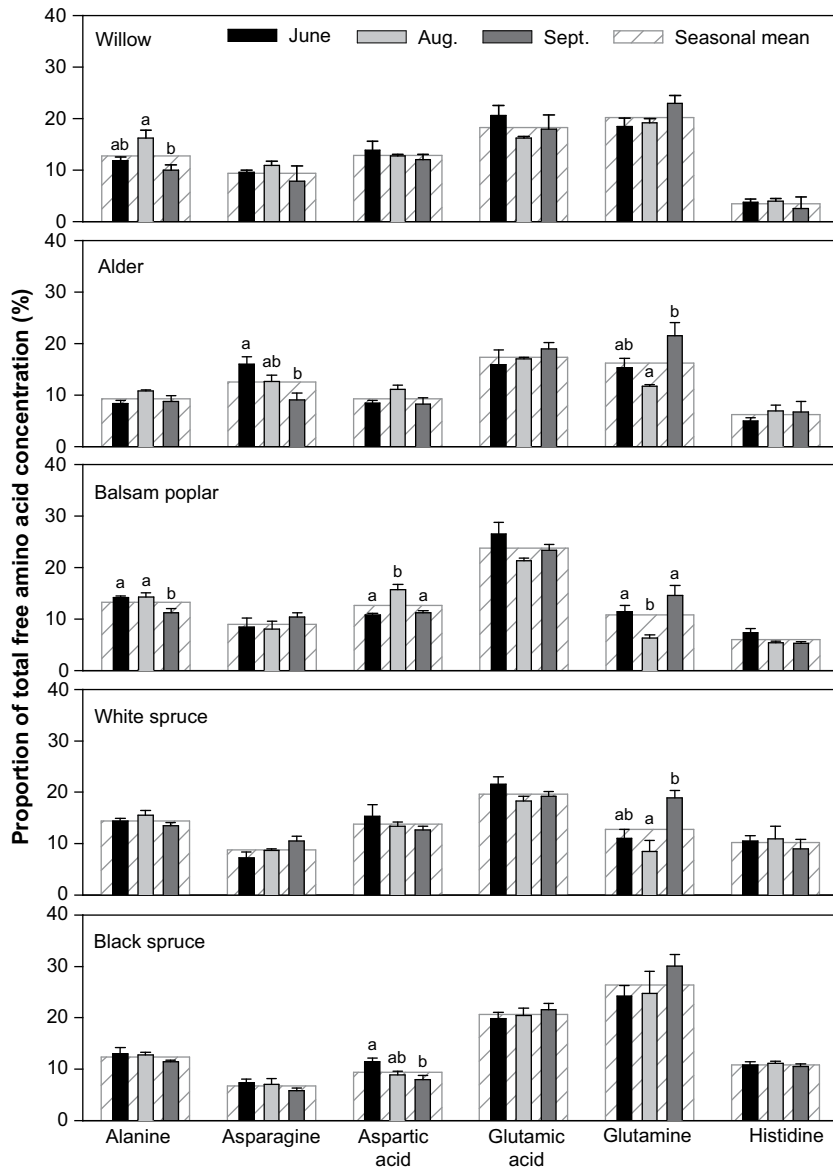


Fig. 2. Six dominant amino acid proportions in soils of each successional stage for June, August, and September. The seasonal mean is shown by the wide hatched bar behind each group of three colored bars. Different lower case letters indicate statistically significant differences among months for a given amino acid of the successional stage. Monthly values are means (+1 SEM), $n = 3$. Seasonal values are means (SEM omitted for simplicity), $n = 9$.

(Flanagan and Van Cleve, 1983), which is another source of amino acids. Kielland et al. (2007) found that soil protease activity increased across succession on the same plots sampled in this study and suggested that the high concentrations of soil amino acids in the later successional stages were sustained through high proteolytic activity.

As organic matter accumulates across succession, there is an associated increase in cation exchange capacity (Van Cleve et al., 1993a), so more amino acids may be adsorbed onto the soil exchange sites of the later successional stages. The basic amino acid histidine should develop a strong positive charge in the low pH conditions of late successional soils, so the increase in histidine proportion and concentration over succession (Fig. 1a–c) may reflect strong adsorption onto a greater number of soil exchange sites in the later successional stages. However, proportions and concentrations of the other basic amino acids, arginine and lysine, did not consistently increase across succession (Table 3), so an explanation for the increased histidine

proportion and concentration remains uncertain. A similar observation was reported for heathland soils (Abuarghub and Read, 1988).

Another possible explanation for the increased concentration of soil amino acids across succession is that the later successional stages have greater belowground allocation in the form of fine roots (Ruess et al., 2006). In the boreal forest, belowground inputs from root turnover are greater and decompose faster than the aboveground litter inputs (Ruess et al., 1996). The large spike in proportion and concentration of glutamine in the black spruce successional stage (Fig. 1a and b) may result from greater quantities of root exudation or lysis from this active root biomass. The pattern of glutamine proportion and glutamine concentration per unit area across succession (Fig. 1a and c) mirrors the pattern of belowground fine root production across succession that Ruess et al. (2006) measured using minirhizotrons. We found high proportions and concentrations of glutamine in the willow and black spruce stages where Ruess et al. (2006) found the greatest allocation of

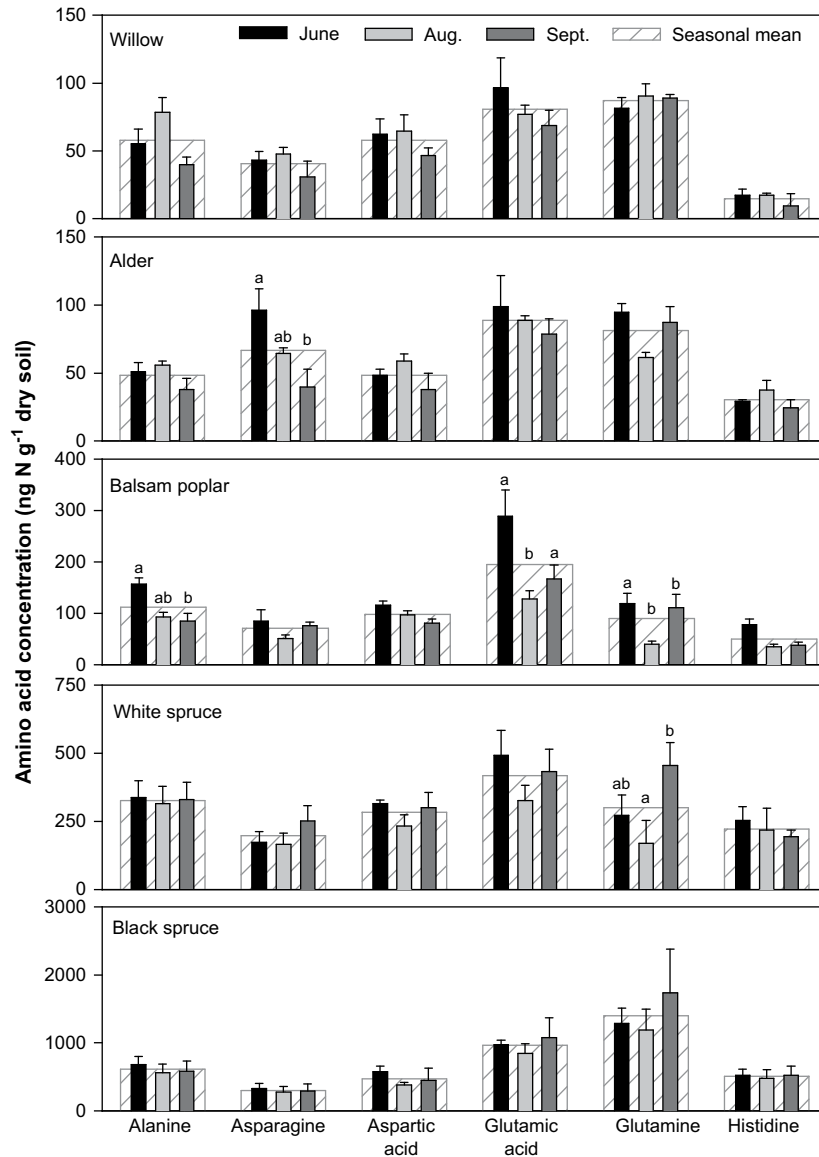


Fig. 3. Concentrations of the six dominant amino acids in soils of each successional stage for June, August, September, and the seasonal mean. Different lower case letters indicate statistical differences among months for a given amino acid of the successional stage. Monthly values are means (+1 SEM), $n = 3$. Seasonal values are means (SEM omitted for simplicity), $n = 9$.

belowground production, whereas we found a low proportion and concentration of glutamine in the balsam poplar stage that had the lowest belowground allocation.

4.3. Seasonal dynamics of soil amino acids

The composition and concentration of the soil amino acid pool appeared relatively constant over the growing season for each successional stage, in contrast to our original hypothesis and despite major seasonal changes in plant productivity, litterfall, microbial freeze–thaw flushes, and soil temperatures. Most studies have found that amino acid concentrations do vary significantly across the season (Abuarghub and Read, 1988; Kielland, 1995; Lipson et al., 1999; Weintraub and Schimel, 2005; though see Nordin et al., 2001; Jones et al., 2005a). Detecting seasonal changes, if present, may be problematic given that soil amino acids can fluctuate from high to low concentrations in less than a week (Weintraub and Schimel, 2005). We sampled soils over three

months at monthly intervals, which may constitute a rather coarse temporal resolution.

4.4. TFAA concentration compared to other studies

The range of TFAA concentrations of this study is within the range of values reported for a variety of ecosystems and climates (Table 4). Our mean TFAA values (20–350 nmol amino acid g^{-1} dry soil, 0.4–5.4 μg amino acid $N g^{-1}$ dry soil, or 40–300 μM) are similar or slightly higher than soil TFAA concentrations in the boreal forest in Sweden (5–455 nmol amino acid g^{-1} dry soil; Persson and Näsholm, 2001), the alpine dry meadows of Colorado (0.3–3.0 μg amino acid $N g^{-1}$ dry soil; Lipson et al., 1999), and the mixed conifer and pygmy forests in northern California (1.6–29.9 μM ; Yu et al., 2002). We caution, however, about making inferences from these comparisons due to differences in soil sampling depth, organic matter content, and extraction methods among the studies (Jones and Willett, 2006).

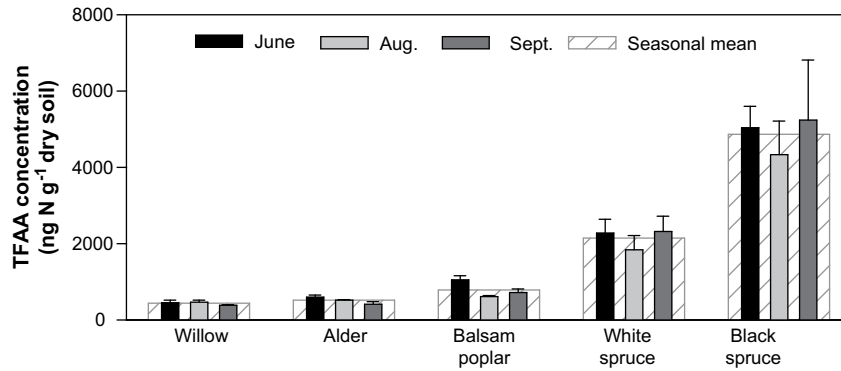


Fig. 4. Total free amino acid (TFAA) concentration in soils of each successional stage for June, August, September, and the seasonal mean. There was no statistical significance among the months for any successional stage. Monthly values are means (+1 SEM), $n = 3$. Seasonal values are means (SEM omitted for simplicity), $n = 9$.

5. Conclusions

Contrary to our original hypothesis, the composition of the soil amino acid pool did not change across succession. Irrespective of successional stage, the soil amino acid pool was dominated by the same six amino acids: glutamic acid, glutamine, aspartic acid, asparagine, alanine, and histidine. However, the concentration of soil amino acids did significantly increase across the successional sequence, and amino acid concentrations were an order of

magnitude higher in the coniferous-dominated late successional stages than in the early deciduous-dominated stages. In addition, despite major seasonal changes in plant productivity, litterfall, microbial freeze–thaw flushes, and soil temperature, the composition and concentration of the soil amino acid pool were generally constant over the growing season for each successional stage. The similar amino acid composition across the successional sequence suggests that amino acids originate from a common source or through similar biochemical processes, yet elucidating which

Table 4

Comparison of soil amino acid composition and total free amino acid (TFAA) concentration in this and previously published studies. For comparability, studies were limited to those that examined water or weak salt extracts of soil or soil pore water. Individual amino acid composition is the proportion of the total free amino acid concentration (%) and rank (1–18). Bold indicates proportions greater than 5.6% (value if all 18 amino acids were found in equal proportion).

Amino acid	Agricultural, Ontario, Canada ^a	<i>Calluna</i> heathland, England ^b		<i>Calluna</i> heathland, England ^c		Arctic tundra, Alaska ^d	Boreal forest, Sweden ^e	Forest, northern California ^f	Fertilized grassland, Wales ^g	Boreal forest, Alaska ^h
	Rhizosphere	A0	A1	Litter	0–5 cm	Organic 0–10 cm	Mor 0–7 cm	Oa	Ah 0–15 cm	0–20 cm
Alanine	9.7 (6)	21.8 (1)	8.4 (3)	16.5 (2)	17.2 (2)	1.3 (12)	13.9 (2)	4.5 (9)	14.1 (2)	12.4 (3)
Arginine	tr (16)	17.5 (3)	52.5 (1)	8.0 (5)	7.3 (6)	14.8 (2)	7.6 (6)	8.7 (5)	6.2 (8)	4.0 (7)
Asparagine	18.3 (1)					1.9 (11)	8.4 (5)	18.4 (2)	4.9 (11)	9.3 (5)
Aspartic acid	10.4 (4)	7.5 (5)	2.4 (9)	4.3 (8)	5.4 (7)	13.9 (4)		5.5 (8)		11.6 (4)
Glutamic acid	10.0 (5)	20.9 (2)	13.6 (2)	11.5 (3)	10.0 (5)	9.2 (5)		14.8 (3)	16.9 (1)	19.9 (1)
Glutamine	4.6 (8)					2.8 (8)	18.1 (1)			17.3 (2)
Glycine	11.9 (3)	5.4 (6)	3.0 (6)	6.5 (6)	11.7 (3)	22.7 (1)	8.9 (4)	9.5 (4)	7.3 (7)	2.6 (11)
Histidine	2.4 (10)			27.7 (1)	21.5 (1)			6.9 (6)	5.3 (10)	7.3 (6)
Isoleucine	1.5 (14)	1.7 (10)	0.6 (12)	1.9 (11)	2.0 (11)			0.9 (12)	5.3 (9)	1.4 (12)
Leucine	1.8 (12)	4.6 (7)	2.6 (7)	5.3 (7)	5.2 (8)	4.2 (7)		0.8 (13)	8.5 (4)	1.3 (13)
Lysine	0.8 (15)	2.5 (9)	6.0 (4)	3.4 (9)	3.9 (9)	2.3 (9)		6.4 (7)	1.7 (14)	0.8 (16)
Methionine	tr (17)					2.3 (10)		0.1 (15)	0.6 (15)	0.5 (18)
Phenylalanine	1.6 (13)	0.0 (12)	1.8 (10)	1.1 (12)	1.2 (12)	0.8 (13)		0.3 (14)	3.7 (12)	0.9 (15)
Serine	16.1 (2)	14.2 (4)	5.9 (5)	9.7 (4)	11.3 (4)	14.4 (3)	10.5 (3)	19.3 (1)	9.6 (3)	3.8 (8)
Threonine	5.9 (7)	w/Ser ⁱ	w/Ser ⁱ	tr ^j (14)	tr ^j (14)	8.5 (6)		w/Gly ⁱ	7.3 (6)	2.7 (9)
Tryptophan								0.1 (16)		1.0 (14)
Tyrosine	1.9 (11)	0.7 (11)	2.5 (8)	0.7 (13)	0.9 (13)			2.0 (10)	2.6 (13)	0.6 (17)
Valine	3.2 (9)	3.2 (8)	0.9 (11)	3.4 (10)	2.2 (10)	0.8 (13)		1.5 (11)	8.4 (5)	2.6 (10)
TFAA	4721 ^k	1521 ^k	1286 ^k	5709 ^k	1933 ^k	2360 ^k	1878 ^k	250 ^l	601 ^l	1752 ^k
TFAA range	2220–7221 ^k					1570–8290 ^k	1158–2305 ^k	33–475 ^l	370–909 ^l	386–5236 ^k

^a Ivarson and Sowden (1969).

^b Read and Bajwa (1985).

^c Abuarghub and Read (1988).

^d Kielland (1995).

^e Nordin et al. (2001).

^f Yu et al. (2002).

^g Jones et al. (2005a).

^h This study.

ⁱ Amino acid is combined with serine or glycine value.

^j Trace amount detected.

^k TFAA concentration expressed as ng N g⁻¹ dry soil.

^l TFAA concentration expressed as ng N ml⁻¹ soil pore water.

ecosystem components or processes regulate a particular amino acid needs further investigation. Results of this study demonstrate that amino acids are important constituents of the biogeochemically diverse soil N pool in the boreal forest of interior Alaska.

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