

Amino acid abundance and proteolytic potential in North American soils

Kirsten S. Hofmockel · Noah Fierer ·
Benjamin P. Colman · Robert B. Jackson

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Abstract Studies of nitrogen (N) cycling have traditionally focused on N mineralization as the primary process limiting plant assimilation of N. Recent evidence has shown that plants may assimilate amino acids (AAs) directly, circumventing the mineralization pathway. However, the general abundance of soil AAs and their relative importance in plant N uptake remains unclear in most

ecosystems. We compared the concentrations and potential production rates of AAs and NH_4^+ , as well as the edaphic factors that influence AA dynamics, in 84 soils across the United States. Across all sites, NH_4^+ and AA-N comprised similar proportions of the total bioavailable N pool ($\sim 20\%$), with NO_3^- being the dominant form of extractable N everywhere but in tundra and boreal forest soils. Potential rates of AA production were at least comparable to those of NH_4^+ production in all ecosystems, particularly in semi-arid grasslands, where AA production rates were six times greater than for NH_4^+ ($P < 0.01$). Potential rates of proteolytic enzyme activity were greatest in bacteria-dominated soils with low NH_4^+ concentrations, including many grassland soils. Based on research performed under standardized laboratory conditions, our continental-scale analyses suggest that soil AA and NH_4^+ concentrations are similar in most soils and that AAs may contribute to plant and microbial N demand in most ecosystems, particularly in ecosystems with N-poor soils.

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K. S. Hofmockel (✉) · R. B. Jackson
Nicholas School of the Environment and Earth Sciences,
Duke University, Durham, NC 27708, USA
e-mail: khof@iastate.edu

K. S. Hofmockel
Department of Ecology, Evolution and Organismal Biology,
Iowa State University, 237 Bessey Hall, Ames, IA 50011, USA

N. Fierer
Cooperative Institute for Research in Environmental Sciences,
University of Colorado at Boulder, 216 UCB, Boulder,
CO 80309-0216, USA

N. Fierer
Department of Ecology and Evolutionary Biology,
University of Colorado at Boulder, 334 UCB, Boulder,
CO 80309-0334, USA

B. P. Colman
Department of Ecology, Evolution, and Marine Biology,
University of California, Mail Stop 9610, Santa Barbara,
CA 93106-9610, USA

B. P. Colman · R. B. Jackson
Department of Biology, Duke University,
Durham, NC 27708, USA

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Introduction

Recent observations of direct amino acid (AA) uptake by plant roots have challenged the paradigm that plants primarily assimilate inorganic nitrogen (N) from soils (Weigelt et al. 2005). In all ecosystems where AA cycling has been studied, plants and soil microorganisms are both capable of assimilating AAs (Bardgett et al. 2003; Hofmockel et al. 2007; McFarland et al. 2002). If this observation is true more generally, then ecosystem nutrient

budgets that do not include AAs may be missing an important source of bioavailable N that can be assimilated by plants and microorganisms (Schimel and Bennett 2004). Models of soil N cycling are also beginning to include the depolymerization and production of simple organic molecules as an important process driving available N supply (Lipson and Näsholm 2001; Schimel and Bennett 2004). Given the amount of soil N found in organic molecules, organic N depolymerization and AA production may be broadly important for the terrestrial N cycle (Kielland 1995; Schulten and Schnitzer 1998).

Most investigations of AA uptake by plants have been conducted in alpine and higher latitude boreal or tundra ecosystems. Such systems often have deep organic horizons and relatively slow rates of mineralization and nitrification, factors that may make AAs a more important N source for plants than inorganic N. In contrast, soil AA dynamics remain largely unstudied in warm temperate or tropical ecosystems, where deep organic horizons are lacking, and where enzymatic reactions often proceed more rapidly (Hofmockel et al. 2007; Weigelt et al. 2005).

The incorporation of AA pools and production rates into the current conceptual model of N cycling has enhanced our understanding of the plant–microbe interactions that regulate N availability in terrestrial ecosystems (Fig. 1). This new conceptual model suggests that in N-poor ecosystems, AAs may provide an important source of bioavailable N. In N-rich systems with high rates of ammonification and subsequent nitrification, direct incorporation of AAs by plants may not be biologically

significant because AAs will be rapidly mineralized to NH_4^+ and subsequently oxidized to NO_3^- prior to plant assimilation (Schimel and Bennett 2004). Instead, “short-circuiting” of the mineralization pathway, i.e., the direct assimilation of AAs by plants, is assumed to primarily occur in soils with low rates of ammonification and nitrification. However, this conceptual model has not to our knowledge been validated with empirical evidence.

The goal of our work was to compare the importance of protein degradation to AAs (proteolysis) relative to mineralization to NH_4^+ (ammonification) for the production of plant-available N (Fig. 1). We examined the concentrations and potential rates of proteolysis and ammonification concurrently in laboratory incubations of 84 soils collected from a range of ecosystems across North America. Using these results, and our characterization of site and soil properties, we addressed the following three questions. (1) Is proteolysis of plant and microbial litter faster than the rate of ammonification, resulting in AA concentrations that permit assimilation by plants? (2) Is the enzymatic potential for protease activity only relevant in ecosystems with low N availability, such as high-elevation/latitude systems, or is proteolysis a more universal mechanism for supplying bioavailable soil N? (3) What differences in edaphic factors and climate might be driving AA pool size and production rates? To our knowledge, this research is the first continental-scale survey of AA cycling and the broad ecological controls on AA dynamics. Understanding the balance between proteolysis and ammonification among terrestrial ecosystems should greatly improve the current understanding of AAs in soil and their role in the terrestrial N cycle.

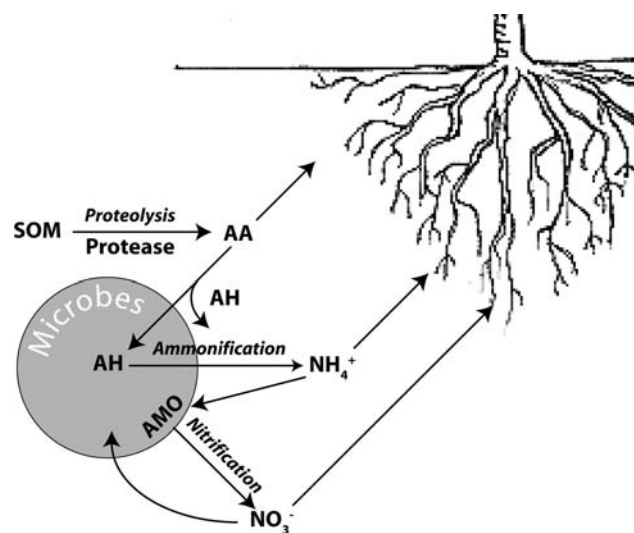


Fig. 1 Emerging conceptual model of the soil N mineralization pathway. Soil pools include soil organic matter (SOM), free amino acids (AA), ammonium (NH_4^+) and nitrate (NO_3^-). *Unlabeled arrows* represent assimilation by plants and microorganisms. *Labeled arrows* depict enzyme-induced transformations. Enzymes include amidohydrolyase (AH), ammonia monooxygenase (AMO) and protease

Materials and methods

Sample collection

We collected 84 soils from 35 different locations across the US as described in Fierer et al. (2006). We restricted our sampling to unsaturated/upland soils found in mid- to late-successional ecosystems (see Appendix A of the “[Electronic supplementary material](#)”). All samples were collected between April and September of 2003 near the height of the plant-growing season for each individual location.

At each site, the top 0–5 cm of mineral soil was collected using a soil corer or hand trowel. We acknowledge that excluding organic horizons underestimates total organic N pools within the soil profile; however, because 32 of the soils included in this study did not have organic horizons, we limited our investigation to mineral horizons to permit cross-site comparisons. Soil was collected from a

minimum of five different places within each site and composited into a single soil sample (~1 kg field-moist soil) representative of that site. Soils were then sieved to 4 mm and homogenized, with root fragments removed by hand.

Site characteristics and soil measurements

The following site characteristics were determined for each location: average O horizon depth, dominant plant species, mean annual temperature (MAT), mean annual precipitation (MAP), and average annual soil moisture deficit (SMD) (see Fierer and Jackson 2006 for additional details). The following soil characteristics were measured on field-moist soils: pH (in distilled water), total organic carbon (C) and N concentration, texture, gravimetric moisture content, and water-holding capacity, using methods described in Fierer and Jackson (2006). Fungal:bacterial ratios were estimated using quantitative PCR assays as described in Fierer et al. (2005b).

We incubated all soils at 20°C and 35% of water-holding capacity. Constant temperature and similar moisture conditions were necessary to allow us to minimize the confounding effects of moisture and temperature on N cycling and enable us to compare potential N dynamics across sites (Zak et al. 1993; Barrett and Burke 2000; Santiago et al. 2005; Jones et al. 2009). We chose 20°C because it was close to the mean monthly temperature average for all sites (17°C), and we chose 35% of water-holding capacity to insure maintenance of aerobic conditions, while keeping the soils moist (Gulledge and Schimel 1998). We adjusted all soils to 35% of water-holding capacity by drying at 20°C or wetting with deionized water, as in Fierer and Jackson (2006). To minimize the impacts of antecedent temperature and moisture conditions, and to minimize the pulse of microbial activity associated with the disturbance of sieving and manipulating moisture, soils were preincubated at 20°C for ten days.

After the ten-day incubation, triplicate soil samples were extracted for 1 h with 0.5 M K₂SO₄. Extractable NH₄⁺ and NO₃⁻ concentrations were then measured on a Lachat 2300 autoanalyzer (Milwaukee, WI, USA), using Lachat methods 31-107-06-5-A and 12-107-04-1-B, respectively. Concentrations of dissolved organic C and total dissolved N (DOC and TDN, respectively) in the K₂SO₄ extracts were measured using a Shimadzu TOC-V CPH (Columbia, MD, USA). The concentration of AA-N in K₂SO₄ extracts was determined fluorometrically by the *o*-phthaldialdehyde and β-mercaptoethanol method (OPAME; Jones et al. 2002).

Potential rates of net N mineralization and microbial CO₂ production were measured on triplicate subsamples over the course of a 50-day incubation at 20°C (Fierer et al.

2006). This duration was chosen because some soils had very little microbial activity, and thus an incubation of this length was necessary to observe measurable rates. Net N mineralization was calculated as the change in total K₂SO₄-extractable NH₄⁺-N and NO₃⁻-N (see above) over the course of the incubations. Because the soils were sieved and incubated at a constant temperature and moisture content, the rates of C and N mineralization represent potential mineralization rates, and as such, the rates cannot be scaled to field rates. However, the rates can be compared across ecosystems, allowing us better insight into how soil edaphic factors influence these rates.

Potential rates of enzyme activity

We measured the activities of amidohydrolase and protease enzymes (Fig. 1) in the presence of ambient and saturating substrate concentrations (sensu Lipson et al. 1999; Watanabe and Hayano 1995). Our approach enabled us to measure maximum rates of enzyme activity as well as rates governed by ambient substrate concentrations. After the ten-day preincubation, soil slurries were prepared by measuring 3 g soil subsamples into 40-ml centrifuge tubes, each with 10 ml of 0.05 M sodium acetate buffer (pH 5.5). Protein-enriched samples received 10 ml of 0.6% casein in 0.05 M sodium acetate buffer. Next, 0.4 ml of toluene were added to inhibit microbial consumption of AAs and NH₄⁺, which enabled us to quantify the combined activity of intracellular and extracellular enzymes (Skujins 1967). Toluene additions are standard protocol for protease (Lipson et al. 1999; Finzi and Berthrong 2005; Kielland et al. 2007) and amidohydrolase assays (Tabatabai 1995). In the absence of a microbial inhibitor, microbial assimilation of reaction products makes it impossible to compare enzyme activity among soils. By inhibiting microbial growth and making microbial cell membranes permeable, toluene additions are believed to stop de novo enzyme synthesis, release intracellular enzymes and products, and inhibit assimilation of reaction products (Skujins 1967; Tabatabai 1995). Although active excretion of NH₄⁺ may decrease with toluene additions, reaction products should be released due to increased permeability of the cell membranes. Samples were shaken lengthwise during incubation periods of 1, 3, 5, 7, and 24 h. At the end of each incubation period, 3 ml of trichloroacetic acid mix were added to arrest enzyme activity, and samples were subsequently centrifuged and filtered. This technique was intended to measure potential enzyme activity in the absence of assimilation, and therefore does not account for plant-microbe interactions that will influence the availability and cycling of AAs under field conditions.

Free AA concentrations were determined by fluorescence using the OPAME method, which has a high degree

of specificity for free AAs and does not react appreciably with other soil solutes (Jones et al. 2002), but may include amino sugars. Concentrations of AA-N were determined by comparing the absorbance of the samples relative to a leucine standard curve (Rosen 1957; Berthrong and Finzi 2006; Weintraub and Schimel 2005; Kielland et al. 2007). Ammonium concentrations were determined by colorimetric analysis (Lachat QuickChem 8000 Series, Milwaukee, WI, USA). Potential rates of proteolysis and ammonification were calculated as the slope of the relationship between AA-N or NH_4^+ -N production and time. For those samples that did not produce any relationship between accumulation of AA-N or NH_4^+ -N and time due to apparent N consumption (despite the addition of toluene), we could not calculate potential rates of enzyme activity. These samples (9% of AA samples and 20% of NH_4^+ samples) were randomly distributed among sites, and no significant relationship with ecosystem type was detected (Fisher's exact test; $P = 0.90$ and 0.10 , respectively).

We measured potential rates of proteolysis relative to ammonification because the cycling of NH_4^+ is well studied and because ^{15}N studies suggest that NH_4^+ -N and AA-N are assimilated at approximately the same rate (Hofmockel et al. 2007; McFarland et al. 2002). Furthermore, because NH_4^+ production precedes NO_3^- production, we focused on ammonification as an index for potential rates of inorganic N production. Similar to our data for N mineralization and microbial CO_2 production, this approach provides a measure of potential enzymatic activity that does not necessarily represent field rates, but rather gives us insight into which soil and ecosystem characteristics might drive amidohydrolase and protease capacity in general when moisture and temperature are held constant.

Methodological considerations

Kinetic measures such as those used in this study are a useful way to understand and capture N dynamics, providing important information about enzymatic potential that cannot be captured in static measures of pool size or assays of net N mineralization in field or laboratory incubations. Nonetheless, developing acceptable methods for measuring depolymerization in soils remains a major challenge (Schimel and Bennett 2004). For example, because our assays were conducted at one pH and temperature and in the absence of plant and microbial assimilation, they are only a relative index of proteolysis and ammonification under field conditions. Similarly, ^{14}C -labeled AA additions to soils incubated at one moisture and temperature (Jones et al. 2009) can provide a relative index of AA turnover that incorporates plant and microbial assimilation but uses substrate additions, and as such does not measure extant AA production and mineralization.

Because such studies relate only to static assay conditions, field measurements of proteolytic enzyme activity at individual sites are required to determine the actual importance of proteolysis relative to ammonification under environmental conditions, where microorganisms and plants interact with the physiochemical conditions to regulate enzyme activity and N cycling. Development of a direct assay of AA cycling would be a valuable tool for enabling a better understanding of the fate of AAs in terrestrial ecosystems.

Statistical analyses

We addressed whether AA availability differs among ecosystems using a one-way analysis of variance (ANOVA) followed by a multiple comparisons of means using a Fisher's protected LSD procedure to compare soil concentrations of N fractions (NH_4^+ , NO_3^- , AA) and potential production rates (AA, NH_4^+ , AA + NH_4^+) between ecosystems. To test if proteolysis exceeds ammonification, the proteolysis:ammonification rate ratio was calculated for each soil sample, and one-way ANOVA was used to examine differences among ecosystems for the ratio. Linear regressions and stepwise multiple regressions were used to evaluate the relationships between AA availability and soil and site characteristics (see Table 1). Variables that were strongly correlated with several site characteristics (dissolved organic N concentration, organic C concentration, and C mineralization rate) were removed from the stepwise regression. We inspected data for departures from normality using the Shapiro-Wilk method, and when needed, data were log-transformed to meet normality assumptions. In all analyses, statistical significance was evaluated at $\alpha = 0.05$.

Results

Pool sizes

Extractable soil N differed substantially across ecosystem types, with ecosystems following the same rank order of decreasing AA-N and NH_4^+ -N concentrations; the highest concentrations were found in humid forests, followed by intermediate concentrations in boreal forests and tundra, humid grasslands and semi-arid forests, and the lowest concentrations in semi-arid grasslands and shrublands (Fig. 2; $P < 0.05$). On average, the sum of AA-N and NH_4^+ -N comprised a substantial pool in all biomes ($40 \pm 4\%$; mean \pm SE), particularly in boreal forest and tundra ecosystems ($76 \pm 13\%$ of bioavailable N) and humid forests ($61 \pm 7\%$ of bioavailable N; Fig. 3). Across all soils, NH_4^+ -N and AA-N comprised on average

Table 1 Correlations (Pearson's r) between edaphic characteristics and AA pools, potential protease activity and the proteolysis:ammonification ratio

Parameter	AA pool size ^a ($\mu\text{mol AA-N g}^{-1}$)		Potential protease activity ^a ($\mu\text{mol AA-N g}^{-1} \text{ h}^{-1}$)		Proteolysis:ammonification ratio ^a (AA:NH ₄)	
	r	n	r	n	r	n
$\mu\text{mol DON-N g}^{-1}(\text{soil})^a$	0.560***	82	0.063	74	-0.309*	56
Mean annual precipitation	0.473***	84	0.073	76	-0.175	56
pH	-0.601***	84	0.217	76	0.602***	56
Organic C ^a (%)	0.560***	84	-0.032	76	-0.465**	56
$\mu\text{mol NH}_4\text{-N g}^{-1a}$	0.376**	84	-0.486***	76	-0.523***	56
TN ^a (%)	0.499***	84	0.093	76	-0.246	56
Microbial biomass (by SIR)	0.338**	84	-0.087	76	-0.342*	56
Net C mineralization ^a	0.354**	84	-0.102	76	-0.443***	56
C:N	0.321**	84	-0.272*	76	-0.569***	56
$\mu\text{mol NO}_3\text{-N g}^{-1}(\text{soil})^a$	0.019	84	0.256*	76	0.429**	56
Net N mineralization	0.116	84	0.103	76	0.109	56
Soil moisture deficit ^a	-0.538***	84	-0.02	76	0.284*	56
Fungal:bacterial ratio ^a	0.13	74	-0.409**	68	-0.462**	56
Net nitrification ^a	0.124	67	0.196	59	0.312*	42
Mean annual temperature	-0.202	84	0.251*	76	0.389**	56
Elevation ^a	-0.029	84	-0.122	76	0.112	56
O horizon depth	-0.072	84	0.314	76	-0.22	56

Samples with nonlinear production rates were not included in analyses

SIR substrate-induced respiration

Bold values are significant with a probability of <0.05 indicated by *, a probability of <0.01 indicated by **, and a probability of <0.0001 indicated by ***

^a Log transformation

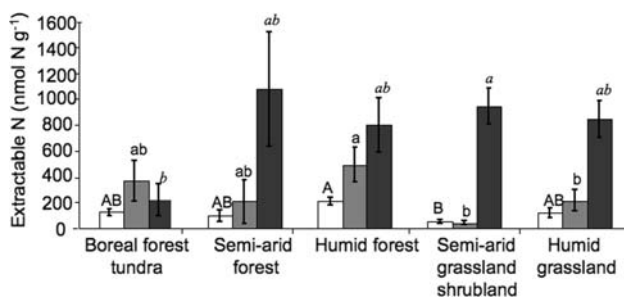


Fig. 2 Average extractable N pool size across all soil samples. Mean (\pm SE) pool size of AA (open bars), NH₄⁺ (gray bars), and NO₃⁻ (black bars). Significant differences are shown in capital letters for AA-N, lower case letters for NH₄⁺-N, and italic letters for NO₃⁻-N

22 ± 2.9 and $18 \pm 2.4\%$ of the total bioavailable N pool, respectively. AA-N and NH₄⁺-N were 3.5 and 6.8 times greater proportions of the bioavailable N pool in boreal forest and tundra than in humid forests and semi-arid grasslands and shrublands, respectively (Fig. 3). Overall, NO₃⁻-N was the dominant form of N in soil solution. On average, the concentration of NO₃⁻-N was three times higher than the concentrations of either NH₄⁺-N or AA-N ($P < 0.0001$). It is important to note that plant-free, aerobic

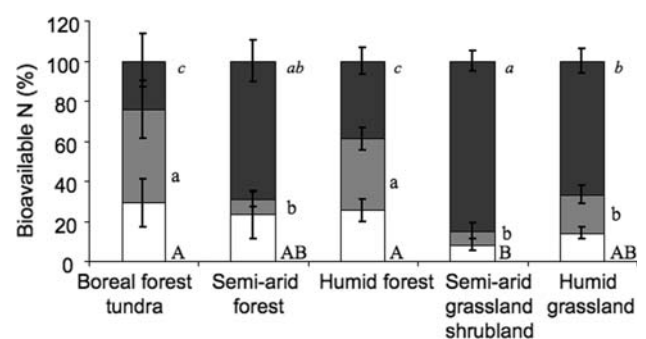


Fig. 3 The proportions of bioavailable N within the extractable soil N pool (AA + NH₄⁺ + NO₃⁻) contributed by AA-N (open bars), NH₄⁺-N (gray bars), and NO₃⁻-N (black bars). Significant differences are shown in capital letters for AA-N, lower case letters for NH₄⁺-N, and italic letters for NO₃⁻-N

incubations promote accumulation of NO₃⁻ because it is an end-product with limited consumption.

Enzyme activity

Rates of proteolysis were slightly higher than rates of ammonification and differed across ecosystems (Fig. 4).

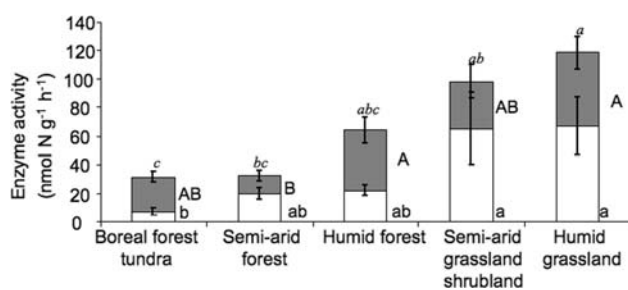


Fig. 4 Average (\pm SE) protease (open bars) and amidohydrolase (solid bars) activity from unenriched samples. Lower case letters correspond to differences in potential rates of proteolysis, capital letters correspond to differences in potential rates of ammonification, and italic letters correspond to differences in the overall enzymatic potential (proteolysis + ammonification)

Across all sites, potential enzyme activity averaged 46 ± 9 nmol N g⁻¹ h⁻¹ (mean \pm SE) for proteolysis and 40 ± 5 nmol N g⁻¹ h⁻¹ for ammonification. Grasslands and humid forests had the greatest enzymatic potential for bioavailable N production. Potential rates of ammonification were four times greater in humid grasslands and three times greater in humid forests compared to semi-arid forests ($P < 0.05$; Fig. 4). Potential rates of proteolysis were nine times greater in humid and semi-arid grassland soils than in boreal forest/tundra ecosystems ($P < 0.05$; Fig. 5). Total potential N production (ammonification + proteolysis) was 3.7 times greater in humid grasslands compared to boreal forest/tundra and semi-arid forests (Fig. 3).

The proteolysis:ammonification ratio provides an index of the activity of proteolytic enzymes compared to amidohydrolase enzymes (Fig. 1). The enzymatic potential for proteolysis in semi-arid grasslands was six times greater than the rate of ammonification ($P = 0.0008$; Fig. 5), suggesting a much greater enzymatic potential for depolymerization to AAs than mineralization to inorganic N. Calculating this ratio for each site and averaging by ecosystem type produced potential proteolysis:ammonification ratios that were close to unity for most ecosystems.

Protein addition experiments

Protein addition experiments were used to evaluate proteolytic potential in the absence of substrate limitation. In the absence of substrate additions, proteolysis ranged from 7 to 67 nmol N g⁻¹ h⁻¹, with the greatest enzyme activities observed in humid grasslands and semi-arid grasslands and shrublands and the lowest activities in boreal forest and tundra soils. Under enriched conditions, potential rates of proteolysis ranged from 27 to 421 nmol N g⁻¹ h⁻¹, and they were 15 times higher in semi-arid grasslands than in boreal forest/tundra systems.

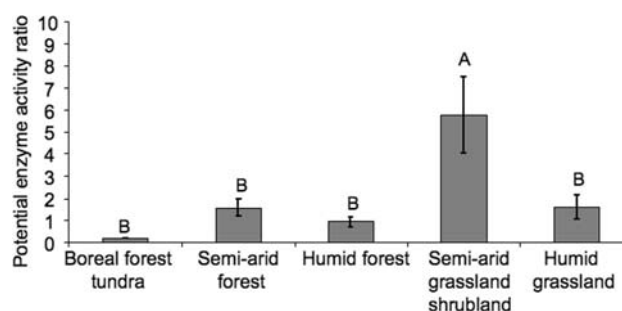


Fig. 5 Proteolysis:ammonification ratios for individual sites, averaged (\pm SE) over ecosystem categories

We used the ratio of enzyme activities under enriched and unenriched conditions to determine the relative extent to which substrate supply or enzyme concentrations limit N production (Weintraub and Schimel 2005). We detected significantly higher enriched:unenriched rates of amidohydrolase activity in semi-arid grassland/shrubland ecosystems relative to humid temperate forests and boreal forest/tundra ecosystems ($P < 0.05$; Fig. 6a). In the presence of additional substrate, no additional ammonification occurred in boreal forest/tundra and humid forest ecosystems (enriched:unenriched ratio ≤ 1 ; Fig. 6a), despite increased proteolysis (Fig. 6b). In boreal forest and tundra soils, enzyme production, rather than substrate availability, appeared to limit NH₄⁺-N production. Among all soils, enriched:unenriched ratios for proteolysis averaged 4.7 (± 0.37), indicating that substrate availability is a major factor limiting potential rates of proteolysis in the samples evaluated here. Enriched proteolysis rates were significantly greater in soils from semi-arid grassland/shrubland compared to humid forests and boreal forest/tundra ($P < 0.05$; data not shown).

Edaphic characteristics and site properties

We also examined the relationships between soil and site characteristics and AA pool size, enzymatic capacity, and the ratio of AA to NH₄⁺ production (Table 1). AA pools were correlated with numerous soil characteristics, including soil pH, organic C and N content, and SMD. In contrast, potential rates of proteolysis were significantly correlated with only a few edaphic parameters, including soil NH₄⁺-N concentration and the fungal:bacterial ratio (Table 1). In stepwise regression analyses, soil AA concentrations were best explained by SMD and pH, producing a cumulative r^2 of 0.45 (Table 2). Proteolytic enzyme activity was negatively correlated with both soil NH₄⁺-N concentration and fungal:bacterial ratio, with a cumulative r^2 of 0.47 (Table 2). The proteolysis:ammonification ratio was positively correlated with soil pH and potential net nitrification and negatively correlated with NH₄⁺

Table 2 Stepwise linear regression results for edaphic factors and AA pools, proteolytic potential and potential proteolysis:ammonification ratio

Dependent variable	Explanatory variable	Partial r^2	Model r^2	p
AA pool size ^a ($\mu\text{mol AA-N g}^{-1}$)	pH	0.41	0.41	<0.0001
	Soil moisture deficit ^a	0.04	0.45	0.047
Proteolytic potential ^a ($\mu\text{mol AA-N g}^{-1} \text{ h}^{-1}$)	$\mu\text{mol NH}_4 \text{ g}^{-1\text{a}}$	0.38	0.39	<0.0001
	Fungal:bacterial ratio ^a	0.08	0.47	0.01
Proteolysis:ammonification ratio ^a	pH	0.47	0.47	<0.0001
	Net nitrification ^a	0.08	0.55	0.01
	$\mu\text{mol NH}_4 \text{ g}^{-1\text{a}}$	0.05	0.6	0.05

^a Denotes log transformations

concentration (Table 1). Together, these parameters had a cumulative r^2 of 0.60 (Table 2).

Discussion

Implications for N cycling

The emerging paradigm of terrestrial N cycling requires an integrated understanding of how proteolysis, ammonification, and N assimilation are related (Fig. 1). Here we provide evidence that the relationship between proteolysis and ammonification may determine the relative availability of AAs for direct assimilation by plants and microorganisms. Our results suggest that, at the peak of plant productivity in mineral soils from boreal forests/tundra and humid forests, microorganisms are not rapidly mineralizing AAs to NH_4^+ (Fig. 6a). This is consistent with a “short-circuiting” of the mineralization pathway, which occurs when the direct assimilation of AAs by plants precludes ammonification by microorganisms (Fig. 1). Soils from boreal forest/tundra ecosystems also have high concentrations of AAs compared to inorganic N forms (Fig. 2), and AAs comprise close to 30% of the bioavailable N pool in soils from these ecosystems (Fig. 3). Low rates of ammonification and high soil AA concentrations support field-based experiments showing that AAs are an important source of bioavailable N for plants in boreal forest/tundra ecosystems (Kielland 1994; Näsholm et al. 1998).

In contrast to other studies (Jones and Kielland 2002), these results suggest that proteolysis and ammonification may be decoupled in some high-latitude soils. It is likely that this decoupling is in part due to diminished microbial mineralization of AA-N in high-latitude ecosystems. Our ecosystem-specific results differ from results derived from weeklong incubation experiments using ^{14}C -labeled AAs in soils from a latitudinal gradient, which demonstrate that on average, microbial mineralization (microbial immobilization and subsequent turnover) accounts for 71% of added AA-C (Jones et al. 2009). Similar to our results,

experiments in high-latitude forest ecosystems using proteolytic enzyme assays and ^{13}C -labeled AAs demonstrate that proteolytic activity could provide the majority of plant N demand, despite significant microbial immobilization of AAs (Kielland et al. 2007). Complementary field studies in high-latitude ecosystems are required to determine if the low rates of ammonification relative to proteolysis are attributable to higher rates of direct AA assimilation by plants and microorganisms near the peak of the plant-growing season.

Proteolysis appears to control ammonification in soils from semi-arid forest, semi-arid grasslands and humid grasslands. In these soils, amidohydrolase enzymes respond to substrate additions (Fig. 6), indicating that ammonification increases with increasing rates of proteolysis, as expected (Schimel and Bennett 2004; Jan et al. 2009; Jones et al. 2009; Fig. 1). In these ecosystems, proteolysis, not ammonification, limits the rate of mineralization to NH_4^+ (Jones et al. 2004). Soils from semi-arid forests and humid grasslands had an average proteolysis:ammonification ratio of about 1.5 (Fig. 5), suggesting that most AAs are rapidly converted to NH_4^+ . In these soils, proteolysis was closely linked to ammonification; therefore, it is unlikely that direct uptake of AAs is a dominant pathway for N assimilation. Field experiments in the presence of roots and organic horizons are required to directly assess the relative importance of AAs for plant and microbial N uptake (Finzi and Berthrong 2005; Hofmockel et al. 2007; Hobbie et al. 2009).

Our enzyme activity results from semi-arid grasslands do not consistently support the paradigm that, in soils with high N availability and cycling, organic N is less important than inorganic N (Nordin et al. 2001; Schimel and Bennett 2004). Although both humid and semi-arid grasslands had similar rates of proteolysis (Fig. 4), the relationship between proteolysis and ammonification differed between these two ecosystems (Fig. 5). Soils from semi-arid grasslands had six times the enzymatic potential for proteolysis compared to ammonification (Fig. 5). This elevated proteolysis potential relative to ammonification

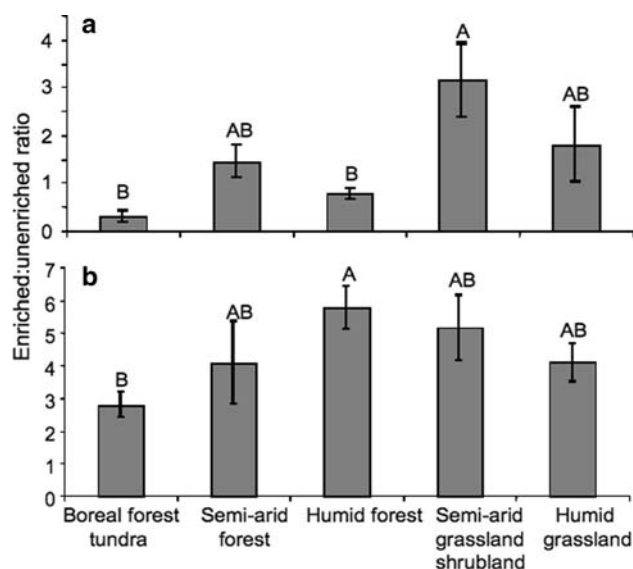


Fig. 6 Ratio of enzymatic potential in protein-enriched incubations compared to unenriched incubations for **a** amidohydrolase and **b** protease

suggests that extant organic N substrate quality (Jan et al. 2009) and quantity may cause ecosystem-specific differences in proteolysis and ammonification that may not be detected in studies based on substrate additions (Jones et al. 2009). Additionally, a high enzymatic potential for proteolysis relative to ammonification suggests that AAs may provide an important source of available N to plants and microorganisms in semi-arid grasslands. If this is the case, the plant-available N pool would be greater than previously thought, potentially altering our understanding of N limitation and cycling in these grassland ecosystems.

Although grasslands can support high rates of nitrification (Austin et al. 2006), it is unlikely that this NH_4^+ -consuming process contributed to the contrasting results in humid versus semi-arid grasslands. Nitrification is much more sensitive to toluene inhibition than ammonification, and under the experimental conditions of our enzyme assays, nitrification was inhibited (Fuller and Scow 1996). Therefore, the high proteolysis:ammonification ratio in semi-arid grassland and shrubland ecosystems is likely driven by high rates of protease activity relative to amidohydrolase activity, not consumption of NH_4^+ by nitrification. Although soil NO_3^- concentration was significantly greater in soils from semi-arid grasslands (Fig. 2), soil N concentrations represent the balance between production and consumption. High NO_3^- concentrations are often associated with preincubation of samples, because in plant-free aerobic soils, consumption of NO_3^- is limited, promoting NO_3^- accumulation (Schimel et al. 1989). Nonetheless, assessing the relationship between proteolysis, ammonification and nitrification in the field is an

important next step in determining the contribution of nitrification to the contrasting results in humid versus semi-arid grasslands.

Our experiments further suggest that bioavailable N is greatest in grassland ecosystems in general. Because we incubated soils from a range of latitudes at one standard temperature, our results may not reflect a standard response under field conditions. Our results demonstrate that the microbial potential to produce protease and amidohydrolase enzymes differs significantly among ecosystems. Using enzymatic potential (Fig. 4) as an index of N availability, we found that boreal forest and tundra ecosystems had the lowest potential for bioavailable N production and grasslands had the highest. Potential proteolysis rates were three times greater in grassland ecosystems compared to boreal forest/tundra ecosystems (Fig. 4). This study did not measure potential enzyme activity in the O horizon. For the 52 sites where an O horizon was present, the average depth was only 3.34 ± 0.45 cm. Although there was no significant relationship between O horizon depth and enzyme potential (Table 1), it is likely that our measurements may underestimate proteolysis in high-altitude sites. Nonetheless, if proteolysis is the key cog in the N cycle, high proteolytic enzymatic potential supports the idea that, in mineral soil, N production is greatest in grassland ecosystems.

Possible drivers of patterns

Microbial community dynamics appear to have an important impact on proteolytic enzyme activity. Although fungi have been shown to dominate N transformations in some ecosystems (Crenshaw et al. 2008; Green et al. 2008), potential rates of proteolytic enzyme activity in this study were greatest in bacterial-dominated soils with low NH_4^+ pools (Table 2). The few field studies that have measured microbial sources of proteolytic enzymes consistently identify bacteria as the dominant source (Hayano 1996; Sakurai et al. 2007; Watanabe et al. 2003). On average, semi-arid grasslands and shrublands had the highest pH, favoring both bacterial communities and proteolytic enzyme activity. Because bacterial biomass and enzymatic activity tend to increase with increasing pH (Baath 1996, 1998; de Vries et al. 2006), and because the optimal pH for protease activity is neutral to alkaline (Ladd 1972), the higher pH conditions in semi-arid grasslands (7.7 ± 0.20) may favor proteolysis. Similarly, high pH has been shown to create optimal conditions for oxidative enzyme activity in desert soils, resulting in enzymatic potentials that were an order of magnitude greater than the oxidative enzyme potential measured in other ecosystems (Stursova and Sinsabaugh 2008; Stursova et al. 2006). Therefore, our results may actually underestimate the differences between

potential proteolytic activities in soils outside of the optimal pH range (e.g., low pH boreal soils and higher pH semi-arid soils; see Appendix A of the “[Electronic supplementary material](#)”).

Large standing pools of NH_4^+ may also contribute to low rates of proteolysis in some soils. The negative correlation between NH_4^+ concentration and AA pools and production rates may be due to NH_4^+ inhibition of proteolysis. Inhibition of protease activity by NH_4^+ has been demonstrated in laboratory studies of *Saccharomyces cerevisiae* (Coffman and Cooper 1997) and *Bacillus* sp. (Liu and Hsieh 1969). Nitrogen inhibition of proteolytic enzyme activity (peptidase) has also been demonstrated in experiments from semi-arid grassland soils (Stursova et al. 2006). In soils with low enzymatic potential for proteolysis (e.g., boreal forests/tundra, humid forest), proportionally higher concentrations of NH_4^+ (Fig. 3) may inhibit proteolytic enzyme activity. By contrast, high enzymatic potential in grasslands may be a consequence of small standing pools of NH_4^+ , thereby alleviating NH_4^+ inhibition of proteolytic enzyme activity.

Due to correlations between edaphic characteristics measured in our study, it is difficult to isolate the precise mechanisms controlling AA availability (Fierer and Jackson 2006). For example, under the conditions of our experiment, it is not possible to directly determine which independent variables caused significantly greater proteolytic enzyme activity and potential proteolysis:ammonification ratios in semi-arid grasslands. Because enzyme potential is a function of the interactions among plant litter biochemistry, microbial community composition, ecosystem N demand, and the exchange of C and N between plant and microbial communities, correlations with multiple edaphic characteristics should be considered in concert to develop testable hypotheses for future field studies. While extracellular enzyme activity can be influenced by a wide variety of biotic and abiotic factors, we found that microbial community structure and soil ammonium concentrations had strong influences on potential rates of proteolysis (Tables 1, 2), and thus are potential drivers of AA availability. There are multiple alternative scenarios that could be put forth to explain these correlations. Such hypotheses need to be tested under field conditions.

Conclusions

Studies investigating N cycling dynamics in terrestrial ecosystems often do not consider proteolysis or the availability of AAs for plant or microbial uptake. As a result, we often lack evidence on the ecological significance of AAs in soil (Jones et al. 2005). Our continental-scale survey of soil AA concentrations and proteolytic potential provides

evidence that proteolysis regulates N availability in a wide range of ecosystems. Our results also suggest that AA availability for plants will be greatest in soils with low amidohydrolase enzyme activity. In ecosystems with higher N cycling rates, ammonification is tightly linked to proteolysis, resulting in ammonification of AAs to NH_4^+ . The much greater capacity for proteolysis compared to ammonification in semi-arid grasslands may be driven by high rates of nitrification, which minimizes NH_4^+ inhibition of protease production. Field validation of the relationship between proteolysis, ammonification and nitrification is needed for soils with high N availability and high plant N uptake. The importance of AAs as a plant-available source of N appears to be greatest in N-poor soils that have low ammonification rates. If proteolysis consistently exceeds ammonification and subsequent nitrification in semi-arid grasslands, however, AAs may also make a biologically significant contribution to plant N demand in relatively N-rich ecosystems.

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References

- Austin AT, Sala OE, Jackson RB (2006) Inhibition of nitrification alters carbon turnover in the Patagonian steppe. *Ecosystems* 9:1257–1265
- Baath E (1996) Adaptation of soil bacterial communities to prevailing pH in different soils. *FEMS Microbiol Ecol* 19:227–237
- Baath E (1998) Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. *Microb Ecol* 36:316–327
- Bardgett RD, Streeter TC, Bol R (2003) Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology* 84:1277–1287
- Barrett JE, Burke IC (2000) Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. *Soil Biol Biochem* 32:1707–1716
- Berthrong ST, Finzi AC (2006) Amino acid cycling in three cold-temperate forests of the northeastern USA. *Soil Biol Biochem* 38:861–869
- Coffman JA, Cooper TG (1997) Nitrogen GATA factors participate in transcriptional regulation of vacuolar protease genes in *Saccharomyces cerevisiae*. *J Bacteriol* 179:5609–5613
- Crenshaw CL, Lauber C, Sinsabaugh RL, Staveland LK (2008) Fungal control of nitrous oxide production in semiarid grassland. *Biogeochemistry* 87:17–27
- de Vries FT, Hoffland E, van Eekeren N, Brussaard L, Bloem J (2006) Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biol Biochem* 38:2092–2103
- Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* 103:626–631

- Fierer N, Craine JM, McLauchlan K, Schimel JP (2005a) Litter quality and the temperature sensitivity of decomposition. *Ecology* 86:320–326
- Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005b) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl Environ Microbiol* 71:4117–4120
- Fierer N, Colman BP, Schimel JP, Jackson RB (2006) Predicting the temperature dependence of microbial respiration in soil: a continental-scale analysis. *Global Biogeochem Cycles* 20:10
- Finzi AC, Berthrong ST (2005) The uptake of amino acids by microbes and trees in three cold-temperate forests. *Ecology* 86:3345–3353
- Fuller ME, Scow KM (1996) Effects of toluene on microbially-mediated processes involved in the soil nitrogen cycle. *Microb Ecol* 32:171–184
- Green LE, Porras-Alfaro A, Sinsabaugh RL (2008) Translocation of nitrogen and carbon integrates biotic crust and grass production in desert grassland. *J Ecol* 96:1076–1085
- Gulledge J, Schimel JP (1998) Moisture control over atmospheric CH₄ consumption and CO₂ production in diverse Alaskan soils. *Soil Biol Biochem* 30:1127–1132
- Hayano K (1996) Characterization and origin of protease activity in cultivated soils. *Jpn Agric Res Q* 30:79–84
- Hobbie JE, Hobbie EA, Drossman H, Conte M, Weber JC, Shamhart J, Weinrobe M (2009) Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: ¹⁵N is the key signal. *Can J Microbiol* 55:84–94
- Hofmockel KS, Schlesinger WH, Jackson RB (2007) Effects of elevated atmospheric carbon dioxide on amino acid and NH₄⁺-N cycling in a temperate pine ecosystem. *Global Change Biol* 13:1950–1959
- Jan MT, Roberts P, Tonheim SK, Jones DL (2009) Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. *Soil Biol Biochem* 41:2272–2282
- Jones DL, Kielland K (2002) Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. *Soil Biol Biochem* 34:209–219
- Jones DL, Owen AG, Farrar JF (2002) Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. *Soil Biol Biochem* 34:1893–1902
- Jones DL, Shannon D, Murphy DV, Farrar JF (2004) Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biol Biochem* 36:749–756
- Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? *Soil Biol Biochem* 37:413–423
- Jones DL, Kielland K, Sinclair FL, Dahlgren RA, Newsham KK, Farrar JF, Murphy DV (2009) Soil organic nitrogen mineralization across a global latitudinal gradient. *Global Biogeochem Cycles* 23:GB1016
- Kielland K (1994) Amino-acid-absorption by arctic plants—implications for plant nutrition and nitrogen cycling. *Ecology* 75:2373–2383
- Kielland K (1995) Landscape patterns of free amino acids in arctic tundra soils. *Biogeochemistry* 31:85–98
- Kielland K, McFarland JW, Ruess RW, Olson K (2007) Rapid cycling of organic nitrogen in taiga forest ecosystems. *Ecosystems* 10:360–368
- Ladd JN (1972) Properties of proteolytic enzymes extracted from soil. *Soil Biol Biochem* 4:227–237
- Lipson D, Näsholm T (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* 128:305–316
- Lipson DA, Schmidt SK, Monson RK (1999) Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology* 80:1623–1631
- Liu PV, Hsieh HC (1969) Inhibition of protease production of various bacteria by ammonium salts—its effect on toxin production and virulence. *J Bacteriol* 99:406
- McFarland JW, Ruess RW, Kielland K, Doyle AP (2002) Cycling dynamics of NH₄⁺ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. *Ecosystems* 5:775–788
- Näsholm T, Ekblad A, Nordin A, Giesler R, Hogberg M, Hogberg P (1998) Boreal forest plants take up organic nitrogen. *Nature* 392:914–916
- Nordin A, Hogberg P, Näsholm T (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* 129:125–132
- Sakurai M, Suzuki K, Onodera M, Shinano T, Osaki M (2007) Analysis of bacterial communities in soil by PCR-DGGE targeting protease genes. *Soil Biol Biochem* 39:2777–2784
- Santiago LS, Schuur EAG, Silvera K (2005) Nutrient cycling and plant-soil feedbacks along a precipitation gradient in lowland Panama. *J Trop Ecol* 21:461–470
- Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85:591–602
- Schimel JP, Jackson LE, Firestone MK (1989) Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. *Soil Biol Biochem* 21:1059–1066
- Schulten HR, Schnitzer M (1998) The chemistry of soil organic nitrogen: a review. *Biol Fertil Soils* 26:1–15
- Skujins J (1967) Enzymes in soil. In: McLaren AD, Peterson GH (eds) *Soil biochemistry*, vol 1. Marcel Dekker, New York, pp 371–414
- Stursova M, Sinsabaugh RL (2008) Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. *Soil Biol Biochem* 40:550–553
- Stursova M, Crenshaw CL, Sinsabaugh RL (2006) Microbial responses to long-term N deposition in a semiarid grassland. *Microb Ecol* 51:90–98
- Tabatabai MA (1995) Enzymes. In: Weaver RW, Augle S, Bottomly PJ, Bezdicsek D, Smith S, Tabatabai A, Wollum A (eds) *Methods of soil analysis, part 2: microbiological and biochemical properties*. Soil Science Society of America, Madison, pp 775–833
- Watanabe K, Hayano K (1995) Seasonal-variation of soil protease activities and their relation to proteolytic bacteria and *Bacillus* spp. in paddy field soil. *Soil Biol Biochem* 27:197–203
- Watanabe K, Sakai J, Hayano K (2003) Bacterial extracellular protease activities in field soils under different fertilizer managements. *Can J Microbiol* 49:305–312
- Weigelt A, Bol R, Bardgett RD (2005) Preferential uptake of soil nitrogen forms by grassland plant species. *Oecologia* 142:627–635
- Weintraub MN, Schimel JP (2005) Seasonal protein dynamics in Alaskan arctic tundra soils. *Soil Biol Biochem* 37:1469–1475
- Zak DR, Grigal DF, Ohmann LF (1993) Kinetics of microbial respiration and nitrogen mineralization in Great-Lakes forests. *Soil Sci Soc Am J* 57:1100–1106