

Soil nitrogen mineralization not affected by grass species traits

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Abstract

Species N use traits was evaluated as a mechanism whereby *Bromus inermis* (*Bromus*), an established invasive, might alter soil N supply in a Northern mixed-grass prairie. We compared soils under stands of *Bromus* with those from three representative native grasses of different litter C/N: *Andropogon gerardii* (*Andropogon*), *Nassella viridula* (*Nassella*) and *Pascopyrum smithii* (*Pascopyrum*); in ascending order of litter quality. Net mineralization (per g soil N) measured in year-long laboratory incubations showed no differences in comparisons of *Bromus* with two of the three native grasses: *Andropogon* and *Nassella*. Higher mineralization in *Pascopyrum* stands relative to *Bromus* was consistent with its higher litter quality. However, an unusually high occurrence of an N-fixing legume in *Pascopyrum* stands, potentially favoring high mineralization rates, confounded any conclusions regarding the effects of plant N use on N mineralization. Instead of an initial flush of net mineralization, as would be expected in laboratory incubation, we observed an initial lag phase. This lag in net N mineralization coincided with high microbial activity (respiration) that suggests strong N limitation of the microbial biomass. Further support for the importance of immobilization initially came from modeling mineralization dynamics, which was explained better when we accounted for microbial growth in our model. The absence of strong differences in net mineralization beneath these grasses suggests that differences in plant N use alone were unlikely to influence soil N mineralization through substrate quality, particularly under strong N control of the microbial biomass.

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1. Introduction

Plant species traits related to N use and allocation to structure directly determine litter quality (Heal et al., 1997; Murphy et al., 2002). Traits such as productivity and mean residence time of N in vegetation have an effect on litter quality in terms of the C/N ratio and the concentration of secondary compounds. The slow decomposition of poor quality litter (Swift et al., 1979) can therefore link species composition with ecosystem nutrient status through soil N supply.

Support for a species effect on soil nutrient supply has been demonstrated for forest ecosystems (Pastor et al., 1984; McClougherty et al., 1985; Pastor and Post, 1986;

Mladenoff, 1987; Zak et al., 1989; Lovett et al., 2004; but also see Verchot et al., 2001). Evidence from grasslands is less consistent. In a comparison of five grass monoculture stands, Wedin and Tilman (1990) found significant differences in field estimates of N mineralization that were supported by laboratory measurements of N mineralization potential (Wedin and Pastor, 1993). Differences in mineralization resulted from differences in the activity of a small but labile fraction of soil organic nitrogen. In semiarid short grass steppe, Vinton and Burke (1995) found ecosystem structural attributes, particularly patterns in plant cover, to be a more important determinant of soil N dynamics than species-driven effects. In other words, the distribution of vegetation was more important than which species were present. Knops et al. (2002) suggested generally strong microbial control over N cycling which results in a microbial bottleneck; thus potential species-driven effects on N mineralization resulting from differences in N use may

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be masked by immobilization and result in no net differences in mineralization.

Our focus in this study is plant N use traits, not plant N input, altering soil N supply. We are investigating how traits such as photosynthetic pathway and stature, with an effect on litter C/N, can influence soil N supply. This relationship is particularly interesting when considering that the N cycle is largely biologically driven and predominantly closed, such that internal N cycling accounts for the majority of N flux. Our goal was to evaluate the effect of an invasive perennial grass, *Bromus inermis* (*Bromus*), on soil N processes following its establishment in a Northern mixed-grass prairie. We wished to evaluate the underlying mechanisms by monitoring microbial activity and modeling N mineralization kinetics. Do differences in litter quality among graminoids of the Northern mixed-grass prairie result in differences in soil N supply? We expected the differences in litter quality between *Bromus* and the native grasses, resulting from differences in plant stature and N use strategies, would alter soil N mineralization potential and would be largely driven by alterations in the size and decomposition rate of a small but labile pool of soil organic nitrogen. A secondary objective was to examine indices of substrate quality, for soil and litter, which correlate with mineralization parameters.

2. Methods

2.1. Site description

The study was conducted on the Fort Pierre National Grassland located in central South Dakota (lat. 43°89' N, long 100°28' W). Mean annual precipitation is 576 mm and mean annual temperature is 8.6 °C, with a 124-day growing season (South Dakota Office of Climatology, unpublished data). Soils of the series Opal sansarc are fine textured vertisols, formed from residuum of shale parent material of montmorillonitic mineralogy (Schumacher, 1986). The high content of smectitic clays results in a relatively high capacity for ammonium fixation and physical protection of soil organic matter. The vegetation is characteristic of the Northern mixed-grass prairie, consisting of a mixture of warm- and cool-season grasses that vary in stature between short, mid-height, and tall grasses.

2.2. Experimental layout

The particular sites selected for this study were dominated by *Andropogon gerardii* (*Andropogon*), alone constituting 23–34% of total canopy cover estimates. Cool season grasses: *Nassella viridula* (*Nassella*), constituted 9–22%, and *Pascopyrum smithii* (*Pascopyrum*) constituted 0.2–5% of total canopy cover. *Bromus inermis* (*Bromus*) was established as a codominant in the ungrazed site, where it constituted 21% of total canopy cover versus only 8% in the grazed site. Also prominent was an exotic N-fixing legume (*Melilotus* sp.) (Table 1).

Table 1

Vegetation composition determined as percentage species cover estimates from five, 20 m transects per site

Site-scale vegetation composition as percent cover		
	Grazed	Ungrazed
<i>Andropogon gerardii</i> —ANGE	33.98 ± 27.1	22.80 ± 22.2
<i>Nassella viridula</i> —NAVI	8.48 ± 10.1	21.62 ± 17.7
<i>Pascopyrum smithii</i> —PASM	5.19 ± 6.9	0.17 ± 0.3
<i>Bromus inermis</i> —BRIN	8.20 ± 17.8	20.62 ± 35.4
Other grasses and sedges	4.65 ± 6.5	1.08 ± 1.5
Legumes	28.00 ± 19.4	21.68 ± 11.9
Forbs	8.04 ± 5.4	6.68 ± 4.8
Shrubs	3.60 ± 7.0	6.02 ± 7.8

Data are means ± SD.

We selected two sites that differed only in grazing history to incorporate land use as a potential source of variability. The ungrazed pasture had not been grazed by cattle for 9 years prior to the study, while the grazed pasture had been grazed at a moderate level every other year. To focus on species-driven differences we sampled relatively homogeneous stands (this was more successful for some species than others), about 5 m in diameter. Plots consisted of a stand of *Bromus* paired with an adjacent stand, within 0–4 m, dominated by one of the three native grasses [*Andropogon gerardii* (*Andropogon*, C₄, tallgrass), *Nassella viridula* (*Nassella*, C₃, mid-height grass) and *Pascopyrum smithii* (*Pascopyrum*, C₃, mid-height grass)]. Paired stands were replicated four times in each of two grazing sites for a total of 24 plots. Comparisons were restricted to paired stands within a plot to control within-site variability. This conservative approach limited the number of possible comparisons, but also the effects of potential confounding factors since comparisons were only made on samples within a few meters of each other.

2.3. Soil and litter sampling and analysis

Five, 2.5-cm-diameter by 20-cm-depth cores were collected at the end of the growing season in August 2002 and composited for each stand. Fresh samples were passed through a 2 mm sieve and allowed to air-dry, removing coarse particles and bulk root biomass. Remaining visible detritus and root material was picked out by hand. A subsample was homogenized by grinding in a ball mill and analyzed for total C and N using a Leco CHN-1000 analyzer (Leco, St. Joseph, Michigan, USA). Total C was corrected for carbonates using a pressure-calimeter method (Sherrod et al., 2002). Field capacity was determined as gravimetric water content of samples that were saturated then allowed to drain overnight. Particle size analysis was determined using the hydrometer method. Senescent aboveground biomass was collected at the end of the growing season in October 2003 from four, 20 × 50 cm quadrats per plot and pooled for analysis. Samples were

dried at 55 °C for at least 3 days, ground in a Wiley mill and analyzed for N and C in a Leco CHN-1000 analyzer.

2.4. Incubations

The mineralization procedure followed Stanford and Smith (1972) for long-term aerobic incubations as modified by Nadelhoffer (1990) to allow for repeated short-term microbial respiration measurements. Controlled moisture and temperature conditions in laboratory incubations isolate substrate quality from other controls over soil N mineralization. Twenty grams of air-dried soil from each stand and equal weights of washed sand were moistened and mixed thoroughly to facilitate leaching. Soil-plus-sand was placed in a filter unit (150 ml Falcon Filter Model 7102, Becton Dickinson Labware, Lincoln Park, NJ), brought to field capacity and the weight of soil plus filter recorded. A dilute salt solution of 0.004 M CaCl₂ plus nutrients allows for minimally disruptive repeated sampling of net N mineralized. For this study, the composition of the nutrient-extract solution was as follows: 4.0 mM CaCl₂, 2.0 mM KH₂PO₄, 1.0 mM K₂SO₄, 1.0 mM MgSO₄, 25 μM H₃BO₃, 2.0 μM MnSO₄, 2.0 μM ZnSO₄, 0.5 μM CuSO₄, and 0.5 μM Na₂MoO₄ (Nadelhoffer, 1990). Extraction efficiency using 0.004 M CaCl₂ is low relative to 2 M KCl (Motavalli et al., 1995), particularly for smectitic clays with high NH₄⁺-N adsorption. We assumed this effect was consistent across treatments since samples had similar mineralogy and clay content (Table 2).

Samples were leached on day 1, 6, 10, 15, 22, 36, 66, 96, 139, 200, 256, and 382. The initial leaching was excluded from analysis to reduce artifacts related to soil preparation. A 100-ml aliquot of nutrient solution was allowed to equilibrate with the soil for 0.5 h before leachates were extracted with a 59 kPa vacuum, applied for 4 h or until the flow of solution ceased. Samples were analyzed colorimetrically using an Alpkem autoanalyzer for NO₃⁻ and NH₄⁺. Solution concentrations were expressed as mg N g⁻¹ soil N to normalize differences in total soil N among samples. All values refer to net N mineralization. Cumulative mineralization for a given period was the sum of N mineralized for that period and the previous periods.

Respiration rates were determined 4–5 days after leaching by short-term measurements of CO₂ that accumulated in the headspace. Filter units were purged with CO₂-free air and a 5 ml sample taken before and after filters

were sealed for ~4 h. The initial concentration was subtracted from the final to account for any background CO₂ left after purging of headspaces. Concentrations of CO₂ in the gas samples were determined using a LI-COR 6252 gas analyzer (LI-COR, Lincoln, Nebraska, USA). Respiration rates were expressed as mg C g⁻¹ soil C d⁻¹.

2.5. Modeling mineralization dynamics

The first-order model presented by Stanford and Smith (1972) and later modified by Bonde and Rosswall (1987) describes the rate of substrate change over time. Potentially mineralizable N (N₀) is characterized by both a labile and a recalcitrant fraction:

$$N_t = N_l(1 - e^{-ht}) + ct, \quad (1)$$

where N_t is the cumulative amount of N mineralized at time *t*; N_l and *h* are the labile fraction and its rate constant, respectively; and *c* denotes the turnover rate of the recalcitrant fraction, a zero-order rate constant.

The mixed-order, two-component model, presented by Brunner and Focht (1984) has been used to describe mineralization dynamics characterized by an initial lag phase (Bonde and Lindberg, 1988). Unlike the Stanford and Smith (1972) model, the mixed-order model accounts for microbial growth and not only the rate of substrate change over time.

We compared Eq. (1) with two forms of the mixed model:

mixed/first-order model:

$$N_t = N_l[1 - \exp(-h_1t - h_2t/2)] + N_r[1 - \exp(-kt)], \quad (2)$$

and a mixed/linear model:

$$N_t = N_l[1 - \exp(-h_1t - h_2t/2)] + ct, \quad (3)$$

where *k* is a first-order rate constant for the recalcitrant pool, N_r, and rate constants *h*₁ and *h*₂ belong to the mixed-order model. When there is no need to account for microbial growth *h*₂ disappears and Eqs. (2) and (3) are simplified to a first-order equation, plus a first-order or a linear term, respectively. Thus no a priori decisions on reaction order are needed. A discussion on the theory behind the mixed model is reported in Brunner and Focht (1984).

The three models were fitted to the N mineralization results, for each plot, using the NLIN procedure in SAS for non-linear curve fitting (SAS Institute, 2000). Model adequacy was evaluated primarily on the basis of convergence and subsequently by comparing residual sum of squares minimized with a computed Akaike's information criterion, modified for small data sets (AIC_c) (Burnham and Anderson, 1998):

$$AIC_c = AIC + 2[P(P + 1)/n - P - 1],$$

Table 2

Soil particle size for distinct homogenous stands in grazed and ungrazed sites of mixed grass prairie

Site	Sand	Silt	Clay
Grazed	6.4 ± 1.7	21.1 ± 1.5	72.5 ± 1.0
Ungrazed	8.3 ± 1.0	19.0 ± 1.4	72.7 ± 0.9

Values are means and SD, *n* = 8.

where n is the sample size and P the number of parameters in the model.

2.6. In situ N availability

The sum of NO_3^- -N and NH_4^+ -N accumulation on ion exchange resin (IER) was used as an index of in situ N availability (Binkley, 1984). Resin bags were prepared with mixed-bed ion-exchange resin (IONAC NM60) placed in nylon stockings (Binkley, 1984). Three bags per plot were buried in the top 5–10 cm of soil and pooled at the end of each month. Bags were extracted with 50 ml of 2 M KCl on a shaker for 1 h. Units were expressed as g N/bag. Soil cores were also collected from the top 10 cm and were extracted with 2 M KCl to determine the composition of mineral N, NO_3^- -N and NH_4^+ -N.

2.7. Statistical analysis

For differences in means of the response variables: soil and litter quality, mineralization potential for a given time period, N_1 , ct , and IER-N the data were analyzed as a split-plot factorial design with site (grazed versus ungrazed) and species (*Andropogon*, *Pascopyrum*, or *Nassella*) as the whole plot factors and invasion status (within plot comparison of *Bromus* versus native) as the subplot factor. When the three-way interaction of species*invasion status*grazing was not significant ($P > 0.05$), then the two-way interaction of species*invasion status, which was used to evaluate the effect of *Bromus* establishment, could be studied by pooling the data from the two sites (i.e. averaging over grazing status). Pairwise differences were analyzed using t -tests to compare *Bromus* with each of the three native grasses. We used the MIXED procedure in

SAS (SAS Institute, 2000), where species, site, and invasion status were treated as fixed effects and plot was treated as a random variable. Model assumptions were examined using residual plots and data were log-transformed in cases where variance increased as a function of the mean. In addition to the three-way interaction (species*invasion status*site), a test of equal variance was used to determine whether the two sites were to be treated as a single population (averaging over grazing), or as separate populations.

Correlation coefficients were calculated to examine relationships between substrate quality indices and mineralization parameters. Correlations were also calculated for field measurements of IER-N and laboratory measurements of net N mineralization ($\text{mg g}^{-1}\text{N}$) to compare laboratory and field estimates of soil N supply.

3. Results

3.1. Total soil pools and litter C and N

Variance in soil pool size was significantly greater in the ungrazed site than the grazed site for both C and N ($P < 0.01$), consequently the two sites were analyzed as separate populations, while values for soil C/N could be averaged over sites. Invasion status had little effect on total soil C and N (Table 3), except for higher soil C/N in *Bromus* stands relative to *Pascopyrum*. Marked differences in litter quality between *Bromus* and native grasses were observed and appeared to be consistent with plant N use traits such as photosynthetic pathway and stature. For example, *Andropogon*, a native C_4 tall grass, exhibited lower tissue N concentration and higher C/N, while the

Table 3
Total C (%) and N (%) soil pools, IER-N (mg N bag^{-1}) and litter quality indices for three grasses in a mixed grass prairie

		<i>Andropogon</i>			<i>Nassella</i>			<i>Pascopyrum</i>		
		B	N	<i>P</i> -diff	B	N	<i>P</i> -diff	B	N	<i>P</i> -diff
<i>Soil</i>										
	C:N	8.63	8.91	0.08	8.72	8.77	0.753	8.21	7.88	0.036
<i>Grazed</i>										
	%N	0.31	0.3	0.125	0.29	0.29	0.288	0.3	0.3	0.378
	%C	2.65	2.6	0.553	2.54	2.51	0.693	2.56	2.34	0.012
	IER-N	0.855	0.745	0.529	1.022	1.324	0.427	1.633	1.996	0.409
<i>Ungrazed</i>										
	%N	0.31	0.31	0.897	0.34	0.3	0.054	0.26	0.23	0.054
	%C	2.75	2.84	0.615	2.97	2.65	0.093	2.06	1.78	0.136
	IER-N	0.846	0.698	0.140	1.302	1.632	0.198	1.187	4.749	<0.001
<i>Litter</i>										
	%N	0.41	0.30	<0.001	0.43	0.52	0.02	0.46	0.58	0.04
	C:N	110.17	154.19	<0.001	104.81	89.14	0.115	94.55	84.01	0.32

Values represent least significant means. Total soil C and N were analyzed separately for the grazed and ungrazed study sites, while values for total soil C:N and litter quality indices are averaged across sites. Results of t -tests for pairwise differences (B = *Bromus*, versus N = Native) are presented as P -values of the differences (P -diff). P -values for litter N and IER-N were calculated for log-transformed values.

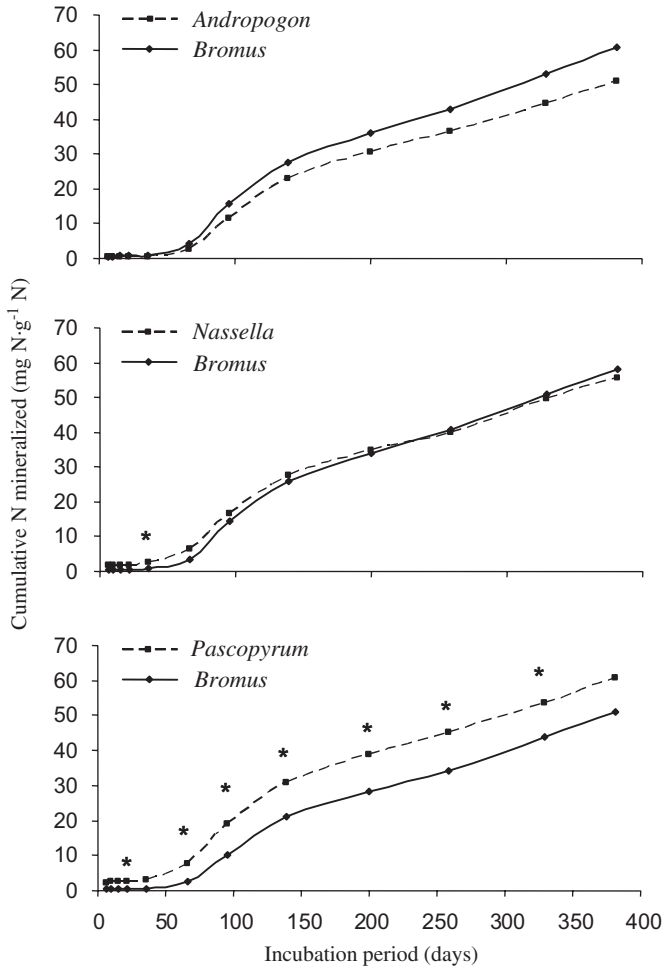


Fig. 1. Pairwise comparisons of net N mineralization during 382-day laboratory incubations of soils collected under homogenous stands of *Bromus inermis* paired with each of three native grasses. Values shown are relative cumulative N mineralized, $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$ (least significant means). Points labeled “*” indicate significant pairwise differences in log-transformed data for a given incubation period at $P \leq 0.05$, $n = 8$.

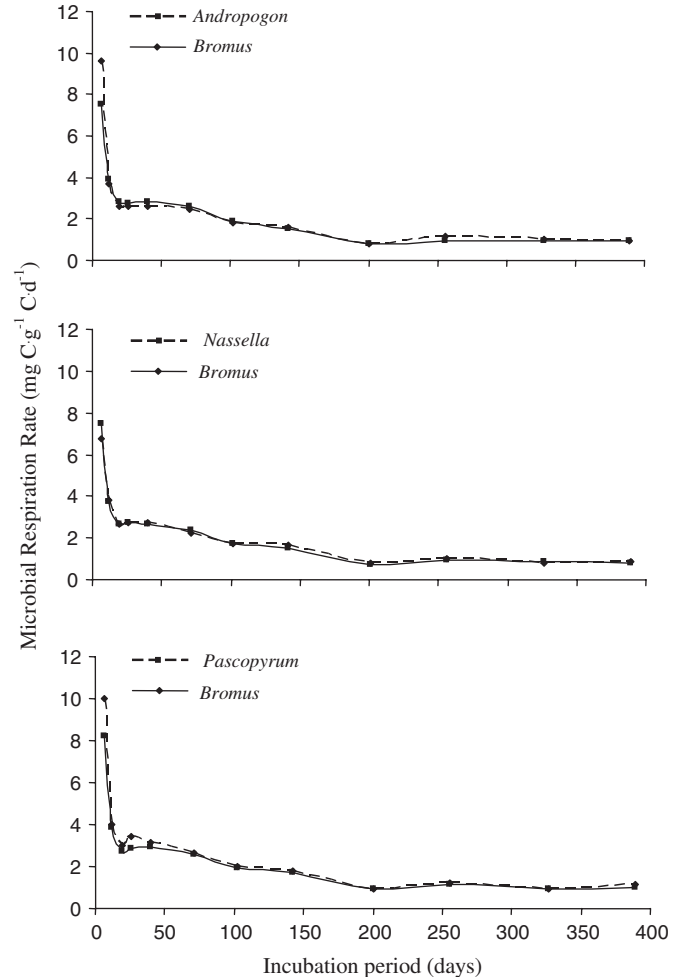


Fig. 2. Pairwise comparisons of microbial respiration rates during 382-day laboratory incubations. Values are daily respiration rates expressed as relative C respired per g soil C (least significant means). High respiration rates initially indicate high microbial activity, coinciding with the lag in net N mineralization during the initial 36 days of incubation.

two native C_3 grasses both had higher tissue N, but similar C/N (Table 3).

3.2. Soil N supply

An initial lag phase in net mineralized N (Fig. 1) and a corresponding flush in respiration (Fig. 2) characterized mineralization dynamics, and persisted until d36 in all soil samples. Site differences in cumulative net N mineralized were significant: the grazed site averaged 14% higher ($P = 0.022$) (Fig. 3). However, since the 3-way interaction (species*invasion status*site) was not significant, the data were pooled across sites.

Net N mineralized ranged from 50.8 to 60.5 $\text{mg g}^{-1} \text{N}$ (Fig. 1), with little or no differences in the case of *Andropogon* and *Nassella* when compared with *Bromus*. *Andropogon* showed little difference ($P > 0.05$) in N mineralization but was consistently lower compared to *Bromus*, while *Nassella* had similar net mineralization

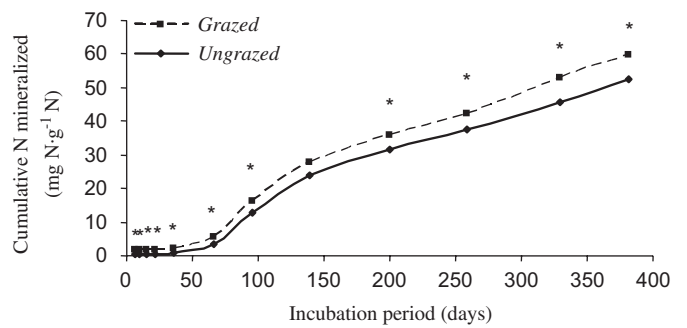


Fig. 3. Site comparisons of net N mineralization during 382-day laboratory incubations of soils collected under homogenous stands of *Bromus inermis*, paired with each of three native grasses. Values shown are relative cumulative N mineralized, $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$ (least significant means). Points labeled “*” indicate significant pairwise differences in log-transformed data for a given incubation period at $P \leq 0.05$, $n = 24$.

throughout most of the incubation except for a higher value on d36. Only in the case of *Paspopyrum* was cumulative N mineralized significantly higher than for

Bromus ($P \leq 0.05$) throughout most of the incubation period.

3.3. Mineralization kinetics

The three models used to describe mineralization kinetics performed differently. The first-order, two-component model (Eq. (1)) indicated a lack of fit with the data as the model simply did not converge on any of the samples. In contrast, the mixed-order, two-component models (Eqs. (2) and (3)) did fit the data, and produced comparable AIC_c values. Based on the sum of squared errors (SSE) reduced Eq. (2) (mixed/first-order model) performed better than Eq. (3) (mixed/linear model) (Table 4). However, only having a reduced SSE is not sufficient for model selection since there is an additional parameter in Eq. (2); this is better accounted for in the AIC_c. Finally, Eq. (2) failed to converge on 11 out of 48 samples throughout the entire time period, while Eq. (3) converged on all the samples (Table 4). Therefore, the mixed-order models (Eqs. (2) and (3)), which accounted for microbial growth, explained mineralization dynamics better than did the first order model which only described change in substrate over time.

Estimates of the labile fraction (N_1), determined using the mixed-order/linear model (Eq. (3)) ranged from 6.6 to 21.2 mg g⁻¹ N (Fig. 4). Pairwise comparisons of N_1 were consistent with the mineralization results, such that significant differences were only observed in the comparison of *Bromus* stands with *Pascopyrum* stands (Fig. 4). In contrast, there were similar pool sizes in the recalcitrant fraction (ct).

3.4. In situ N

Variability in IER-N was significantly greater in the ungrazed site than the grazed site, such that the data were analyzed separately by site. Cumulative IER-N was significantly higher in *Pascopyrum* stands compared with *Bromus* stands only in the ungrazed site (Table 3), but no differences were observed for paired stands of either *Andropogon* or *Nassella*. The majority of the N accumulated in IER-bags (79%) occurred as NO₃⁻-N while the reverse pattern was observed for the mineral N pool in direct 2 M KCl extractions (13.6% NO₃⁻-N; 86.4% NH₄⁺-N). This highlights the sensitivity of IER bags to mineral mobility and the role of water movement in such measurements. Consequently, NO₃⁻, being highly mobile, is overrepresented in samples relative to NH₄⁺, the bulk of which is adsorbed to soil cation exchange sites and not in soil solution.

3.5. Correlations

Soil C/N was significantly correlated with both mineralization parameters, N_1 and ct , but litter C/N was not. Soil C/N was negatively correlated with N_1 and positively

correlated with ct (Table 5). Field measurements of IER-N were positively correlated with N_1 ($P = 0.02$) but not with ct ($P = 0.16$).

4. Discussion

Despite differences in species traits related to litter quality, such as functional type (C₃ versus C₄), structure (tall versus mid-height), and litter N concentrations (Murphy et al., 2002), our data suggest that overall *Bromus* stands differed little in effect on net mineralization potential, at least in comparison with two of the three native grasses. Total soil C and N pools showed little to no response to species composition, which is consistent with other studies (Wedin and Pastor, 1993; Vinton and Burke, 1995; Mack and D'Antonio, 2003). For fine textured soils, as is the case here, this was not surprising considering their greater capacity to “fix” soil organic matter and create large pools of C and N with relatively slow turnover (Schimel et al., 1985b; Schimel, 1986).

The case of *Pascopyrum* stands out in more than one way. *Pascopyrum* plots had the highest prevalence of an N-fixing legume (*Melilotus* sp.). This species alone constituted about 50% of total aboveground production in *Pascopyrum* stands compared to <16% in *Andropogon*, *Nassella*, or *Bromus* stands (data not shown). Therefore, the observed differences in substrate quality leading to significant differences in mineralization in *Pascopyrum* plots were likely affected by N₂ fixation by the legume and not N use alone. By sparing soil N and transfer of fixed N to the soil, forage legumes contribute significantly to other plant N use (Carlsson and Huss-Danell, 2003). Rates of biological N fixation for *Melilotus* spp. range between 4 and 123 kg N ha⁻¹ yr⁻¹ (Sparrow et al., 1995). When grown in combination with grasses, legume species may accumulate a net 24–29% of aboveground harvested fixed N₂ (Kristensen et al., 1995).

An initial lag phase in net mineralization during incubation of all soil samples suggests strong N control of the soil microbial biomass. Modeling mineralization kinetics based only on the rate of substrate change over time (Eq. (1)) did not sufficiently explain the observed dynamics, unlike the case in other incubations, both for cultivated and undisturbed soil (Stanford and Smith, 1972; Campbell et al., 1984; Bonde and Rosswall, 1987; Wedin and Pastor, 1993). The two models that accounted for microbial growth as part of the mineralization dynamics (Eqs. (2) and (3)) performed better, consistent with findings by Bonde and Lindberg (1988). Together, these observations suggest N control of the microbial biomass.

Mineralization results were consistent with model estimates of the labile fraction (N_1). Similar values of N_1 were observed for *Andropogon* and *Bromus* stands; higher for *Nassella* compared to *Bromus* stands but not significantly different; and significantly higher for *Pascopyrum* relative to *Bromus* stands (Fig. 4). The labile fraction, averaged across all species, constituted about 1.1% of soil

Table 4

Model performance based on convergence, sum of squared errors reduced (SSE) and AIC_c based on fitting the model to 48 incubations (3 plots: *Andropogon*, *Pascopyrum*, *Nassella*; 2 stands: invaded, native; 4 replicates: rep; and 2 sites: grazed, ungrazed)

Plot	Stand	Rep	Convergence		SEE		AIC_c	
			Linear	1st order	Linear	1st order	Linear	1st order
<u>Grazed site</u>								
<i>Andropogon</i>	Invaded	1	Yes	Yes	15.55	12.98	17	21
<i>Andropogon</i>	Invaded	2	Yes	Yes	2.36	1.12	-6	-8
<i>Andropogon</i>	Invaded	3	Yes	Yes	23.79	11.87	22	20
<i>Andropogon</i>	Invaded	4	Yes	Yes	35.19	32.53	27	32
<i>Andropogon</i>	Native	1	Yes	Yes	7.27	1.60	8	-4
<i>Andropogon</i>	Native	2	Yes	Yes	12.42	8.66	14	16
<i>Andropogon</i>	Native	3	Yes	Yes	17.36	2.71	18	2
<i>Andropogon</i>	Native	4	Yes	—	14.44	55.73	16	38
<i>Pascopyrum</i>	Invaded	1	Yes	—	7.54	57.35	8	39
<i>Pascopyrum</i>	Invaded	2	Yes	Yes	8.96	3.70	10	6
<i>Pascopyrum</i>	Invaded	3	Yes	Yes	39.10	35.74	28	33
<i>Pascopyrum</i>	Invaded	4	Yes	—	42.74	143.50	29	50
<i>Pascopyrum</i>	Native	1	Yes	—	41.08	40.57	28	35
<i>Pascopyrum</i>	Native	2	Yes	Yes	32.72	15.21	26	23
<i>Pascopyrum</i>	Native	3	Yes	Yes	49.29	5.67	31	11
<i>Pascopyrum</i>	Native	4	Yes	—	90.42	113.80	38	47
<i>Nassella</i>	Invaded	1	Yes	Yes	4.15	0.50	1	-18
<i>Nassella</i>	Invaded	2	Yes	Yes	39.08	25.06	28	29
<i>Nassella</i>	Invaded	3	Yes	Yes	7.09	7.08	7	14
<i>Nassella</i>	Invaded	4	Yes	Yes	13.45	8.81	15	16
<i>Nassella</i>	Native	1	Yes	Yes	18.85	7.76	19	15
<i>Nassella</i>	Native	2	Yes	Yes	37.38	11.97	27	20
<i>Nassella</i>	Native	3	Yes	Yes	19.71	13.58	20	21
<i>Nassella</i>	Native	4	Yes	Yes	33.60	25.05	26	29
<u>Ungrazed site</u>								
<i>Andropogon</i>	Invaded	1	Yes	—	10.77	45.83	9.39	103.30
<i>Andropogon</i>	Invaded	2	Yes	Yes	7.51	0.68	8	-14
<i>Andropogon</i>	Invaded	3	Yes	Yes	5.95	4.08	5	7
<i>Andropogon</i>	Invaded	4	Yes	Yes	11.36	7.02	13	14
<i>Andropogon</i>	Native	1	Yes	Yes	15.46	6.91	17	13
<i>Andropogon</i>	Native	2	Yes	Yes	8.60	4.85	10	9
<i>Andropogon</i>	Native	3	Yes	Yes	12.29	4.29	14	8
<i>Andropogon</i>	Native	4	Yes	—	4.58	62.52	2	40
<i>Pascopyrum</i>	Invaded	1	Yes	—	11.65	73.18	13	42
<i>Pascopyrum</i>	Invaded	2	Yes	Yes	15.22	6.85	17	13
<i>Pascopyrum</i>	Invaded	3	Yes	Yes	10.44	2.83	12	3
<i>Pascopyrum</i>	Invaded	4	Yes	—	11.99	87.26	14	44
<i>Pascopyrum</i>	Native	1	Yes	Yes	39.33	10.83	28	19
<i>Pascopyrum</i>	Native	2	Yes	Yes	20.56	12.49	20	20
<i>Pascopyrum</i>	Native	3	Yes	—	11.05	124.30	13	48
<i>Pascopyrum</i>	Native	4	Yes	Yes	8.10	0.96	9	-10
<i>Nassella</i>	Invaded	1	Yes	Yes	7.71	4.30	8	8
<i>Nassella</i>	Invaded	2	Yes	—	16.77	63.29	18	40
<i>Nassella</i>	Invaded	3	Yes	Yes	13.84	5.95	15	12
<i>Nassella</i>	Invaded	4	Yes	Yes	1.58	0.62	-11	-16
<i>Nassella</i>	Native	1	Yes	Yes	45.81	45.90	30	36
<i>Nassella</i>	Native	2	Yes	Yes	13.33	2.99	15	3
<i>Nassella</i>	Native	3	Yes	Yes	7.31	3.97	8	7
<i>Nassella</i>	Native	4	Yes	Yes	27.00	30.96	23	31

The two models fitted to the data were Eq. (2), the mixed/first-order model (first order), and Eq.(3), the mixed/linear model (linear).

organic nitrogen, but about 2.1% for *Pascopyrum* alone. The size of N_1 estimated for *Pascopyrum* is comparable to values reported by Wedin and Pastor (1993), where N_1 constituted about 3% of soil organic nitrogen and had a significant effect on soil N mineralization.

Considering that, by definition, the activity of the labile fraction of N_0 is limited to the short term, it is likely that the initial immobilization-dominated phase of the incubations masked some differences in gross mineralization. Although gross mineralization was not measured in this

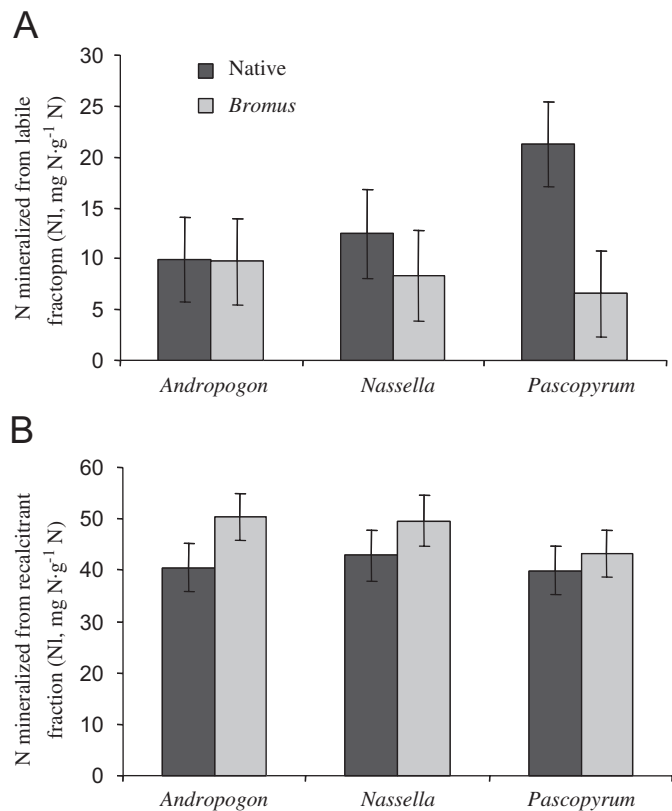


Fig. 4. Pairwise comparisons of estimates of the labile (A) and the recalcitrant fractions (B) of N_0 from fitting Eq. (3) to cumulative net N mineralization data. Paired bars represent pairwise comparisons of *Bromus* versus natives and lines above bars are standard errors for pairwise differences. $P \leq 0.05$, $n = 8$.

Table 5

Correlations between mineralization parameter estimates (N_i , or ct , determined using the mixed order/linear model (Eq. 3)), and in situ IER-N, soil C and N, and litter quality (C:N, %N) ($n = 8$)

	N_i		ct	
	R^2	P	R^2	P
<i>Soil</i>				
C:N	-0.234	0.001	0.109	0.023
%N	-0.015	0.409	0.04	0.176
%C	-0.074	0.064	0.009	0.525
<i>IER-N</i>	+0.117	0.020	-0.044	0.164
<i>Litter</i>				
C:N	-0.044	0.168	0.036	0.215
%N	0.049	0.143	-0.044	0.167

study, there is some support for this in the observed patterns. For example, in the case of *Paspopyrum* stands, where significantly higher net mineralization was observed, differences only became significant by d22. Alternatively, Verchot et al. (2001) noted that net mineralization values were more indicative of subtle differences resulting from

species composition than measurements of gross mineralization.

Soil IER-N was consistent with the mineralization results in that significant pair-wise differences were detected only in *Paspopyrum* plots. Of all the potential correlates for mineralization, N_i was the only parameter that was positively correlated with IER-N (Table 5), suggesting that mineralization dynamics were largely driven by an active fraction of soil organic nitrogen (Wedin and Pastor, 1993). Measurements of IER-N can be affected by other factors unrelated to substrate quality that include soil water movement, accordingly, NO_3^- which is more labile, yet six times less abundant than NH_4^+ , is better represented (79% of IER-N). Also plant N uptake of soil available N is not accounted for when estimating soil N supply using IER bags. These considerations limit the use of IER-N to an index of soil N supply.

Weak correlations between litter quality and mineralization parameters were observed. This lack of stronger relationships might be reflective of the notion that controls over decomposition change with time (Heal et al., 1997). For example, Berg and Staaf (1980) showed a shift from nutrient control, over pine litter decomposition during the early stages of decomposition, to lignin control during the later stages. Similarly, for soil N mineralization we are considering the later stages of decomposition, where soil organic matter composition is most reflective of substrate quality. Consequently, mineralization parameters correlated well with soil parameters, such that C/N ratio was negatively correlated with the labile N fraction.

Other potential reasons why we found few species effects on net mineralization might be related to the highly buffered nature of fine textured soils. There is reason to suggest that a different conclusion might have been reached for coarse textured soils where N_0 is a larger portion of the total pool (Schimel et al., 1985a; Schimel, 1986). High CEC represents another mechanism whereby these fine-textured soils can mask differences in mineralization as mineralized $\text{NH}_4\text{-N}$ is adsorbed onto CEC sites. Finally, the observed initial phase of immobilization may be an artifact of the method employed to process the samples, resulting in significant amounts of protected organic matter being made available with soil disturbance preparation.

In addition to the restriction of comparisons within plots, and elimination of land history as sources of confounding factors, support for the assumption that the observed dynamics in N cycling was primarily species driven is suggested here. (1) In contrast to lithophilic nutrients, N cycling is largely biologically driven and less subject to geochemical control; (2) stands were selected to be of a minimum size to ensure the presence of established plants.

In conclusion *Bromus* did not alter soil N mineralization when compared with native grasses with differing N use traits that might affect litter quality, if we exclude the situation with *Paspopyrum*. Our results suggest that a strong feedback between species composition and soil N

dynamics is unlikely in this grassland, particularly under strong N-limitation of the microbial biomass. Consequently, a feedback loop between plant N use and mineralization is an unlikely mechanism whereby *Bromus* can alter soil N supply.

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