



Landscape versus ungulate control of gross mineralization and gross nitrification in semi-arid grasslands of Yellowstone National Park

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Abstract

Grazers have marked effects on decomposition and N cycling processes, generally resulting in increased net N mineralization. Within landscapes, topographic and edaphic gradients also affect these microbial processes. The objective of this study was to evaluate the effects of grazers on N cycling processes in a landscape context that encompassed a wide range of environmental conditions in order to increase our mechanistic understanding of these processes and provide a stronger basis for management and assessment of grassland ecosystems. This study was conducted on a series of 37–41-year-old grazing exclosures on the northern winter range of Yellowstone National Park. We measured gross and net mineralization, nitrification and immobilization in grazed and ungrazed plots in upland and bottomland landscape positions by ¹⁵N pool dilution in a laboratory incubation with intact cores. There were no significant differences in either gross mineralization, immobilization, gross nitrification or NO₃⁻ immobilization between grazed and ungrazed plots in a paired *t*-test using all plots ($P = 0.52$, $P = 0.32$, $P = 0.91$ and $P = 0.93$, respectively). These results were unexpected, because previous reports indicated that grazers increased soil N dynamics, including net N mineralization, in Yellowstone grassland, and suggest that herbivore regulation of N processes may accumulate over a longer time scale than was measured in this study (24 h). Instead, we found that landscape position was the dominant factor controlling both mineralization and immobilization rates, with higher rates located at the bottoms of the slopes (32.9 and 33.4 μg N g soil⁻¹ d⁻¹, respectively), compared to the upland sites (4.4 and 3.2 μg N g soil⁻¹ d⁻¹, respectively). Gross mineralization and immobilization rates were highly correlated with soil C and N content, while gross nitrification was not. Turn over times for NH₄⁺ and NO₃⁻ pools averaged 1.2 days for the NH₄⁺ pool and 2.25 days for the NO₃⁻ pool. There was no apparent effect of grazers on turnover times, nor were there any significant correlations between turnover times and soil C and N pools, slope position, or soil water content. Previous modeling to understand how herbivores affect N cycling suggested that stimulation of net mineralization was most likely due to grazers suppressing immobilization by reducing belowground primary productivity and organic matter inputs to the soil. Our results do not suggest that grazers suppress immobilization. If anything, they suggest that grazers enhance this process, especially in the more mesic components of the landscape. Landscape position effects on N cycle processes were much stronger than grazer effects in this study, and appear to be driven by soil C and N contents. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Grasslands represent approximately one-third of the earth's terrestrial surface, are subject to varying degrees of management by humans, and are highly susceptible to climate change (Barnard and Frankel, 1964; Williams et al., 1968; Gitay et al., 2001). As recently as 200 years ago, most of these ecosystems supported large populations of

migrating ungulates. However, global expansion of cropland and cattle ranching has greatly decreased the area of grassland that follows a 'natural' grazing cycle. Given that there are complex interactions between grazers and grasslands that strongly influence the sustainability of these ecosystems (McNaughton et al., 1988; Tracy and McNaughton, 1997), there is much to be learned by studying these interactions in ecosystems with native grazers. Yellowstone National Park, the subject of this study, is one of the last remaining temperate vestiges of grassland ecosystems where the effects of migrating ungulates on

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biogeochemical cycles can be studied in conjunction with the effects of climate and topography. There is particular interest in understanding the factors that regulate nitrogen (N) cycling and retention in grasslands, given that N is an important constraint on productivity and given concerns about N transfers to aquatic ecosystems and the atmosphere (Vitousek and Howarth, 1991).

An important theme in grassland biogeochemistry research has been to analyze the landscape context of grazer effects on ecosystem N cycling processes (Schimel et al., 1986; Burke et al., 1988; Turner et al., 1993; Frank and Groffman, 1998). Grazers can affect the amount and allocation of plant production, the chemical quality of plant material and the soil microenvironment, all of which influence microbial decomposition and N cycling processes, generally resulting in decreased microbial immobilization and increased net mineralization (Weaver and Zink, 1946; Jameson, 1963; Caldwell et al., 1981; Ingham and Detling, 1984; Richards, 1984; McNaughton et al., 1988; Seagle et al., 1992; Miller and Rose, 1992; Holland et al., 1992; Milchunas and Lowenroth, 1993). Within landscapes, topographic and edaphic factors are associated with gradients of soil texture, moisture, redox potentials and nutrient content, that also affect these microbial processes (Frank et al., 1994; Turner et al., 1993; Frank and Groffman, 1998; Barrett and Burke, 2000). Evaluating N cycling processes in a landscape context that encompasses a wide range of environmental conditions increases our mechanistic understanding of these processes and provides a stronger basis for management and assessment of grassland ecosystems.

In a previous study, Frank and Groffman (1998) examined net rates of mineralization and soil respiration in grazed and ungrazed grasslands in upland and bottomland landscape positions in Yellowstone National Park. In the study presented here, we measured rates of gross and net mineralization, nitrification and immobilization at the same sites. Our hypothesis was that grazed grasslands and bottomland landscape positions have high rates of both gross and net rates of N cycling relative to upland and ungrazed areas. We reasoned that grazer-induced increases in labile C should increase both net and gross rates of N cycling and that accumulation of C in bottomland areas should have the same effect. We also tested the hypothesis that immobilization rates increase as mineralization and nitrification rates increase. Understanding these internal changes should be important to management and assessment of N cycling, availability, and loss in grasslands.

2. Methods

2.1. Site description

This study was conducted on the northern winter range of Yellowstone National Park, USA (44°55′–45°10′N and

110°10′–110°50′W), which consists of 100,000 ha of grassland and shrub-grassland grazed by herds of elk (*Cervus elaphus*) and bison (*Bison bison*) during the winter (approximately November–April). Recent northern elk and bison population estimates for this area are 21,000 and 300–700, respectively (Singer and Mack, 1993). Smaller numbers of pronghorn (*Antilocarpa americana*), bighorn sheep (*Ovis canadensis*), and mule deer (*Odocoileus hemionus*) also graze the winter range.

Climate of Yellowstone's northern winter range is cool and dry, with mean annual temperatures ranging from ~7 °C at lower elevations to 2 °C at the higher elevations. Mean annual precipitation increases from ~28 to 32 cm along the same gradient (Houston, 1982). Soils for much of the winter range were derived from glacial till deposited during the Pleistocene. Those at one of our sites (Stephens Creek) were exceptional, having been formed from a bentonite clay-rich substrate deposited during a Pleistocene landslide (Keefer, 1987). More detailed soil descriptions can be found in Frank and Groffman (1998).

A series of 2 ha exclosures (100 × 200 m²) that have fenced out all ungulates since 1958 or 1962 provided an opportunity to examine the effects of ungulates on soil N dynamics (Frank and Groffman, 1998). We located seven sites, at four exclosures, which were Stephens Creek (S1 and S2), Mammoth (M1 and M2), Blacktail (B), and Lamar (L1 and L2). The Stephens Creek (1620 m elevation) and Lamar (2000 m elevation) sites were 45 km apart, and all other sites were located between these sites. Thus these sites represented a climate gradient from relatively warm and dry to wet and cool. At each site, we sampled 8 × 8 or 10 × 10 m² paired plots inside and outside fences with the same slope and aspect. All sites were part of the original park, except Stephen's Creek, which was added in 1932.

Study sites were selected to include the widest range of topographic positions and as much of the dry to mesic habitat gradient as possible. S1 was an alluvial outwash area dominated by *Stipa comata* and *S. viridula*. S2, M2, and L2 were slopes and B was a hilltop; S2, L2, and B were grasslands dominated by *Festuca idahoensis* and *Pseudoroegneria spicata*, and M2 was a shrub-grassland site with *Artemisia tridentata*, *F. idahoensis*, and *P. spicata* dominating. M1 and L1 were slope-bottom sites dominated by *Poa pratensis*. Grazing pressure at these sites was moderate to low and there were no significant changes in vegetation with the suppression of grazing (Houston, 1982; D. Frank, personal communication).

2.2. Soil sampling

The previous study suggested that maximum net rates of mineralization occurred early in the growing season. Thus, in May 1999, each plot was divided into quarters and we collected five soil cores in close proximity to each other, in each quarter. Cores were collected using 3.8 cm diameter × 10 cm long, schedule 40 PVC pipes, which

were driven into the soil with a rubber mallet. The lower end of each pipe was sharpened to permit insertion with a minimum of compaction. Cores were wrapped in aluminum foil and transferred to our laboratory in New York for labeling and incubation.

2.3. Laboratory procedures

We measured gross rates of mineralization, nitrification and microbial consumption of NO_3^- and NH_4^+ on intact cores using the pool dilution method adapted for laboratory incubations (Davidson et al., 1991; Hart et al., 1994b). In the laboratory, each group of five cores was separated: two were used for determination of gross mineralization and immobilization, two were used for determination of gross nitrification and NO_3^- immobilization, and the fifth core was used for determination of initial NH_4^+ , NO_3^- , total C and total N. For inorganic-N a 15 g subsample of the soil was extracted using 60 ml 2 M KCl and analyzed on an AlpKem autoanalyzer (Wilsonville, OR) using a modified Griess-Illosvay procedure for determination of $\text{NO}_3^- + \text{NO}_2^-$, which was reported as NO_3^- (Bundy and Meisinger, 1994), and a salicylate–hypochlorite procedure for NH_4^+ (Kemper and Zweers, 1986). Total C and N were measured with a Carlo Erba Model 1500 CN analyzer (Carlo Erba Instruments, Milano, Italy).

For determination of gross mineralization and nitrification rates, we adapted the ^{15}N pool dilution technique (Davidson et al., 1991; Hart et al., 1994b). Incubations were begun on all cores within 3 days of collection. In the laboratory, each core received 6 ml of a solution containing either 30 mg l^{-1} ^{15}N as $(^{15}\text{NH}_4)_2\text{SO}_4$ (99 at.% ^{15}N), for measurement of gross mineralization, or 30 mg l^{-1} ^{15}N as K^{15}NO_3 (99 at.% ^{15}N), for measurement of gross nitrification. The solutions was injected through spinal needles with lateral holes. The needles were slowly drawn through the core during the injection to ensure even distribution of the label throughout the core. The addition of the solution increased gravimetric soil water content on the average by <6% and never more than 15%. For each pair of cores, one core was incubated in the laboratory for 24 h and the second was homogenized and extracted immediately after injection for determination of initial N pools and initial at.% ^{15}N enrichment. Following incubation, the second core was homogenized and inorganic-N was extracted using 2 M KCl as above. Inorganic-N concentrations in the extracts were measured on the AlpKem autoanalyzer as described above. Samples were prepared for ^{15}N analysis using the PTFE trap diffusion procedure of Stark and Hart (1996). Blank correction was done using the calculated blank method of Stark and Hart (1996). This method uses diffused and non-diffused isotope standards to determine the amount of dilution that occurs during diffusion. An isotope dilution equation is used to compute the amount of N in the blank as

follows:

$$M_b = \frac{M_{\text{std}}(E_m - E_{\text{std}})}{(E_b - E_m)}$$

where M_b is the mass of N in the blank, M_{std} is the mass of N in the standard, E_{std} is the ^{15}N enrichment measured in the non-diffused standards, E_m is the enrichment measured in the diffused standards, and E_b is the enrichment of the blank (assumed to be 0.366%). The mass of N in the blank is then used to compute the corrected ^{15}N enrichment for the sample as follows:

$$E_s = E_m + \frac{M_b(E_m - E_b)}{M_s}$$

where E_s is the corrected ^{15}N enrichment of the sample, and M_s is the mass of N in the sample prior to diffusion. Gross rates of mineralization, nitrification and microbial consumption were calculated following Hart et al. (1994b):

$$m = \frac{[\text{NH}_4^+]_0 - [\text{NH}_4^+]_t}{t} - \frac{\log(\text{APE}_0/\text{APE}_t)}{\log([\text{NH}_4^+]_0/[\text{NH}_4^+]_t)}$$

where m is gross mineralization rate ($\text{mg N kg soil}^{-1} \text{d}^{-1}$), t is the length of the incubation in days, $[\text{NH}_4^+]_0$ and $[\text{NH}_4^+]_t$ are the respective concentrations (mg N kg soil^{-1}) of NH_4^+ at time 0 and t , and APE_0 and APE_t are the respective at.% excesses of the NH_4^+ pool ($\text{APE} = E_s - 0.366$) at times 0 and t . Microbial consumption is then computed as follows:

$$c_A = m - \frac{[\text{NH}_4^+]_t - [\text{NH}_4^+]_0}{t}$$

where c_A is the rate of microbial NH_4^+ consumption ($\text{mg N kg soil}^{-1} \text{d}^{-1}$). Gross nitrification and microbial nitrate consumption are calculated with the above equations by substituting the concentration of NO_3^- for the concentration of NH_4^+ and the APE of the NO_3^- pool for the APE of the NH_4^+ pool.

2.4. Statistical analysis

Differences between sites for inorganic-N stocks and for each of the measures of mineralization, immobilization or nitrification were tested using an analysis of variance (ANOVA) test; means separation was done using the Student–Newman–Keuls multiple range test. Grazing effects were examined with paired t -tests. Regression analysis was used to test for relationships between gross and net rates of mineralization and immobilization and to test the hypothesis that microbial immobilization increases with rates of mineralization and nitrification. All statistical tests were done with SAS statistical software (SAS Institute, 1992).

Table 1

Soil properties for study sites (mean, one standard error in parentheses) at study sites in Yellowstone National Park

Site	Grazed (G) or ungrazed (U)	C (%)	N (%)	C/N	NO ₃ -N (μg N g soil ⁻¹)	NH ₄ -N (μg N g soil ⁻¹)	Gravimetric moisture (%)	pH
S1	U	1.94 (0.31)	0.17 (0.03)	11.1	2.26 (1.16)	3.31 (0.68)	25.2 (2.9)	7.0
	G	1.48 (0.15)	0.14 (0.02)	10.9	1.71 (0.19)	1.91 (0.20)	19.7 (1.2)	7.1
S2	U	0.77 (0.00)	0.07 (0.00)	11.2	0.54 (0.11)	1.66 (0.08)	20.4 (1.9)	7.3
	G	1.01 (0.09)	0.08 (0.00)	12.3	0.67 (0.10)	1.44 (0.26)	17.1 (2.0)	7.2
M1	U	16.62 (1.44)	0.87 (0.03)	19.1	1.28 (0.45)	20.29 (5.25)	90.6 (1.42)	7.9
	G	16.42 (1.44)	1.01 (0.13)	16.2	0.71 (0.19)	37.44 (8.85)	97.4 (9.6)	7.8
M2	U	3.30 (0.11)	0.29 (0.01)	11.4	2.06 (0.41)	8.78 (1.76)	26.8 (1.0)	6.5
	G	3.58 (0.19)	0.32 (0.01)	11.3	2.00 (0.24)	10.99 (2.30)	32.4 (1.3)	6.3
B	U	2.30 (0.56)	0.15 (0.01)	15.2	0.46 (0.18)	3.92 (0.60)	17.8 (1.0)	6.7
	G	2.38 (0.15)	0.22 (0.01)	10.7	2.25 (0.75)	6.04 (0.40)	19.3 (1.9)	6.7
L1	U	7.21 (1.32)	0.69 (0.14)	10.4	0.86 (0.21)	13.75 (4.59)	64.0 (10.7)	6.6
	G	8.22 (0.71)	0.77 (0.07)	10.7	0.80 (0.11)	15.79 (2.49)	59.3 (7.8)	6.8
L2	U	3.11 (0.47)	0.16 (0.01)	19.4	2.78 (1.07)	4.97 (0.37)	18.3 (2.3)	7.6
	G	3.04 (0.39)	0.15 (0.02)	20.3	1.67 (0.73)	5.68 (1.16)	19.2 (1.0)	7.7

3. Results

Soil properties varied greatly among the sites, but there were few differences between soils inside and outside the exclosures (Table 1). Slope position strongly influenced soil properties, where sites at the bottom of the slope (M1 and L1) had higher gravimetric moisture content, total N, NH₄⁺ and total C content than the other

mid- and upper-slope sites ($P < 0.05$). Climatic influences were also apparent; the drier and warmer site (Stephens Creek) had lowest concentrations of C, N, NH₄⁺, and NO₃⁻. The variation in soil properties among sites was large, ranging by an order of magnitude for C, N and NH₄⁺, and by a factor of 4 for NO₃⁻ and gravimetric moisture content. Thus, the sites studied cover a wide spectrum of soil conditions.

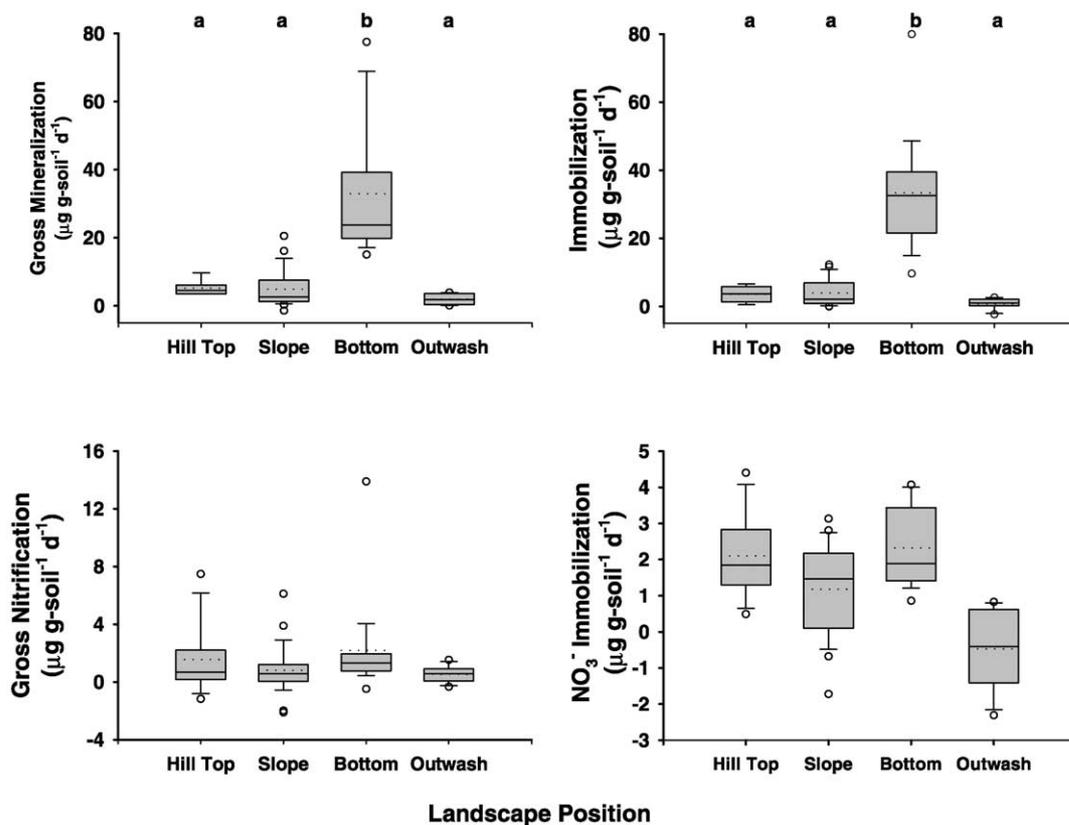


Fig. 1. Box and whisker plot with box showing 25th, 50th (median), and 75th percentiles and error bars showing 10th and 90th percentiles. Mean is shown as a dotted line and outliers are shown as open circles. Letters above the boxes indicate significant differences ($P < 0.05$) for gross mineralization and immobilization.

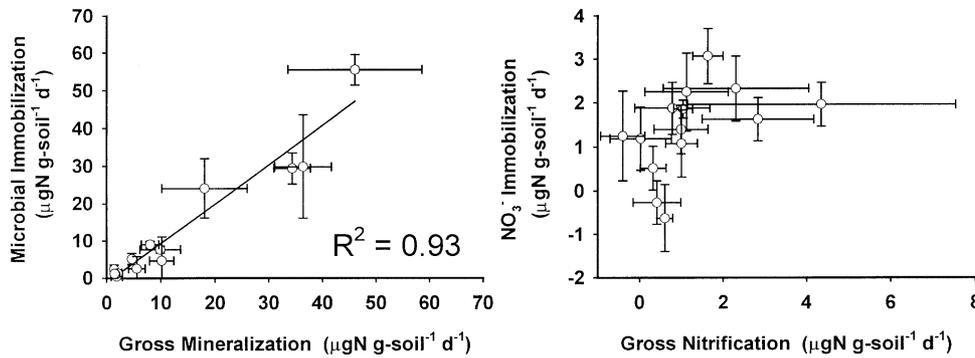


Fig. 2. Relationships between gross flux rates of mineralization and nitrification and the corresponding immobilization processes. The relationship for gross nitrification was insignificant. Error bars show ± 1 SE.

Slope position influenced the magnitude of both mineralization and immobilization rates (Fig. 1), with the highest rates at the bottoms of the slopes (M1 and L1). Microbial immobilization rates ranged between 20 and 170% of mineralization rates and increased as gross mineralization rates increased (Fig. 2). There were no significant differences in either gross mineralization or immobilization between grazed and ungrazed plots in a paired *t*-test using all plots ($P = 0.52$ and $P = 0.32$, respectively). Gross rates of mineralization and immobilization varied among sites by an order of magnitude (Table 2). A plot of gross mineralization and immobilization in grazed versus fenced areas (Fig. 3) suggests an interaction between topography and grazing, with stimulation of both processes in the mesic, bottomland sites (M1, L1). An analysis of variance where sites were classified as either slope bottom sites or upland sites suggested that the interaction was not highly significant ($P = 0.1044$) for gross mineralization, but highly significant for immobilization ($P = 0.0086$).

Gross rates of nitrification and gross NO_3^- immobilization varied among sites in this study by two orders of magnitude (Table 2). There was no significant difference between grazed and ungrazed plots for either parameter in a *t*-test using all plots ($P = 0.91$ and $P = 0.93$, respectively).

Slope position was not an important factor for either gross nitrification or NO_3^- immobilization (Fig. 1). Microbial NO_3^- immobilization ranged between 45 and 4000% of gross nitrification, and while the two were positively correlated (Fig. 2), the correlation was not significant. The data suggest no interaction between topography and grazing for these processes (Fig. 3).

Correlation analysis showed that gross mineralization and immobilization were significantly correlated with total C, total N, gravimetric water content, NH_4^+ (Table 3). Interestingly, neither was correlated with C/N ratio, despite high ratios at some sites that would have suggested high immobilization. Only NO_3^- immobilization was positively correlated with total C, total N, gravimetric water content, and NH_4^+ (Table 3). Gross nitrification was not significantly correlated with any soil parameter.

Turnover times for soil inorganic N pools were calculated assuming steady state of the inorganic N pools, which is reasonable for such a short-term incubation. All observations with negative gross rates of either mineralization or nitrification were eliminated, and then the NH_4^+ or NO_3^- pool was divided by the rate of gross mineralization or gross nitrification, respectively. Turnover times for both pools were fairly rapid (Table 4), averaging a little more

Table 2

Mineralization and nitrification rates ($\mu\text{g N g soil}^{-1} \text{d}^{-1}$) for study sites in Yellowstone National Park (mean, one standard error in parentheses)

Site	G or U	Gross mineralization	Gross immobilization	Net mineralization	Gross nitrification	Gross NO_3^- immobilization	Net nitrification
S1	U	1.88 (0.97)	0.41 (1.04)	1.47 (0.49)	0.61 (0.19)	-0.63 (0.77)	1.24 (0.77)
	G	2.05 (1.57)	1.36 (0.70)	0.69 (0.87)	0.42 (0.57)	-0.27 (0.50)	0.69 (0.73)
S2	U	1.66 (0.58)	1.01 (0.45)	0.65 (0.14)	0.33 (0.31)	0.52 (0.50)	-0.19 (0.21)
	G	1.50 (0.51)	1.15 (0.47)	0.35 (0.27)	1.01 (0.38)	1.08 (0.76)	-0.06 (0.75)
M1	U	36.42 (13.71)	29.86 (5.28)	6.57 (13.75)	1.64 (0.36)	3.07 (0.63)	-1.43 (0.35)
	G	46.06 (11.43)	55.59 (12.52)	-9.53 (4.06)	1.13 (1.00)	2.25 (0.89)	-1.12 (0.12)
M2	U	10.17 (5.56)	4.65 (2.27)	5.52 (6.43)	-0.40 (0.53)	1.25 (1.02)	-1.65 (1.41)
	G	8.04 (2.34)	8.98 (1.59)	-0.94 (0.90)	1.00 (0.65)	1.40 (0.55)	-0.41 (1.16)
B	U	4.68 (0.14)	5.15 (1.47)	-0.46 (1.61)	2.31 (1.74)	2.33 (0.74)	-0.03 (1.09)
	G	5.57 (2.07)	2.52 (1.53)	3.05 (3.28)	0.79 (0.90)	1.88 (0.59)	-1.09 (0.80)
L1	U	18.10 (1.24)	24.12 (7.92)	-6.02 (7.93)	4.35 (3.19)	1.97 (0.50)	2.38 (3.38)
	G	34.38 (6.05)	29.41 (3.42)	7.23 (4.18)	1.05 (0.23)	1.86 (0.20)	-0.81 (0.37)
L2	U	9.89 (3.93)	7.64 (3.75)	2.25 (0.94)	0.03 (0.74)	1.19 (0.72)	-1.16 (0.19)
	G	1.40 (0.97)	2.33 (0.58)	-0.93 (1.25)	2.84 (1.33)	1.63 (0.49)	1.21 (1.07)

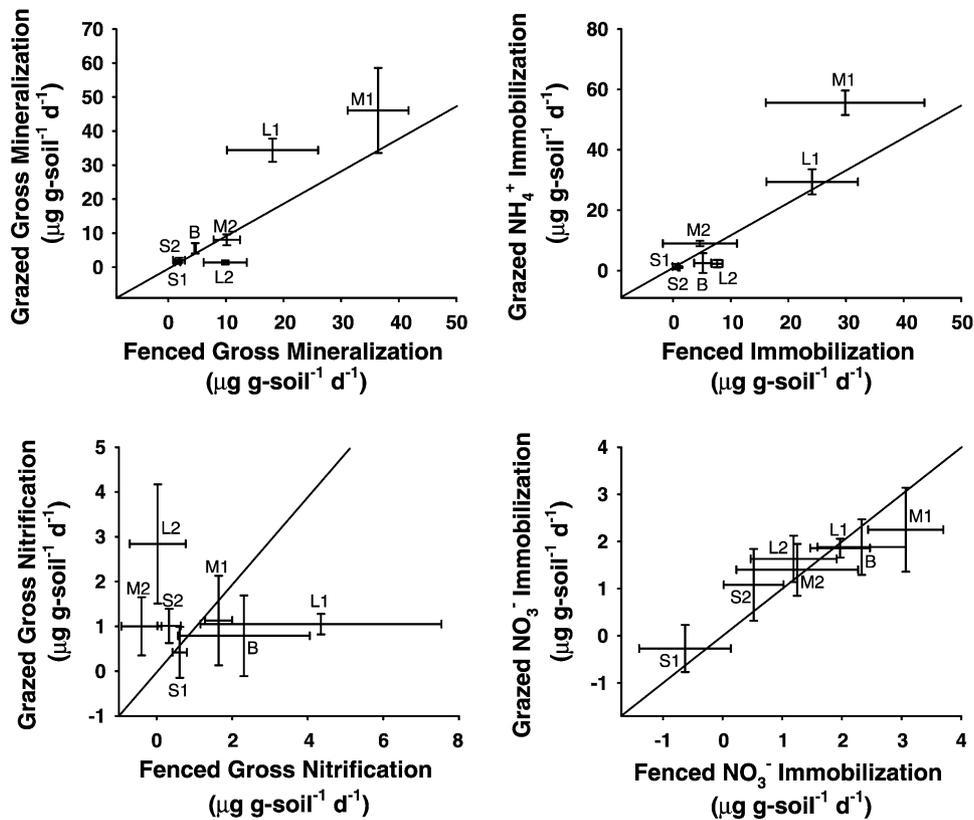


Fig. 3. Relationships between gross fluxes of mineralization, immobilization, nitrification and NO_3^- immobilization between pairs of fenced and unfenced plots. Plotting symbols are the site names; error bars show ± 1 SE; lines show the 1:1 relationship.

than 1 day for the NH_4^+ pool and around 2.25 days for the NO_3^- pool. There was no apparent effect of grazers on turnover times, nor were there any significant correlations with soil C and N pools, slope position, or soil water content.

Recovery of ^{15}N in the incubations showed wide variation and was not correlated with turnover times (Table 4). Recovery was particularly low at the slope bottom sites and higher at the mid-slope sites.

4. Discussion

Grazing maintains vegetation in a young, N-rich condition, removes high C/N material, and creates low C/N waste products, e.g. urine, dung (McNaughton et al., 1988; Holland et al., 1992; Frank and Evans, 1997). While

these factors all increase rates of decomposition, N mineralization, nitrification, denitrification, ammonia volatilization and N leaching, other factors appear to retain N in grazed ecosystems. In Yellowstone, previous work on these paired plots has shown that grazing appears to increase both N availability and retention (Frank et al., 2000). Annual in situ net N mineralization in grazed grassland was greater than that in the exclosures, indicating that N availability to plants was higher in the presence of large herbivores. However, there were no differences in soil C stocks between grazed and ungrazed plots after 35 years of grazing exclusion. Moreover, grazing did not significantly lower C/N ratios and in a few cases actually raised the ratio. Grazing did appear to create differences in soil C quality. In a 28-week laboratory incubation, microbial respiration in grazed soils was enhanced early and depressed late in the incubation. Thus, grazing increased the labile C

Table 3

Pearson correlation coefficients with *P* values in parentheses for significant correlations between mineralization and nitrification processes and soil parameters

Process	C (%)	N (%)	C/N	NO_3^-	NH_4^+	Gravimetric moisture (% H_2O)
Gross mineralization	0.95 (0.0001)	0.97 (0.0001)	0.23	0.32	0.73 (0.0029)	0.94 (0.0001)
Immobilization	0.93 (0.0001)	0.96 (0.0001)	0.21	-0.38	0.64 (0.0145)	0.95 (0.0001)
Gross nitrification	0.23	0.30	0.17	-0.42	0.15	0.30
NO_3^- immobilization	0.66 (0.0102)	0.62 (0.0175)	0.44	-0.34	0.62 (0.0108)	0.58 (0.0288)

Table 4

Inorganic N pool turnover times calculated from the mean of the pool size divided by the mean of the mineralization or nitrification rate, respectively, and recovery of ^{15}N after 24 h of incubation

Site	G or U	Turnover times (d)		^{15}N recovery (%)	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-
S1	U	1.8	3.7	57.7 (15.7)	58.0 (9.5)
	G	1.1	2.2	45.5 (5.9)	61.0 (14.2)
S2	U	1.1	0.8	61.9 (6.6)	60.3 (16.3)
	G	1.0	0.5	46.0 (4.1)	77.8 (13.9)
M1	U	2.6	0.8	20.6 (6.7)	2.9 (0.3)
	G	0.7	0.5	24.0 (1.1)	2.4 (0.7)
M2	U	0.9	15.1	51.8 (3.6)	47.7 (0.0)
	G	1.4	1.5	33.4 (2.3)	39.1 (8.1)
B	U	0.7	0.2	28.5 (4.5)	36.6 (13.6)
	G	1.0	1.1	59.1 (10.7)	51.2 (5.9)
L1	U	0.7	0.2	20.1 (4.4)	3.1 (0.6)
	G	0.4	0.6	19.9 (4.6)	4.7 (1.4)
L2	U	0.5	4.0	46.4 (6.8)	55.3 (11.4)
	G	2.3	0.6	46.5 (8.8)	57.2 (8.2)

fraction, and as Frank et al. (2002) later showed, this was probably done by increasing belowground productivity at these sites.

We did not find a systematic effect of grazers on net or gross mineralization or immobilization during this study. It is perhaps not surprising to find no grazer effects as the grazing pressure on the winter range is moderate to low, and the heaviest grazing occurs on senescent grasses during the winter. However, this result is not consistent with the previous work on these sites. It is important to note that the time scale over which these processes were measured in this study was much shorter than in the previous study. Given the rapid turnover times of the inorganic-N pools, the rates measured in the previous study were undoubtedly the results of several cycles of mineralization–microbial uptake–remineralization, which may account for the difference in the outcomes of the measurement. Our data do show, however, that there is a significant immobilization sink for N in the Yellowstone landscape. Moreover, this sink appears to be increased by grazers in the most mesic, bottomland components of the landscape (Fig. 3).

The importance of soil C as a regulator of grazing effects on N cycling, availability and loss is amplified in a landscape context. Levels of plant productivity and soil C tend to be higher in lower topographic positions that accumulate soil water and fine soil particles (Schimel, 1995; Burke et al., 1988; Frank et al., 1994). Frank and Groffman (1998) found higher ratios of respiration/N mineralization in C-rich bottomland components of the Yellowstone landscape, suggesting an enhanced potential for N immobilization in these areas. We found higher mineralization and immobilization rates at these landscape positions. These landscape analyses support the idea that there are strong

relationships between C and N cycling (Zak et al., 1993; Hart et al., 1994a) and that these relationships may interact with grazer effects to affect N cycling and loss.

Analysis of links between C and N cycling is facilitated by measurement of both gross and net rates of N cycling processes. Field and laboratory assays of net mineralization represent the net product of offsetting processes—mineralization and immobilization—which are often an order of magnitude greater than net rates and are directly controlled by the nature and extent of C availability (Schimel et al., 1986; Hart et al., 1994a; Verchot et al., 2001). Given that grazing affects both the amount and quality of soil C, changes in gross rates of mineralization and nitrification may be critical regulators of N cycling and loss in grazed ecosystems. This regulation likely varies strongly with soil C levels across the landscape.

Carbon and N cycling are tightly coupled in semi-arid temperate grasslands and landscape factors are generally considered to be the primary determinants of C and N cycling these ecosystems (Schimel et al., 1985; Burke et al., 1988; Frank et al., 1994). However, previous work in Yellowstone suggested that grazers might play a more important role than suspected. Frank and Groffman (1998) found grazer enhancement of net mineralization that approached the magnitude of the landscape effect on this process in ungrazed plots. Their study reinforced work by Holland and colleagues (Holland and Detling, 1990; Holland et al., 1992) who showed that large herbivores stimulated net mineralization in a mixed-grass prairie in South Dakota. Frank et al. (2000) also observed that grazer stimulation of mineralization was not associated with marked increases in N loss, suggesting that grazers also stimulate N conservation mechanisms. These observations were a primary motivation for the measurements of gross rates of mineralization and immobilization reported here.

Modeling work with CENTURY (Parton et al., 1987, 1988) to try to understand how herbivores impact N cycling suggested that stimulation of net mineralization was most likely due to grazers suppressing immobilization (Holland et al., 1992). This effect was attributed to grazers lowering the C/N ratio by indirectly reducing belowground primary productivity and thus organic matter inputs to the soil. However, as Frank and Groffman (1998) pointed out, no decrease in soil C has been observed at these Yellowstone sites despite 37 years of grazing exclusion and in many cases C/N ratios were higher on grazed plots. Our results also do not suggest that grazers suppress immobilization. If anything, they suggest that grazers enhance this process in the more mesic components of the landscape. We hypothesize that these results, which are contrary to the modeling work described above, are due to grazer stimulation of belowground productivity and root mortality, resulting in increased labile C availability (Frank et al., 2002).

Landscape position effects on N cycle processes were much stronger than grazer effects in this study. Rates of

mineralization and nitrification were strongly determined by landscape position and were highly correlated with soil C and N (Table 3), similar to results reported by Barrett and Burke (2000). As Frank and Groffman (1998) and Frank et al. (2000) suggested, soil C accumulation at mesic, slope-bottom sites was responsible for the markedly higher rates of immobilization. Our results confirm that higher rates of immobilization were associated with these sites and that there was a strong correlation between immobilization and both soil C and N. However, our results show that there was also much greater gross mineralization at these sites. Our results also suggest an interaction between grazing and slope position. This result is not robust for mineralization, but is statistically strong for immobilization.

There was no consistent grazer effect on nitrification, and while there did appear to be a pattern of enhancement at the slope sites, a paired *t*-test showed that this effect was not significant ($P = 0.12$). There was also no significant grazer effect for NO_3^- immobilization (paired *t*-test, $P = 0.09$) at the slope sites. Gross nitrification was not significantly correlated with any soil parameter, while immobilization was correlated with soil organic matter, the NH_4^+ pool and soil water content. At most sites, gross rates of NO_3^- immobilization were higher than gross rates of nitrification suggesting that microbial demand for NO_3^- is strong enough to make it difficult to discern environmental controls on nitrification in this landscape.

The comparison between gross and net rates of N cycle processes also illustrates strong landscape position control of N dynamics. In the drier, upland sites in this study, gross and net rates of mineralization and nitrification were of the same order of magnitude, while in the more mesic, bottomland sites, gross rates were an order of magnitude higher than net rates. In forest ecosystems, gross rates of mineralization and nitrification generally exceed the net rates by an order of magnitude (Verchot et al., 2001; Stark and Hart, 1997; Hart et al., 1994a,b; Davidson et al., 1992). Despite lower gross rates, and by virtue of generally smaller inorganic N pools in these grasslands than in forests, the inorganic-N turnover times observed were similar to turnover times of forests and did not vary with landscape position (Verchot et al., 2001). Thus, N is moving rapidly through the inorganic-N pools of these grasslands. However, the more mesic, bottomland components of the landscape have much greater fluxes of N through the microbial biomass.

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