Plant Soil (2007) 300:289–300
DOI 10.1007/s11104-007-9416-0

REGULAR ARTICLE

The pattern between nitrogen mineralization and grazing intensities in an Inner Mongolian typical steppe

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Received: 22 February 2007 / Accepted: 4 September 2007 / Published online: 19 September 2007

Abstract Ungulate grazing is known to play a crucial role in regulating energy flow and nutrient cycling in grassland ecosystems. However, previous studies of the effect of grazing on soil N dynamics have showed controversial results. Some studies indicate that grazing stimulates N mineralization while others report that grazing suppresses N mineralization. In order to reconcile these contrasting results, we investigated the response pattern of nitrogen transformation to multiple grazing intensities in an Inner Mongolian steppe. In our study, we measured net nitrogen mineralization rates and nitrification rates during a whole growing season in a 17-year field experiment that had five grazing intensities (0.00, 1.33, 2.67, 4.00 and 5.33 sheep ha^{-1}). Primarily because of changes in temperature and moisture conditions, net N mineralization rates varied substantially during the growing season with higher values occurring in late July. No consistent differences in net N mineralization rates were observed between grazing intensity treatments at the monthly time scale. Compared to mineralization rates, net nitrification rates were generally low with slightly higher values occurring in late July and late August. Ungulate grazing stimulated the cumulative net N transformations (mineralization, nitrification and ammonification) at the annual time scale, and the most stimulation occurred at a moderate grazing intensity of 4.00 sheep ha^{-1}, whereas the highest grazing intensity of 5.33 sheep ha^{-1} and the lighter grazing intensity of 1.33 sheep ha^{-1} stimulated less. The general response of net N mineralization to grazing intensity gradient is roughly in the form of a normal distribution at the annual time scale. Our study demonstrated that grazing intensity in concert with soil moisture and temperature conditions imposed significant controls on soil N transformation and availability in this Inner Mongolian steppe.

Keywords Ammonium · Nitrate · Nitrogen transformation · Grassland · Grazing intensity · Inner Mongolia

Responsible Editor: Lars S. Jensen.

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Introduction

In terrestrial ecosystems, N availability is often a limiting factor that controls primary production (Vitousek and Howarth 1991), C storage (Shaver et al. 1998), and N trace gas emissions (Davidson et al. 1993). Nitrogen mineralization rates and the total quantity of soil N are two important indicators of N availability, because they directly control soil $NH_4^+ - N$ and $NO_3^- - N$ concentrations and the availability of inorganic N to plants (Owen et al. 2003). By controlling NO_3^- concentration and availability to denitrification, nitrification is an important microbial process that regulates N losses, either through NO_3^- leaching or gaseous emissions from denitrification (Vitousek and Matson 1985). Because of the critical role of N mineralization and nitrification, understanding how different management practices such as ungulate grazing influence these processes is important for proper use and management of grassland ecosystems.

Herbivores play a major role in regulating the nitrogen cycle and primary production in grassland ecosystems (De Mazancourt et al. 1998). Grazers may simultaneously increase N availability and N conservation by stimulating N mineralization and reducing N losses (Frank et al. 2000). Nitrogen mineralization is often stimulated by grazing primarily because of the dropping of readily decomposable feces that are rich in nitrogen (Tracy and Frank 1998). Ungulate trampling also facilitates the incorporation of plant litter into the soil organic matter (Zacheis et al. 2002) and increases labile soil C and N pools that often result in accelerated soil N transformation processes (McNaughton et al. 1997). Furthermore, grazing reduces aboveground plant biomass (van Wijnen et al. 1999), alters plant species composition (Olofsson et al. 2001), and induces changes in the N concentration of plant tissues (Epstein et al. 2001). These changes also indirectly influence N mineralization by affecting plant litter decomposition or soil microbial activities (Bardgett et al. 1997; Tracy and Frank 1998).

However, published results about the effect of grazing on nitrogen mineralization were controversial. Some studies indicated that ungulate grazing enhanced N mineralization (Groffman et al. 1993; Le Roux et al. 2003), while other studies reported that grazing suppressed N mineralization and reduced N availability (Biondini et al. 1998; Bardgett and Wardle 2003). Most of these studies only compared the ungrazed treatment with the grazed treatment of a varying level of grazing intensity. These controversial results could potentially be explained by differential responses to varying levels of grazing intensity, if there was a threshold of grazing intensity at which N mineralization rate was the highest, other grazing intensities either lower or higher than the threshold would result in a lower N mineralization rate. In this study, we tested this possible pattern in an Inner Mongolian steppe using five defined grazing intensities.

The effect of grazing on soil N is often dependent on the type of ecosystem. For example, a study of the central US grasslands indicated that long-term grazing decreased soil N accumulation beneath *Scizachyrium scoparium* plants in tall- and mid-grass prairies, but increased soil N accumulation beneath *Bouteloua gracilis* plants in shortgrass prairies (Derner et al. 1997). Another study in Serengeti, Tanzania, reported that grazing stimulated net N mineralization in shortgrass regions, but suppressed net N mineralization in tallgrass regions (Seagle et al. 1992). Because of the lack of adequate studies, little is known about how grazing may regulate N dynamics in the shortgrass steppes in Inner Mongolia of China. The central Asian steppe is a vast grassland ecosystem that has been home to nomadic pastoralists and their herds for thousands of years. Unlike most other grassland ecosystems in the world, most plant species found in these steppes are adapted to domesticated ungulate grazing. As hypothesized by Milchunas et al. (1988), ecosystems that have been adapted to grazing by animals for thousands of years may have developed unique functional relationships between grazing and soil resources such as nitrogen. One of our objectives of this study is to investigate this relationship in Inner Mongolian steppes.

Nitrogen mineralization is highly dependent upon environmental conditions (Wang et al. 2006). Some studies have shown that net N mineralization rates are positively correlated with seasonal temperature fluctuation, but less sensitive to soil water content (Hatch et al. 1991; Sierra 1997). Whereas results from other studies indicate that soil moisture is more important in regulating rates of net N transformations than soil temperature (Owen et al. 2003; Isaac and Timmer 2007). The effect of temperature and soil moisture on N mineralization processes must also be investigated if one aims to understand the relationship between grazing intensity and soil N transformation.

Sheep grazing is the main type of land-use in Inner Mongolia of China, where nomadic pastoralists used to roam for thousands of years. However, recent population increases coupled with poor management have left the grasslands of Inner Mongolia facing severe degradation and desertification (Chen and Li 1999). Understanding the effects of grazing on N cycling in these grassland ecosystems is critical for better management and reversing the trend of degradation. In this study, we took advantage of an existing long-term (17 years) grazing manipulations, and examined how grazing intensities affect soil inorganic N pools and N transformation rates in Inner Mongolian steppes during an entire growing season (140 days). Our main objectives were: (1) to test the afore-mentioned pattern about the relationship between grazing intensity and net N mineralization, (2) to investigate the seasonal dynamics of net N mineralization and nitrification, and (3) to explore how soil moisture and temperature control N dynamics under different grazing intensities.

Materials and methods

Site description

The experiment was conducted within the grazing enclosures of the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences, located in the central part of Inner Mongolia Autonomous Region. The latitude of the experimental site is 43°50′N, the longitude is 116°34′E, and the elevation is 1,100 m above sea level. The mean annual precipitation at the site is 350 mm, with most rain events occurring in July and August. It is a semi-arid climate with cold, dry winters and mild, wet summers. Average annual air temperature is −0.4°C. Mean monthly temperature ranges from 17.9°C in July to −23°C in January as recorded by a nearby meteorological station. When the experimental plots were established in 1989, *Artemisia frigida* was the dominant plant species. Vegetation is characterized as typical steppe, which is dominated by *Artemisia frigida*, *Kochia prostrate* and *Potentilla acaulis*. Soils are coarse textured with a mean of 71% sand, 15% silt, and 9% clay across all experimental plots (Barger et al. 2004). The aboveground plant biomass and soil properties (soil texture, structure and chemical characteristics) were relatively uniform among all experimental plots before the initiation of the grazing treatments. Five grazing intensity treatments were maintained for 17 years from 1989 to 2005, the year of this study. There were 0, 4, 8, 12 and 16 Inner Mongolia fine wool sheep grazing rotationally in three replicated 1-ha plots, representing the grazing intensities of 0, 1.33, 2.67, 4.00 and 5.33 sheep ha^{-1}, respectively. Every year, grazing started on May 20th and ended on October 5th. Each plot was rotationally grazed three times per year, each time for 15 days with a rotation interval of 30 days. The total grazing period of each grazed plot was thus 45 days per year. During grazing periods, the sheep were driven to the enclosures at 5:00 A.M. and back home at 8:00 P.M. The sheep used for this experiment drunk water twice every day in Xilin River nearby, once before being driven to the enclosures and once after being driven out of the enclosures. No fertilizer was used in any treatment.

Soil sampling and incubation

Within each replicated plot, five 2×2 m quadrats were randomly demarcated at the first sampling date and used for later repeated soil sampling. Net N mineralization rates were measured using the in situ soil core incubation method (Raison et al. 1987; Hook and Burke 1995). After aboveground vegetation was clipped at the ground level and removed together with litter, two PVC cylinders, 12 cm in length and 5 cm in diameter, were driven into the soil within each quadrat to a depth of 10 cm. One soil core was removed for determining initial concentrations of KCl-extractable NH_4^+ and NO_3^-. The other soil core was incubated in situ after sealing the top of the core with a piece of plastic film that prevented water permeation and allowed gas exchange. The incubation period was about 15 days during the warm and wet season in July and August, and was about 30 days in other months in 2005. All soil cores (initial and incubated) were hand-sorted to remove stones and coarse roots, and sieved through a 2 mm screen. Five initial soil cores, one from each of the five quadrats within each plot, were combined to form a single sample. Incubated soil cores were processed in the same way as the initial cores. All the initial and incubated soil samples were sent to the laboratory, temporarily stored in a refrigerator at 4°C, and extracted within 24 h.

Soil extraction and analysis

A 10 g subsample from each composite sample was extracted with 50 ml of 2 M KCl on a rotary shaker for 1 h. The soil suspension was filtered using Whatman no. 1 filter paper. The filtrate was analyzed for $NH_4^+ - N$ and $NO_3^- - N$ using a flow injection auto-analyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark).

Net N mineralization during the incubation period was calculated from the difference of inorganic N concentrations between the initial and incubated samples. Cumulative net N mineralization was calculated by summing the net amount of nitrogen mineralized (or immobilized) during the entire growing season. Net rates of ammonification, nitrification and mineralization were calculated using the following equations:

For a time interval $\Delta t = t_{i+1} - t_i$,

$$A_{\mathrm{amm}} = c\left(\mathrm{NH}_4^+ - N\right)_{i+1} - c\left(\mathrm{NH}_4^+ - N\right)_i \tag{1}$$

$$A_{\mathrm{nit}} = c\left(\mathrm{NO}_3^- - N\right)_{i+1} - c\left(\mathrm{NO}_3^- - N\right)_i \tag{2}$$

$$A_{\min} = A_{\mathrm{amm}} + A_{\mathrm{nit}} \tag{3}$$

$$R_{\min} = A_{\min}/\Delta t \tag{4}$$

$$R_{\mathrm{nit}} = A_{\mathrm{nit}}/\Delta t \tag{5}$$

$$R_{\mathrm{amm}} = A_{\mathrm{amm}}/\Delta t \tag{6}$$

Where t_{i} and t_{i+1} are the initial and post incubation times, respectively; $c\left(NH_4^+ - N\right)_i$ and $c\left(\mathrm{NH}_4^+ - N\right)_{i+1}$ are the mean concentrations of ammonium N in the initial and incubated samples, respectively; $c\left(NO_3^- - N\right)_i$ and $c\left(\mathrm{NO}_3^- - N\right)_{i+1}$ are the mean concentrations of nitrate N in the initial and incubated samples, respectively; A_{amm}, A_{nit} and $A_{\min}$ are the accumulation of $\mathrm{NH}_4^+ - \mathrm{N}$, $\mathrm{NO}_3^- - \mathrm{N}$ and total inorganic $\mathrm{N}\left(\mathrm{NH}_4^+ - \mathrm{N} + \mathrm{NO}_3^- - \mathrm{N}\right)$, respectively; $R_{\min}$, R_{nit} and R_{amm} are the net N mineralization rate, net nitrification rate and net ammonification rate, respectively.

Measurement of environmental factors

Water content of the composite sample from each plot was determined gravimetrically by oven-drying at 105°C for 24 h. The air-dried soil samples were used for measuring pH, organic C content and organic N content. The pH values were determined in water (water/soil=2.5:1) suspension. Soil organic C contents were analyzed using a H_2SO_4–$K_2Cr_2O_7$ oxidation method (Nelson and Sommers 1982). Soil organic N contents were measured using the Kjeldahl digestion method followed by NH_4^+ determination on an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden).

Daily rainfall, air temperature and soil temperature (10 cm depth) were recorded at a permanent meteorological station belonging to the Inner Mongolian Grassland Ecosystem Research Station, the Chinese Academy of Sciences. The meteorological station was located at 1.5 km east of our study site. Soil temperature at 10 cm depth in each replicate plot was measured during a 28-day period in a pre-experiment. No significant difference in daily mean soil temperature was found either between treatment plots or between the values at the experiment site and the values at the meteorological station. Accordingly temperature at the experiment site was not measured during the experiment. Instead, the soil temperature from the meteorological station was used.

Soil bulk density was measured using a coring method. Peak aboveground plant biomass (in August) was determined by clipping all plants in five randomly located 1×1 m quadrats in each plot, and oven-drying the plant materials at 70°C for 48 h.

Statistical analysis

We tested for the differences of inorganic N concentrations and net N transformation rates between various grazing intensities using analysis of variance, and we also tested for the correlations of net N transformation rates with soil temperature and moisture under different grazing intensities.

Repeated measures ANOVA in a generalized linear model was used to examine the differences of inorganic N concentrations and net N transformation rates under different grazing intensities for the growing season, using grazing intensity and sampling date (incubation period) as main effects. One-way ANOVA was used for the comparison of the differences from grazing intensity for each incubation period. LSD values were used to test the significance of the differences, and linear contrasts at a significant level of 0.05 were used to compare group means. Analysis of co-variance, or ANCOVA, was used to distinguish the direct effects of grazing from its indirect effect on environmental factors. Pearson's rank correlations were used to determine correlations between net N transformation rates and soil temperature and moisture values. All statistical analyses were performed using SPSS version 11.5 software package.

Results

Environmental conditions, plant biomass, and soil properties

Monthly rainfall and air temperature during 2005 were shown in Fig. 1 using the format of Biondini et al. (1998). Soil temperature ranged from 1.7 to 27.7°C during the study period. This was a much drier year, with annual precipitation less than 50% of the 50-year average. Gravimetric soil moisture under various grazing intensities showed similar seasonal patterns, i. e., the values were relatively higher in July and October and lower in other months. Average soil moisture in the ungrazed treatment was 38% higher than that under the grazing intensity of 5.33 sheep ha^{-1} and 28% higher than that under the other three grazing intensities, but there were no significant differences in soil moisture between the four grazed treatments (Fig. 2). One-way ANOVA showed that soil bulk density, pH, organic C, N and C:N ratios of the top 10 cm soil layer were not significantly different between different grazing intensities (P>0.05, Table 1). The aboveground biomass of the dominant species, *Kochia prostrate*, was significantly affected by grazing intensity ($F_{4,\ 10}$=4.466, P=0.025), and was highest under the moderate grazing intensity (4.00 sheep ha^{-1}, Table 1).

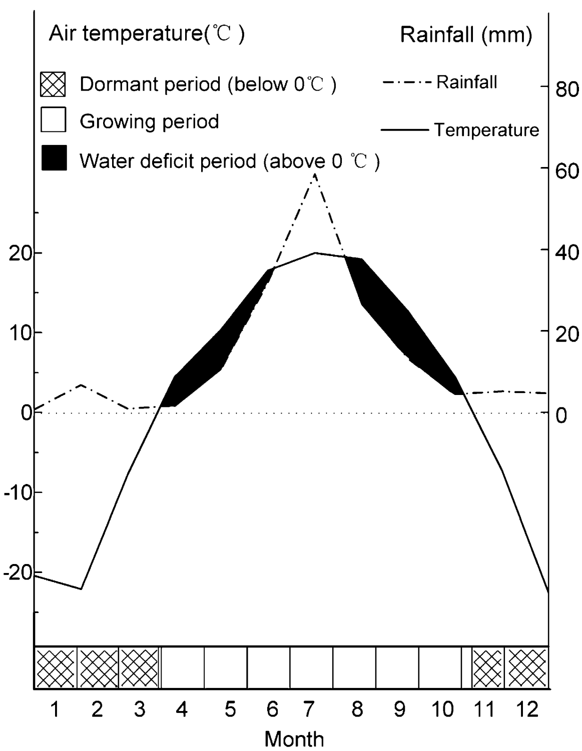

Fig. 1 A diagram of climatic data collected at the meteorological station close to the study site (ca. 1.5 km) during 2005. This *diagram* was drawn using the format of Biondini et al. (1998)

Seasonal dynamics of inorganic N concentration and N mineralization

Soil $NH_4^+ - N$ concentrations ranged from 1.71 to 9.45 μg N g^{-1} dry soil and peaked in early August for all grazing intensities (Fig. 3a). There were no significant differences in the $NH_4^+ - N$ concentrations between different grazing intensities. However, the ungrazed treatment had a significantly higher $NH_4^+ - N$ concentration than all grazed treatments at the August 4 sampling date (Fig. 3a). Soil $NO_3^- - N$ concentrations ranged from 0.27 to 11.21 μg N g^{-1} dry soil and showed a gradually increasing trend throughout the summer with a peak in early September (Fig. 3b). The $NO_3^- - N$ concentration in the ungrazed treatment was 131, 125, 137 and 176% higher than in the grazed treatments of 1.33, 2.67, 4.00 and 5.33 sheep ha^{-1}, respectively (P<0.05). But there were no significant differences between the four grazed treatments, even though moderate grazing intensities (2.67 and 4.00 sheep ha^{-1}) tended to have higher $NO_3^- - N$ concentrations than the heaviest grazing intensity (5.33 sheep ha^{-1}, Fig. 3b).

The inorganic N concentrations ranged from 2.69 to 14.57 μg N g^{-1} dry soil (mainly from 2.69 to 4.14 μg N g^{-1} dry soil) accounting for 0.49–2.6% of the total N in the ungrazed treatment, and ranged from 2.49 to 8.66 μg N g^{-1} dry soil (mainly from 2.49 to 4.10 μg N g^{-1} dry soil) accounting for 0.35–1.21% of

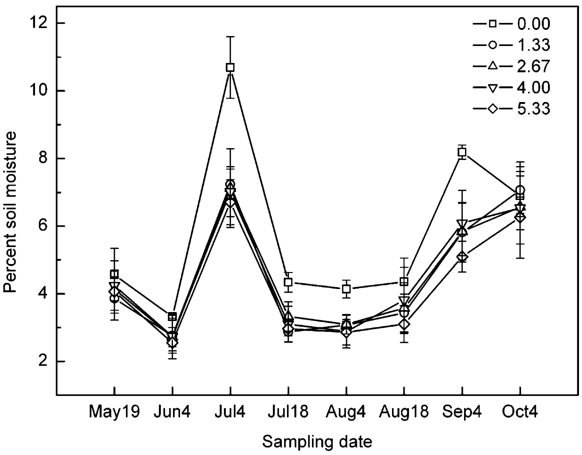

Fig. 2 Percentage gravimetric soil moisture (g H_2O 100 g^{-1} dry soil) in the top 10 cm soil layer under different grazing intensities. *Each point* is the mean from three replicated plots. *Error bars* represent ±1 SE. 0.00, 1.33, 2.67, 4.00 and 5.33 sheep ha^{-1} denote that there were 0, 4, 8, 12 and 16 sheep grazing rotationally in three replicated 1-ha plots. *Each plot* was rotationally grazed three times per year, each time for 15 days with a rotation interval of 30 days

the total N in the grazed treatments. Soil inorganic N pools in the ungrazed treatment were significantly higher than in all grazed treatments during the summer season from July to the end of August. But there were no statistically significant differences between the four grazed treatments, even though moderate grazing intensities (2.67 and 4.00 sheep ha^{-1}) tended to have higher inorganic N concentrations than the heaviest grazing intensity (5.33 sheep ha^{-1}, Fig. 3c).

Net N mineralization rates ranged from −0.61 to 0.27 μg N g^{-1} dry soil day^{-1} and varied significantly during the growing season with higher values occurring in late July (Fig. 4a). There was net N immobilization during August and September. No consistent differences in net N mineralization rates were observed between grazing intensity treatments at the monthly time scale (Fig. 4a). Net nitrification rates were generally low and varied from −0.32 to 0.16 μg N g^{-1} dry soil day^{-1}, with slightly higher values appearing in late July and late August (Fig. 4b). Nitrate N immobilization mainly occurred during September, especially in the ungrazed treatment (Fig. 4b). Compared to the ungrazed treatment, nitrate N immobilization rates in all grazed treatments were significantly lower ($P<0.05$). However, there were no significant differences between the four grazed treatments ($P>0.05$; Fig. 4b).

Repeated measures ANOVA indicated that grazing intensity significantly affected soil mineral nitrogen dynamics, e.g., net N mineralization rates, ammonification rates, and the concentrations of soil mineral nitrogen (Table 2). Inorganic N concentrations and net N transformation rates were also significantly affected by sampling date and the interaction of grazing intensity and sampling date (Table 2). Further simple main effects analysis showed that the grazing effects on net N mineralization rates were significant during early August ($P=0.027$), late August ($P=0.006$) and September ($P<0.001$; Fig. 4a). Net nitrification rates were significantly affected by grazing treatments during early July ($P<0.001$), early August ($P=0.001$), late August ($P=0.005$) and September ($P<0.001$; Fig. 4b). Grazing treatments significantly affected net ammonification rates during early July

Table 1 Dominant species, aboveground biomass and soil physical and chemical properties under different grazing intensities (GI) in August

GI (sheep ha^{-1})		0.00	1.33	2.67	4.00	5.33
Dominant species aboveground biomass (g m^{-2})	*Artemisia frigida*	40.44 (25.14)	43.63 (9.30)	52.33 (7.29)	37.35 (17.45)	46.16 (13.72)
	Kochia prostrata	18.12^a (10.68)	19.25^a (3.18)	22.82^a (6.10)	51.58^b (23.70)	25.78^a (5.58)
	Potentilla acaulis	3.06 (3.45)	4.83 (2.18)	5.75 (3.59)	3.31 (2.27)	10.53 (4.68)
Total aboveground biomass (g m^{-2})		92.10 (10.16)	114.86 (26.79)	100.49 (11.55)	106.68 (15.00)	91.29 (3.34)
Soil bulk density (g cm^{-3})		1.42 (0.06)	1.44 (0.11)	1.47 (0.04)	1.45 (0.05)	1.47 (0.06)
pH value		6.74 (0.06)	6.87 (0.10)	6.76 (0.14)	6.79 (0.14)	6.77 (0.09)
Total organic C (g C kg^{-1})		12.85 (0.27)	10.90 (1.03)	11.27 (2.28)	10.88(1.18)	9.38 (1.40)
Total organic N (g N kg^{-1})		0.71 (0.01)	0.69 (0.06)	0.65 (0.10)	0.66 (0.16)	0.55 (0.08)
C/N ratio		18.10 (0.16)	17.65 (0.21)	17.34 (1.32)	16.90 (2.47)	17.42 (0.32)

Values in parenthesis are standard errors. Numbers within rows followed by different letters in superscript are statistically significant at $P=0.05$ (least significant difference test following ANOVA). $n=3$ for all measurements.

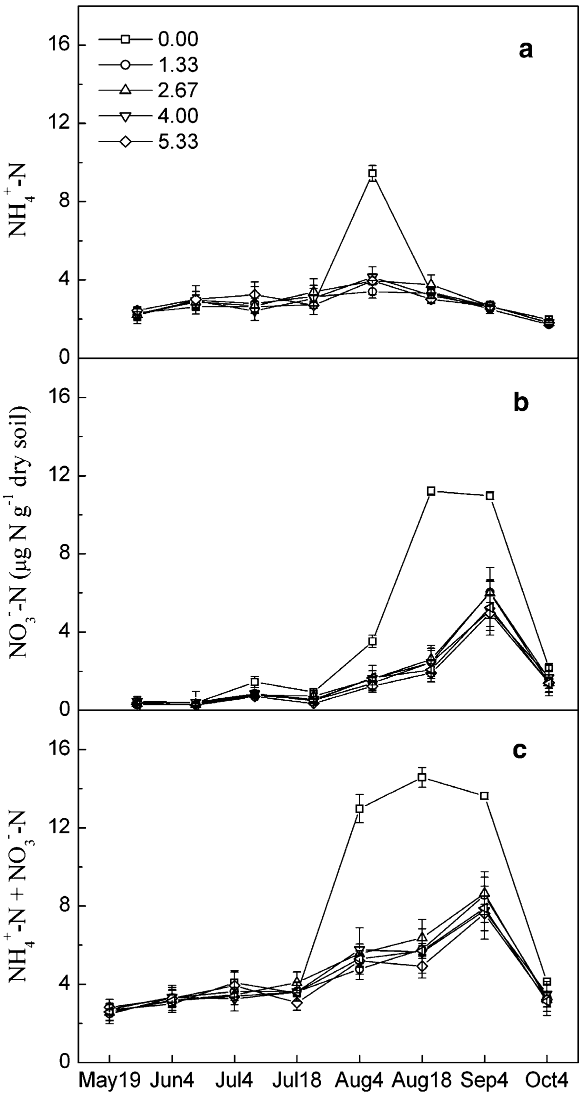

Fig. 3 Seasonal patterns of the concentrations of (**a**), (**b**) and total mineral N (**c**, , μg N g^{-1} dry soil) in the top 10 cm soil layer under different grazing intensities. Each *point* is the mean from three replicated plots. *Error bars* represent ±1 SE. See Fig. 2 for grazing intensity abbreviations

(P=0.011) and early August (P<0.001; Fig. 4c). Our ANCOVA analysis showed that the effect of grazing intensity on seasonal net N transformation rates was no longer significant if soil water content and temperature were used as co-variates of grazing intensity (P>0.05), while the effects of both soil temperature and soil moisture were significant (P< 0.05). This indicated that grazing intensity indirectly affected N mineralization rates by modifying soil moisture and temperature conditions.

Cumulative N mineralization

The general response of cumulative net N mineralization to different levels of grazing intensity at the annual time scale is roughly in the form of a normal distribution. Cumulative net N mineralization over the whole growing season under the moderate grazing intensity of 4.00 sheep ha^{-1} was significantly higher than under the ungrazed treatment (P<0.05), the lighter grazing treatment of 1.33 sheep ha^{-1} (P< 0.1), and the heavier grazing intensity of 5.33 sheep ha^{-1} (P<0.05; Fig. 5). Cumulative net nitrification rates over the whole growing season were generally negative (nitrate immobilization) for most treatments except for the grazing intensities of 4.00 sheep ha^{-1} which resulted in a positive cumulative net nitrification rate. A low level but significant net nitrate immobilization occurred in the ungrazed treatment. Cumulative net ammonification rates were higher

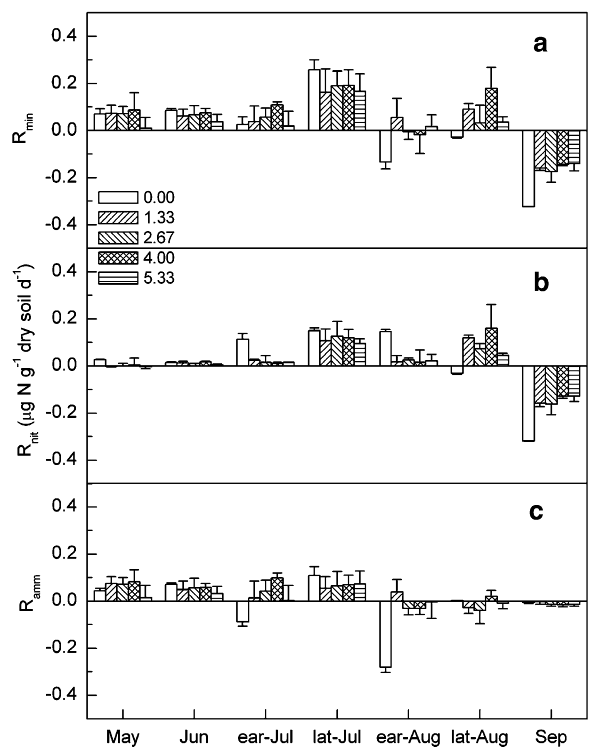

Fig. 4 Seasonal patterns of the rates of net N mineralization (**a**), nitrification (**b**) and ammonification (**c**, R_{min}, R_{nit} and R_{amm}, respectively, μg N g^{-1} dry soil day^{-1}) in the top 10 cm soil layer under different grazing intensities. Each *column* is the mean from three replicated plots. *Error bars* represent ±1 SE. Treatments with *different letters* are statistically different at P< 0.05 level. See Fig. 2 for grazing intensity abbreviations

Table 2 Results of F tests based on repeated measures ANOVA for soil inorganic N concentrations (μg N g^{-1} dry soil) before and after incubation, and for the rates of net N mineralization, nitrification and ammonification (R_{min}, R_{nit} and R_{amm}, respectively, μg N g^{-1} dry soil day^{-1}) under different grazing intensities (GI) for the growing season

Measurements	GI		Season		GI×Season	
	F	P	F	P	F	P
$c\left(NH_4^+ - N\right)_i$	8.910	0.002	329.354	<0.001	3.910	0.003
$c\left(NO_3^- - N\right)_i$	56.812	<0.001	183.597	<0.001	5.664	<0.001
$c(N_{mineral})_I$	41.024	<0.001	897.397	<0.001	13.574	<0.001
$c\left(NH_4^+ - N\right)_{i+1}$	1.090	0.413	48.076	<0.001	3.426	0.004
$c\left(NO_3^- - N\right)_{i+1}$	25.594	<0.001	122.275	<0.001	3.757	0.002
$c(N_{mineral})_{i+1}$	14.205	<0.001	82.482	<0.001	3.738	0.003
R_{min}	4.663	0.022	255.580	<0.001	3.557	0.003
R_{nit}	1.075	0.419	168.377	<0.001	4.092	0.001
R_{amm}	6.016	0.010	55.100	<0.001	2.998	0.009

than the cumulative net nitrification rates. The general pattern of cumulative net ammonification rates was similar to that of cumulative net N mineralization rates under different grazing intensities (Fig. 5).

Influence of grazing intensity and climate on soil net N mineralization

Both monthly net N mineralization and net nitrification rates were significantly positively correlated with soil temperature ($P<0.001$) for all the grazing intensities (Table 3). However, monthly net ammonification rates were not significantly correlated with soil temperature (Table 3). Significant negative correlation between all the initial soil moistures and grazing intensities ($P=0.011$) indicated that there was a significant decreasing trend in soil moisture with increasing grazing intensity, even though grazing intensity only accounted for less than 10% of the variability in soil moisture for the growing season. In general, monthly net N mineralization, nitrification and ammonification rates were negatively correlated with initial soil moisture ($P<0.001$, $P=0.001$, and $P=0.118$, respectively) for all the grazing intensities (Table 3). Because there was a significant negative correlation between grazing intensities and soil water contents, grazing intensity might have directly affected monthly N transformation rates and/or indirectly impacted N transformation via alteration of soil moisture. Results from our analysis of co-variance (ANCOVA) showed that the effect of grazing intensity on monthly net N transformation rates was no longer significant if soil water content and temperature were used as co-variates of grazing intensity. This result indicated that the effect of grazing intensity on monthly net N transformation rates was mostly indirect, primarily by modifying soil moisture and temperature.

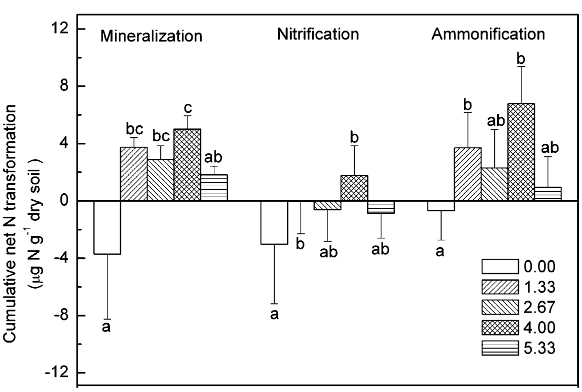

Fig. 5 Cumulative net N mineralization, nitrification and ammonification in the top 10 cm soil layer under different grazing intensities for the growing season. *Error bars* represent ± 1 SE. Treatments with *different letters* are statistically different at $P<0.05$ level. See Fig. 2 for grazing intensity abbreviations

Discussion

Grazing intensities and cumulative net N mineralization

The relationship between grazing intensities and net N mineralization rates in the Inner Mongolian steppe at the annual time scale roughly showed a pattern of a normal distribution, i.e., net N mineralization rate was the highest at an intermediate grazing intensity, while higher or lower grazing intensities reduced net N mineralization rates. However, this pattern was not

Table 3 Correlation coefficients (r) and significant level (P) of the rates of net N mineralization, nitrification and ammonification (R_{min}, R_{nit} and R_{amm}, respectively, μg N g^{-1} dry soil day^{-1}) for different grazing intensities (GI) with soil temperature of corresponding period and the initial soil moisture (n=21)

GI (sheep ha^{-1})	R_{min} r	P	R_{nit} r	P	R_{amm} r	P
Soil temperature						
0.00	0.372	0.090	0.740	<0.001	−0.391	0.080
1.33	0.483	0.026	0.613	0.003	−0.063	0.785
2.67	0.454	0.039	0.631	0.002	−0.082	0.723
4.00	0.409	0.066	0.483	0.027	0.055	0.814
5.33	0.562	0.008	0.683	0.001	0.157	0.497
Soil moisture						
0.00	−0.348	0.122	−0.252	0.270	−0.177	0.443
1.33	−0.509	0.019	−0.459	0.036	−0.265	0.245
2.67	−0.287	0.206	−0.449	0.041	0.126	0.585
4.00	−0.174	0.451	−0.349	0.121	0.231	0.313
5.33	−0.395	0.076	−0.429	0.052	−0.184	0.425

apparent for the monthly net N mineralization rates. Soil temperature and moisture exerted much stronger control of the monthly N dynamics than grazing intensity. These results demonstrated that studies of N transformation patterns require appropriate attention paid to temporal scales. Considering the abnormally low precipitation (only 48% of the long-term average annual precipitation) during the year of this study, multi-year studies were likely needed to further test this normal distribution pattern.

Understanding the exact mechanisms responsible for this pattern is challenging. Grazing stimulation of soil net N mineralization has been attributed to enhanced rhizosphere microbial activities under light grazing (McNaughton et al. 1997; Tracy and Frank 1998; Frank et al. 2000). This mechanism stipulates that light grazing of plant shoots leads to an increased production of root exudates that stimulate rhizosphere microbial metabolism and result in an enhanced N mineralization. Whereas, heavy grazing over the grazing threshold may reduce the production of root exudates due to over defoliation, and therefore, result in a reduced N mineralization (Holland et al. 1996). The adaptation of plant community to grazing at different intensities may explain phenomena in the long-term (Rossignol et al. 2006). In the case of our present study, significantly greater aboveground biomass of the dominant species, *Kochia prostrate*, was found under the intermediate grazing intensity (4.00 sheep ha^{-1}; Table 1) than under other grazing intensities. The aboveground biomass of *Potentilla* spp. (Table 1), which is an indicator for overgrazing in grassland ecosystems (Li et al. 1999), was higher under the heaviest grazing intensity treatment than under other grazing intensities. These differences in plant community structure are a potential cause of the different cumulative net N mineralization rates under different grazing intensities.

Seasonal dynamics

The seasonal dynamics of soil N in this Inner Mongolian steppe were characterized with a low level of net N mineralization rate during the spring and early summer, a peak of N mineralization in the mid-summer time, and a substantial amount of net N immobilization in the autumn when soil water contents were high and soil temperatures were low (Fig. 4). The net N mineralization during the spring and early summer coincided with spring warming up and relatively adequate soil moisture condition for microbial activities (Fig. 1). The peak of net N mineralization occurred during the warmest period and mainly in the form of nitrification. The input of high C:N ratio substrates from root turnover and aboveground litter from senescence, coupled with high soil water availability, might have caused the net N immobilization in the autumn, because labile C with high C/N ratio can stimulate rapid microbial growth and the assimilation of mineral N into microbial biomass (Luizão et al. 1992).

While soil $NH_4^+ - N$ concentrations of all grazing intensity treatments remained relatively stable during the growing season (Fig. 3a), soil nitrate concentrations tended to increase through out the entire growing season except for the last sampling in early

October when soil nitrate concentration declined due to a marked increase in nitrate immobilization in the autumn. Similar increasing trend in nitrate concentrations throughout the summer for all treatments was also reported in a published study (Amatya et al. 2002). In their study, the increase in nitrate concentration was attributed to NH_4^+ accumulated in the bare ground plots due to the lack of plant uptake. While in our study, the unusually dry condition has substantially reduced the amount of plant uptake during the studying period, resulting in the accumulation of nitrate in the soil. This nitrate N was later immobilized by soil microbes and plant roots at the last sampling time when soil moisture recovered from the late summer drought (Fig. 3b). The relatively higher nitrate concentration in the ungrazed treatment than grazed treatments during the dry period from mid-July to September responded to the higher soil water content in the ungrazed treatment. Average soil water content in the ungrazed treatment was approximately 33% higher than that in grazed treatments (Fig. 2). This difference in soil water content may have allowed more microbial nitrification, but not more plant uptake in the ungrazed treatment, resulting in higher level of nitrate concentration (Fig. 3b). The average inorganic N concentration in our study ranged from 2.49 to 14.57 μg N g^{-1} dry soil, accounted for less than 2.6% of total N, and was generally lower than the values reported for a native man-made Russian wild rye grass pasture in North China (6.95–37.93 μg N g^{-1}, Bai et al. 1999), for pastures in the western Brazilian Amazon basin (5–20 μg N g^{-1}, Neill et al. 1997), and for a grazed Bermuda grass pasture (14.0–100.4 μg N g^{-1}, Wright et al. 2004). This suggested that Inner Mongolia steppe generally maintains a relatively low level of N availability.

Our results indicate that grazing intensities had much less influence on the monthly dynamics of soil N transformation than temperature and moisture conditions (Table 3). Previous studies have shown that soil temperature and moisture substantially control the rate of N mineralization (Wang et al. 2006). One of the important processes that control the relationship between soil moisture fluctuation and N transformation is soil drying and re-wetting (Neill et al. 1997; Fierer and Schimel 2002). In general, the temperature and moisture sensitivities of soil N transformation are complex. Different ecosystems behave differently. Oxygen supply and temperature usually play more significant roles in regulating soil N transformations than soil moisture itself in wetter ecosystems (Paul et al. 2003). Whereas water availability to soil microbial communities and drying–rewetting cycles exert stronger controls on soil N processes in relatively dryer ecosystems (Van Gestel et al. 1993).

Surprisingly, soil water content during the growing season was significantly and negatively correlated with grazing intensities, mostly due to the relatively high gravimetric water content in the ungrazed treatment. Ordinarily, soil water loss through plant transpiration should be reduced under higher grazing intensities because more leaf tissues should be removed under higher grazing intensities. Although the exact causes of this negative correlation are difficult to know, the higher soil organic matter content in the ungrazed treatment (Table 1) might have made the gravimetric water content apparently higher than the grazed treatments, but not higher water availability in reality.

Methodological issues inherent with the core incubation method can be a potential concern, even though we followed the procedure specifically recommended for semiarid grassland ecosystems (Hook and Burke 1995). Often mentioned methodological issues include denitrification losses, altered soil moisture inside the core, and induced immobilization by the damaged roots inside the core. In our study, N loss via denitrification should have little effect on our results because denitrification rates were extremely low (data not shown) mostly due to the low soil moisture condition. Soil moisture contents were similar between the soil inside PVC tubes and the surrounding soil outside PVC tubes, similar to results reported by Subler et al. (1995). According to the study of Hook and Burke (1995), damaged roots inside each core did not significantly influence N transformation processes in a semiarid grassland when the incubation period was reasonably short (15–30 days). Also because of the short incubation period employed in our study, we did not notice any root regrowth through the core bottom during sampling after incubation. Overall, there is no major methodological bias that may seriously compromise our results.

In summary, grazing intensity over the long-term (e.g., 17 years) is an important variable in regulating

N dynamics in the Inner Mongolian Steppe. There are significant differences in net N mineralization rates between different grazing intensity treatments. There is a pattern that cumulative net N mineralization rates at the annual time scale tend to be higher under the moderate grazing intensity of 4.00 sheep ha^{-1} than under either lower or higher grazing intensities, showing a normal distribution pattern. Grazing intensities play a lesser role in regulating monthly N transformation rates than soil moisture and temperature conditions. Long-term grazing under the highest intensity (5.33 sheep ha^{-1}) substantially reduces soil C and N storage, which indicate that the system under the highest grazing intensity cannot be sustained. Given these grazing effects, many other ecosystem attributes such as total aboveground plant biomass and soil properties are not significantly modified by different grazing intensities, which suggests that the Inner Mongolian steppe ecosystem is highly adapted to grazing and that it has a reasonably high level of tolerance to grazing of moderate intensities.

Acknowledgment We thank the staff of the permanent meteorological station of Inner Mongolia Grassland Ecosystem Research Station, the Chinese Academy of Sciences (CAS) for providing the temperature and precipitation data, Dr. Shiping Wang for the maintenance of the field experiment plot, and Mr. Yan Zhichun for his assistance in the field. We appreciate the critical and constructive comments from two anonymous reviewers. The study was supported by a general grant (no. 30670347) and an innovative group grant (no. 30521002) from the Natural Science Foundation of China, the Overseas Outstanding Scientists Team project, a key innovation project from CAS (KZCX2-XB2-01-03), and some funding from the Committee on Research of University of California-Santa Cruz.

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