

## Effects of livestock grazing on soil nitrogen mineralization on Hulunber meadow steppe, China

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### ABSTRACT

Soil nitrogen (N) cycling is an important factor in terrestrial ecosystems, including grasslands. Understanding the effects of grazing on nitrogen cycling in grassland ecosystems is critical for better management and for improving knowledge of the mechanisms underlying grassland degradation and can provide basic information for sustainable development in grassland ecosystems. In this study, *in situ* incubation in intact soil cores was used to measure seasonal changes in soil nitrogen mineralization and nitrification in the meadow steppe of the Hulunber grasslands of northeastern China. Soil plots were subjected to varying intensities of cattle grazing, and soil characteristics including several aspects of the nitrogen cycle were analysed. The findings demonstrate that soil inorganic N pools and nitrogen mineralization peaked in August and that moderate grazing intensity produced higher seasonal mean net N mineralization ( $A_{\min}$ ); net nitrogen mineralization rate ( $R_{\min}$ ); net ammonification rate ( $R_{\text{amm}}$ ) and net nitrification rate ( $R_{\text{nit}}$ ). Seasonal mean net mineralization rate was increased by 6–15% in the lightly and moderately grazed plots (0.34–0.46 AU cow/ha) and by 4–5% in the heavily grazed plots (0.69–0.92 AU cow/ha). Also it was found that soil moisture was significantly positively correlated with inorganic N,  $A_{\min}$ ,  $R_{\text{amm}}$  and  $R_{\min}$  and significantly negatively correlated with  $R_{\text{nit}}$ , while soil temperature exhibited the opposite effect. The obtained results demonstrated net nitrogen mineralization and ammonium rates, which were strongly linked to grazing intensity, soil temperature and soil moisture.

**Keywords:** inorganic nitrogen; terrestrial ecosystem; climate; grazing intensity; nutrient cycling; environmental factors

Soil nitrogen (N) mineralization and nitrification are the dominant microbial processes affecting inorganic N availability in terrestrial ecosystems and are an important component of N cycling (Liu et al. 2010). Soil nitrogen mineralization shows high spatial and temporal variability and is regulated by several environmental factors, including soil temperature, moisture and pH, and by anthro-

pogenic activities (Augustine and McNaughton 2006). However, the effects of these factors may vary in different ecosystems.

Grassland ecosystems are an important component of terrestrial ecosystems. Nitrogen availability and transformation caused by seasonal and inter-annual climate variation have a significant effect on N mineralization and the grassland productivity

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(Liu et al. 2010). Nutrient cycling is different in grassland ecosystems than in forest ecosystems, due to the disturbances and inputs caused by livestock, including grazing, trampling and the deposition of excrement. These changes can directly or indirectly influence nitrogen mineralization by affecting plant litter decomposition rates and soil microbial biomass (Tracy and Frank 1998).

Grazing is the most common human activity in grassland ecosystems. Some studies have indicated that an absence of grazing reduces the net soil nitrogen mineralization rate, while light grazing promotes soil nitrogen mineralization, and overgrazing inhibits soil nitrogen mineralization (Biondini et al. 1998). Other studies have shown that moderate grazing can enhance microbial decomposition, which is beneficial for soil nitrogen mineralization (Xu et al. 2007). However, the range of grazing intensities in different grassland types can have different influences on soil nitrogen mineralization processes such as mineralization and nitrification (Zhao et al. 2010). On a low grass steppe, moderate grazing was shown to be most favourable for soil N mineralization, while on Serengeti grassland, net soil N mineralization gradually increased with an increasing grazing intensity (Seagle et al. 1992). Research on nitrogen cycling in meadow grassland ecosystems in China is an emerging field, and many knowledge gaps remain. Few studies have explored nitrogen mineralization in grassland ecosystems. Therefore, the present study on the effects of grazing on soil N mineralization in a meadow steppe region offers an important contribution to the understanding of grassland biogeochemical cycles.

The Hulunber meadow steppe in northeastern China is a representative part of the Eurasia grassland ecosystem. Cattle grazing is the primary use of grasslands in the regional farming system. Therefore, understanding the effects of grazing on nitrogen cycling in these grassland ecosystems is critical for better management and for improving knowledge of the mechanisms underlying grassland degradation and can provide basic information for sustainable development in grassland ecosystems. The objectives of the present study were as follows: (1) to investigate the seasonal variation of the inorganic nitrogen pool and the net nitrogen mineralization rate *in situ* in surface soils; (2) to compare the differences in soil nitrogen mineralization rates among the different grazing intensi-

ties over a growing season; and (3) to reveal the relationships between the soil inorganic nitrogen pool, the net nitrogen mineralization rate and other soil characteristics.

## MATERIAL AND METHODS

**Study site.** This study was conducted in the centre of the Hulunber meadow steppe (49°19'349"49°20'173"N, 119°56'521"119°57'854"E), in the northeastern region of Inner Mongolia, China. The elevation at the research station varies from 666–680 m a.s.l. The climate is temperate, semi-arid and continental, with an annual average of 110 frost-free days. The average annual precipitation ranges from 350–400 mm, approximately 80% of which falls between July and September. The annual mean air temperature ranges from –5°C to –2°C, with a maximum monthly mean of 36.17°C in July and a minimum of –48.5°C in January. The monthly average temperature and precipitation at the study site in 2014 are shown in Figure 1. The soil is chernozem or chestnut soil by type, and the vegetation is characterized as a typical *Leymus chinensis* and forbs meadow steppe. The main species are *L. chinensis* Stipa. *baicalensis*, *Carex pediformis*, *Galium verum*, *Bupleurum scorzonnerifolium* and *Filifolium sibiricum*.

**Experimental design.** The grazing experiment was established in 2009 with 18 paddocks each of 5 ha (300 × 167 m) with six stocking densities (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU/ha; where 1 AU = 500 kg of adult cattle). Each stocking rate was replicated three times in a randomised block design (Figure 2). The stocking rates were achieved by using 0, 2, 3, 4, 6 or 8 young cows (250–300 kg) per plot. Continuous grazing lasted for 120 days between June and October on an annual basis from 2009–2014. The grazing cattle were kept in the grazing plots day and night, and their drinking water was supplied from an outside water source. Before being fenced, the site was part of a larger area, under long-term free-ranging cattle grazing.

The baseline measurements of vegetation and soil traits were carried out using the 50 m × 50 m grid method before the grazing experiment. It was found that the soil profile is composed of humus layer, leaching layer and deposition layer, and the thickness of each soil layer is 0–48, 49–75 and 76–100 cm. The study areas were classified

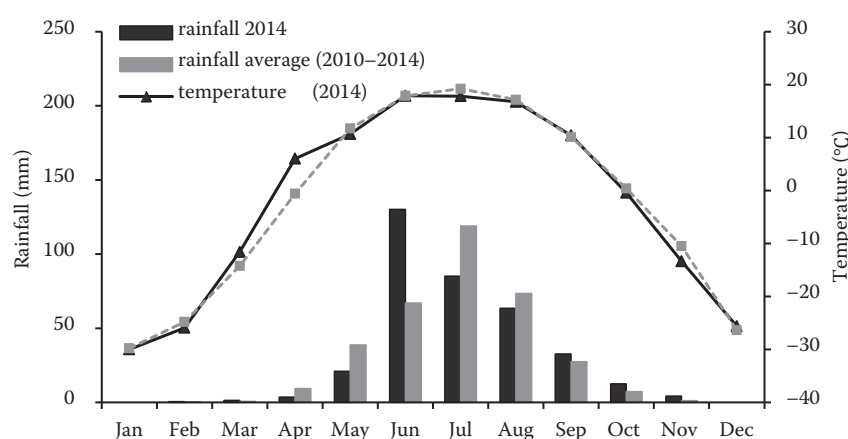


Figure 1. Monthly rainfall and temperature in 2014 and averages from 2010–2014 for the grazing experimental sites. Values on each panel are total annual rainfall and mean temperature

as chernozem or chestnut soil and loam in terms of texture. The plant roots were decreased with the increase of soil depth. The soil pH value was 6–7. The total soil nitrogen was 3.73–4.08 g/kg and the organic carbon concentration of the surface soil was 36.4–39.5 g/kg; these values did not vary significantly among the plots with different grazing intensities.

**Soil sampling and analysis.** Net N mineralization rates were measured using an *in situ* soil core incubation method. Within each replicated plot, five 2 × 2 m quadrats were randomly demarcated on the first soil sampling date and were repeatedly sampled throughout the experiment. Two PVC tubes (7.5 cm in diameter and 12 cm in length) were driven into the soil 10 cm from where the plants and litter were clipped and removed. Two soil cores were obtained. One soil core was returned to the laboratory and stored in a refrigerator at 4°C for later analysis of the initial concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and inorganic N. 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 core was incubated for approximately 30 days during the warm, wet season from June to October in 2014.

In the laboratory, following the removal of roots and stones, each soil core was well mixed by hand to form a homogenous sample and then passed through a 2 mm sieve. To analyse inorganic nitrogen, a 10-g subsample was taken from both the initial and incubated soil cores, and 50 mL of a 2 mol KCl solution was added. The soil and extractant were shaken for 1 h in a reciprocal shaker. After shaking, the soil suspension was filtered through No. 1 filter paper. The concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were determined using a flow injection autoanalyzer (Fiastar 5000 Analyzer, Foss Tecator, Sweden). The net mineralization and nitrification rates were expressed on a dry mass basis.

The soil moisture content and bulk soil density of the composite sample from each plot were determined gravimetrically via oven-drying at 105°C for 24 h. Rainfall and temperature data were collected from an automatic meteorological station

(AU/ha)		(AU/ha)		(AU/ha)	
Herd road	0.00	Herd road	0.23	Herd road	0.00
	0.46		0.69		0.34
	0.92		0.46		0.92
	0.34		0.92		0.46
	0.69		0.34		0.69
	0.23		0.00		0.23
Replication 1		Replication 2		Replication 3	

Figure 2. Experimental design diagram of different cattle grazing stocking rate

(Milos 520, Vaisala, Finland) at intervals of 30 min. The soil temperature (at a 10-cm depth) was recorded using an automatic soil temperature and humidity sensor (Beta Them10K3A1 Thermistor, Campbell Scientific Inc., USA) placed in each plot.

**Calculations and data analysis.** Net N mineralization (nitrification) rates during the incubation period were calculated from the balance between the inorganic N contents of the initial and incubated samples. Cumulative N mineralization was determined by summing the amount of net N mineralization at each incubation stage during the growing season. Net N mineralization and nitrification rates were calculated using the following equations:

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

$$A_{\text{amm}} = [\text{NH}_4^+\text{-N}]_{i+1} - [\text{NH}_4^+\text{-N}]_i;$$

$$A_{\text{nit}} = [\text{NO}_3^-\text{-N}]_{i+1} - [\text{NO}_3^-\text{-N}]_i;$$

$$A_{\text{min}} = A_{\text{amm}} + A_{\text{nit}};$$

$$R_{\text{min}} = A_{\text{min}}/\Delta t;$$

$$R_{\text{amm}} = A_{\text{amm}}/\Delta t;$$

$$R_{\text{nit}} = A_{\text{nit}}/\Delta t.$$

Where:  $t_i$  and  $t_{i+1}$  – initial and final incubation dates, respectively;  $c [\text{NH}_4^+\text{-N}]_i$  and  $c [\text{NH}_4^+\text{-N}]_{i+1}$  – mean  $\text{NH}_4^+\text{-N}$  contents of the initial and final incubated samples, respectively;  $c [\text{NO}_3^-\text{-N}]_i$  and  $c [\text{NO}_3^-\text{-N}]_{i+1}$  – mean  $\text{NO}_3^-\text{-N}$  contents of the initial and final incubated samples, respectively;  $N_{\text{amm}}$  – N ammonium amount ( $A_{\text{amm}}$ );  $N_{\text{nit}}$  – N nitrification amount ( $A_{\text{nit}}$ ); and  $N_{\text{min}}$  – N mineralization amount ( $A_{\text{min}}$ ) – accumulation of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and total inorganic N; and soil net N mineralization rates ( $R_{\text{min}}$ ); soil net N ammonium rates ( $R_{\text{amm}}$ ) and soil net N nitrification rates ( $R_{\text{nit}}$ ) are the net N mineralization, ammonium and nitrification rates. For each selected sampling date, means and standard error (SE) of soil nitrogen mineralization and other factors were calculated and the plot values represent means ( $n = 3$ )  $\pm$  SE. One-way ANOVAs and the least significant difference (LSD) tests were used to examine the effects of grazing treatments on seasonal and mean soil nitrogen mineralization and other factors, with effects of  $P < 0.05$  being significant (ANOVA; SPSS software, Version 21.0, IBM company, USA).

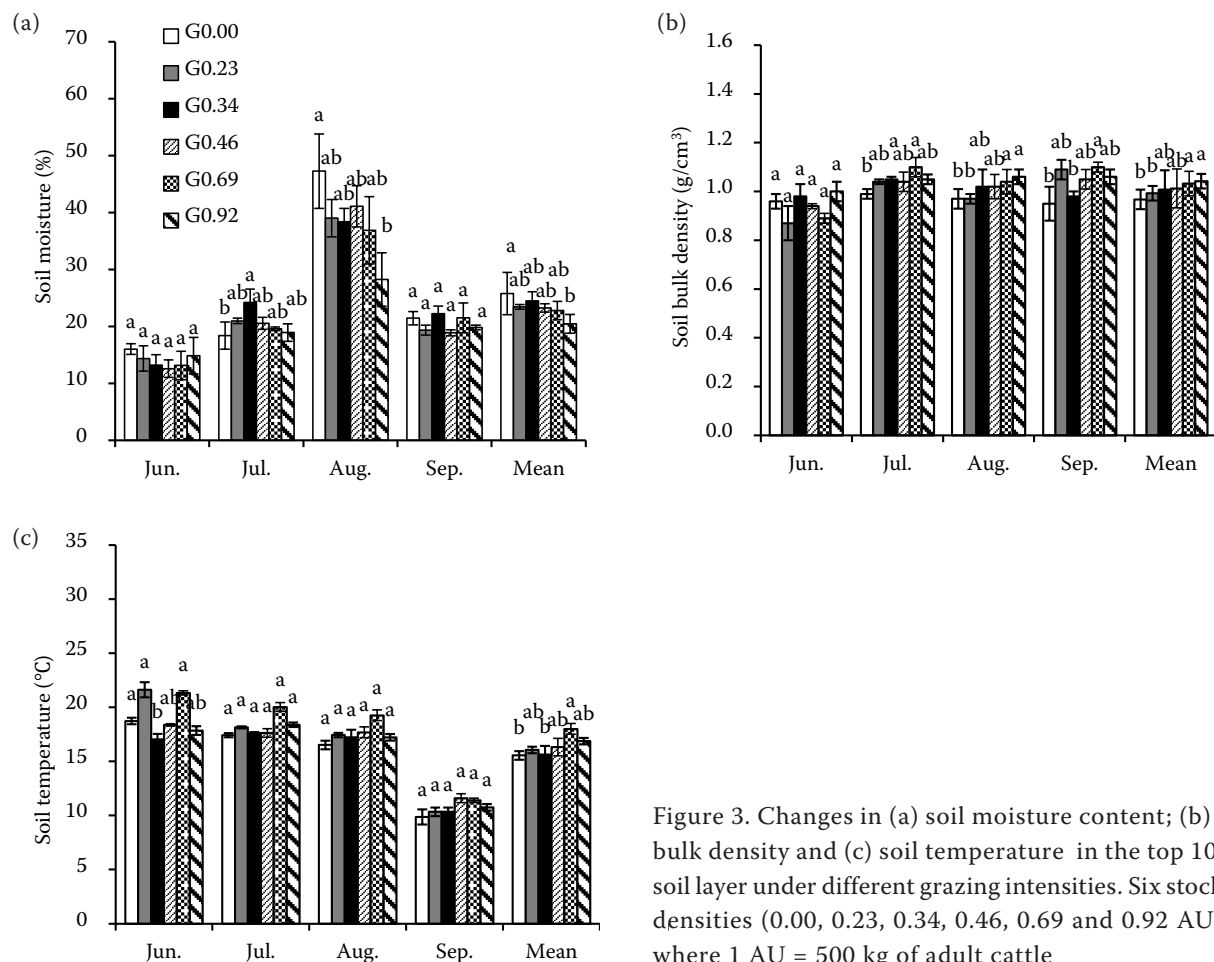


Figure 3. Changes in (a) soil moisture content; (b) soil bulk density and (c) soil temperature in the top 10 cm soil layer under different grazing intensities. Six stocking densities (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU/ha, where 1 AU = 500 kg of adult cattle

## RESULTS AND DISCUSSION

**Change of soil environmental factor.** The seasonal dynamics of soil environmental factor are shown in Figure 3. The soil moisture content showed similar patterns under all grazing intensities, peak soil moisture content occurred in August, but the mean soil moisture content in the ungrazed treatment was 14% higher than in the G0.92 treatment ( $P < 0.05$ ). Soil bulk density showed a gradual positive trend with an increasing grazing intensity throughout the incubation period (Figure 3b). The monthly soil temperature was highest in June and July and lowest in August and September in all of the grazing treatments (Figure 3c). Long-term grazing was associated with a significantly higher soil temperature compared with the ungrazed plots ( $P < 9.95$ ).

**Seasonal variation of soil inorganic N pools under different intensity.** During the incubation period, soil  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and soil inorganic N showed similar temporal patterns under different grazing intensities, increasing through August, then declining in September (Figure 4a). These

results are consistent with the conclusions of Liu et al. (2011), who showed that in semi-arid grazing grassland on the Loess Plateau of northern China during a growing season, a peak of nitrogen mineralization occurred in August, and a substantial amount of nitrogen immobilization in September when soil moisture content and temperature were low (Gleeson et al. 2008, Liu et al. 2011). Compared with the ungrazed plots, the light grazing treatment G0.34 resulted in a higher concentration of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and a larger soil inorganic N pool ( $P < 0.05$ ), while the intermediate grazing treatment G0.46 and the heavy grazing treatment G0.92 led to lower concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and a smaller soil inorganic N pool ( $P < 0.05$ ) (Figure 4).

**Change of soil net N mineralization and nitrification rates under different grazing intensity.** The soil net N mineralization, ammonium and nitrification rates exhibited significant temporal variations during the growing season. The net N mineralization rates ranged from 0.73 to 5.33 mg/kg/day, and the net N mineralization rates and net ammonification rates were highest in August and lowest in June and July (Figure 5a).

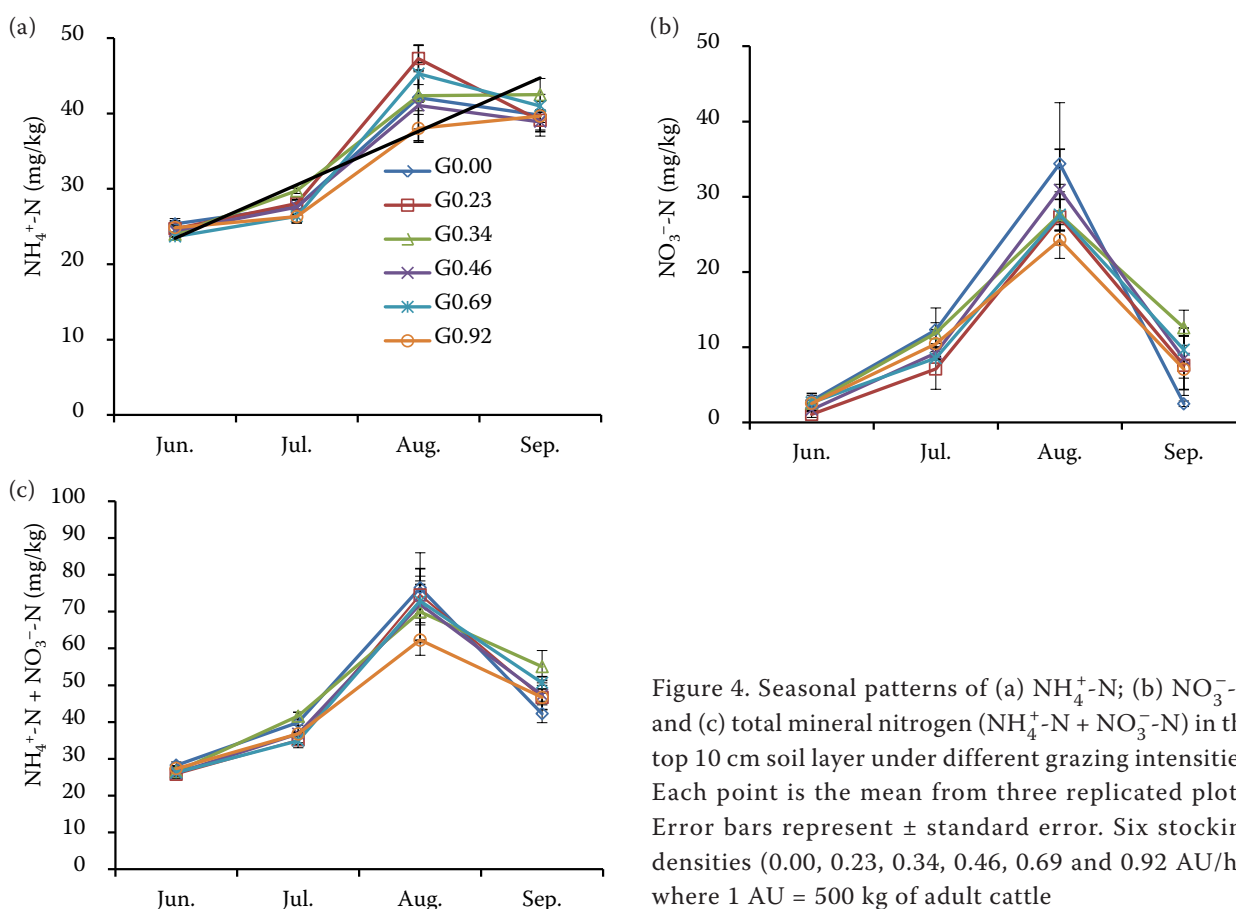


Figure 4. Seasonal patterns of (a)  $\text{NH}_4^+\text{-N}$ ; (b)  $\text{NO}_3^-\text{-N}$  and (c) total mineral nitrogen ( $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$ ) in the top 10 cm soil layer under different grazing intensities. Each point is the mean from three replicated plots. Error bars represent  $\pm$  standard error. Six stocking densities (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU/ha, where 1 AU = 500 kg of adult cattle



Nitrate nitrogen immobilization was observed in August in all treatments, with the greatest net nitrate nitrogen immobilization occurring in the ungrazed treatment G0.00 and the light grazing treatment G0.23 ( $P < 0.05$ ). It was observed that the grazing treatments tended to decrease both  $R_{\min}$  and  $R_{\text{amm}}$  compared with the ungrazed control plots in the early growing season, in June and July. However, in August, grazing increased both  $R_{\min}$  and  $R_{\text{amm}}$  compared with the ungrazed control plots and decreased  $R_{\text{nit}}$  ( $P < 0.05$ ). These results are consistent with others demonstrating that grazing decreases N mineralization in the early growing season (Augustine and McNaughton 2006). However, these results are opposite to those of previous works showing that light grazing positively affected net N mineralization in the early growing season (Le Roux et al. 2003, Shan et al. 2011). On average, during the study period, intermediate grazing treatment G0.46 produced higher seasonal mean  $A_{\min}$ ,  $R_{\min}$ ,  $R_{\text{amm}}$  and  $R_{\text{nit}}$  (Figure 5), which is consistent with a number of previous studies (Seagle et al. 1992, Xu et al. 2007). For example, Xu et al. (2007) showed 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 than under either lower or higher grazing intensity, supporting intermediate disturbance hypothesis (Connell 1978). The reason may be that moderate grazing could further enhance microbial degradation, which was favourable for soil N mineralization (Shariff et al. 1994). But in different types of grasslands, the effects of grazing on soil N mineralization have been found to be different. For example, in low grass prairie, moderate grazing was shown to be the most conducive to soil N mineralization, whereas in tall grass prairie, the soil net N mineralization rate gradually increased with an increasing grazing intensity (Seagle et al. 1992). This study confirms their findings and provides a possible explanation for differences between the results obtained and the results from studies in which the N mineralization rate increased in response to grazing (McNaughton et al. 1997). The role of soil N mineralization warrants further investigations.

**Relationships between soil characteristics and N mineralization and nitrification.** Pearson's correlation analysis demonstrated that soil mois-

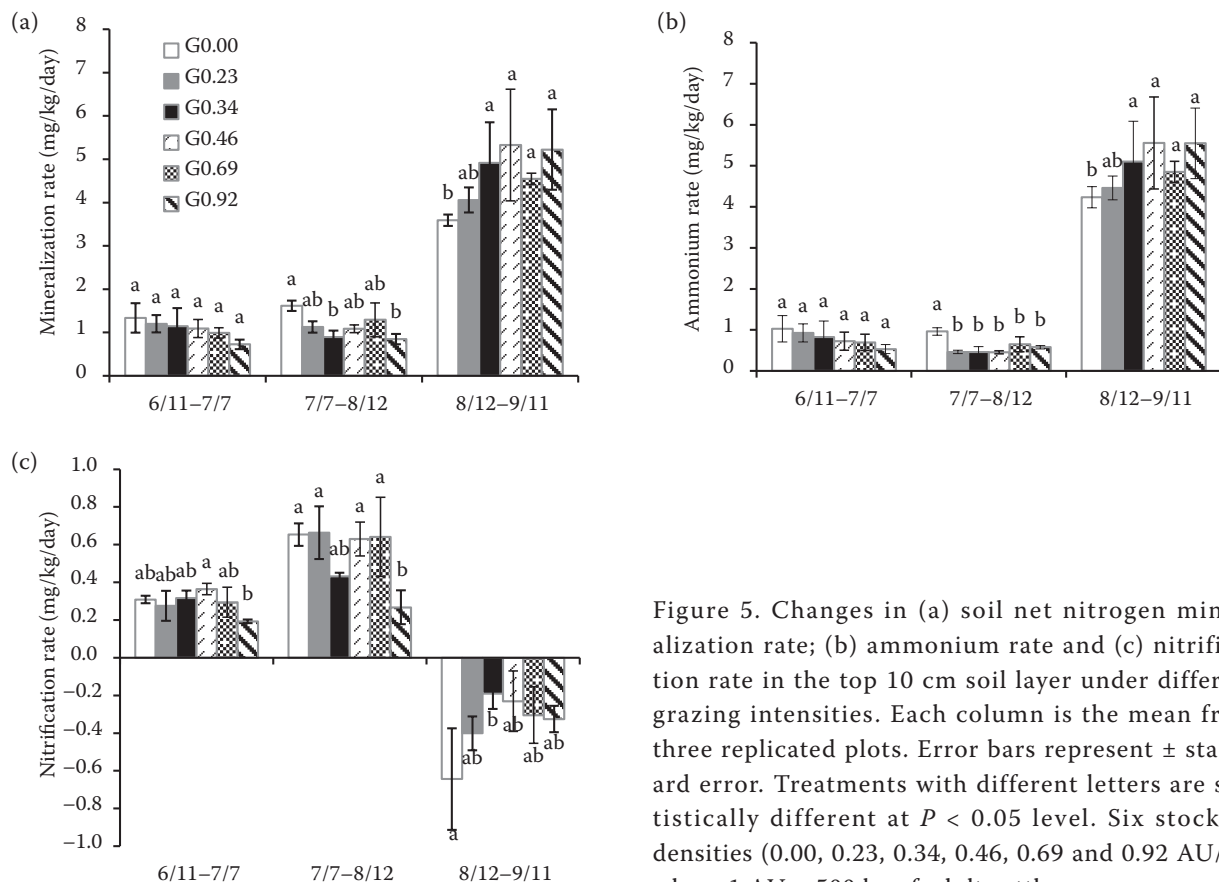


Figure 5. Changes in (a) soil net nitrogen mineralization rate; (b) ammonium rate and (c) nitrification rate in the top 10 cm soil layer under different grazing intensities. Each column is the mean from three replicated plots. Error bars represent  $\pm$  standard error. Treatments with different letters are statistically different at  $P < 0.05$  level. Six stocking densities (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU/ha, where 1 AU = 500 kg of adult cattle

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Table 1. Pearson correlation coefficients ( $r$ ) of season soil inorganic nitrogen (N); soil net N mineralization ( $A_{\min}$ ); soil net ammonium rates ( $R_{\text{amm}}$ ); soil net nitrification rates ( $R_{\text{nit}}$ ) and soil net N mineralization rates ( $R_{\min}$ ) with other soil characteristics including soil moisture, soil temperature and soil bulk density on Hulunber meadow steppe

$r$	Soil moisture	Soil temperature	Soil bulk density
Inorganic N	0.819**	−0.384**	0.296*
$A_{\min}$	0.736**	−0.273*	0.141
$R_{\text{amm}}$	0.756**	−0.268*	0.078
$R_{\text{nit}}$	−0.510**	0.131	0.161
$R_{\min}$	0.725**	−0.256	0.099

\* $P < 0.05$ ; \*\* $P < 0.01$

ture was significantly positively correlated with inorganic N,  $A_{\min}$ ,  $R_{\text{amm}}$  and  $R_{\min}$  and significantly negatively correlated with  $R_{\text{nit}}$ , while soil temperature exhibited the opposite effect (Table 1). The results indicate that the net N mineralization rate was significantly affected by soil temperature and soil moisture, with a relatively low soil temperature and relatively wet soil being related to greater soil mineralization, an increased soil inorganic N content and soil N mineralization. These findings are consistent with the results of other grassland studies (Shan et al. 2011) but do not agree with the results of previous studies showing that high temperature and relatively dry environment encourage more soil mineralization and increase the soil inorganic N content and that between 5 ~ 25°C, the soil net N mineralization rate and nitrification rate increase with an increasing temperature and humidity. The soil net mineralization and nitrification rates decline when the soil temperature exceeds 25°C and soil shows a high water content (Wang et al. 2006). Some studies have indicated that net N mineralization rates are positively correlated with seasonal temperature and are less sensitive to soil moisture (Sierra 1997). Other researchers have reported that soil N transformation is generally sensitive to temperature in wet ecosystems (Paul et al. 2003) but sensitive to soil moisture in arid and semi-arid ecosystems (Xu et al. 2007). These observations could be explained by the different seasonal patterns of temperature and precipitation between the

different study regions, which may directly affect soil temperature and moisture dynamics and lead to different seasonal patterns of soil inorganic N and net N mineralization. However, cumulatively, these results suggest that abiotic factors (e.g., soil moisture and temperature) could regulate the seasonal patterns of soil inorganic N and soil N mineralization. Therefore, the temporal scaling applied in this study provides theoretical references for how soil conditions affect the soil inorganic N pool and net N mineralization under different grazing pressures, which may have important implications for grazing management.

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