

SHEEP GRAZING STIMULATED PLANT AVAILABLE SOIL NITRATE ACCUMULATION IN A TEMPERATE GRASSLAND

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Abstract

We investigated the effects of increasing grazing intensities on N availability (inorganic N, In-N, for plant and microbial growth, e.g., NO_3^- -N, NH_4^+ -N) and variability in soils of 0–10 cm depth during 2009–2010, within a framework of a long-term grazing study. The results showed that the relationship for grazing intensity with respect to soil NO_3^- -N ($R^2 = 0.988$, $P = 0.006$) was well depicted by curvilinear equations. Moreover, soil NO_3^- -N, NH_4^+ -N and inorganic N varied significantly with sample date, year, and soil water content (SWC, %). There were also significant correlation between date and grazing intensities on soil NO_3^- -N, NH_4^+ -N and In-N. SWC and temperature had more impact on soil available N than grazing, especially with respect to the seasonal dynamics of the soil N pool. Grazing intensity, in combination with SWC (precipitation) and temperature, controlled soil N availability and, therefore, affect the N cycles and plant growth within semiarid grasslands.

Key words: Grassland; N availability; N cycle; Sheep; Soil moisture; Stocking rate.

Introduction

Grazing is the major land use type of the Inner Mongolian grasslands. Understanding how grazing affects soil nitrogen (N) availability is critical for the sustainable management of these grasslands. Nitrogen (N) is an essential element that affects the structure and function of terrestrial ecosystems (Vitousek *et al.*, 1997; Pan *et al.*, 2011). Soil N availability (inorganic N for plant growth and microbial growth, e.g., NO_3^- -N, NH_4^+ -N) plays an important role in plant growth, primary productivity, and species composition in many terrestrial ecosystems (Harpole & Tilman, 2007) by altering the efficiency of plant N use (Vitousek, 1982; Yuan *et al.*, 2006) and plant-soil microbe competition (Verhagen *et al.*, 1994). Topsoil N is also critical for regional carbon (C) budgets and greenhouse gas emissions (Davidson *et al.*, 1993; Luo *et al.*, 2004; Zhang *et al.*, 2013). In temperate grasslands, increasing inorganic N (In-N) input in soils can significantly influence aboveground productivity (Zhang *et al.*, 2015), plant and soil microbe composition (Zhang *et al.*, 2011), enzyme activities (Chander *et al.*, 1997), N mineralization rates (Rao *et al.*, 2009), and other abiotic factors (Zhang *et al.*, 2014), such as soil pH, soil moisture, and microelement availability (Tilman & Olff, 1991; Stevens *et al.*, 2009). To date, most studies have been focused on changes in soil N availability of the plant-soil system (Burke *et al.*, 1998), whereas there has been limited research into how the animal-plant-soil system affects soil N availability within temperate grasslands.

In grassland ecosystems, ungulate grazing activity affects light availability, evapotranspiration, and the diversity of soil microbes by the removal and trampling of aboveground vegetation (Teague *et al.*, 2011). These actions, in turn, influence soil bulk density (Steffens *et al.*, 2008; He *et al.*, 2011), temperature, and moisture (Zhao *et al.*, 2011), which consequently affect N availability, soil microbial activities, plant growth, and N mineralization rates (Wolf *et al.*, 2010; Schönbach *et al.*, 2011; Wu *et al.*, 2011). In the Inner Mongolian region, grazing intensity has been increased sharply since the 1980s due to rapid increases in human meat consumption (Jiang *et al.*, 2011). Therefore, understanding how grazing affects the availability of soil N is critical for the sustainable management of Inner Mongolian grasslands. Numerous studies have addressed the effects of grazing intensity on soil N availability (Jarvis & Barraclough, 1991; Lavado *et al.*, 1996; Biondini *et al.*, 1998; Henry & Jefferies, 2003; Xu *et al.*, 2007; Giese *et al.*, 2011; Shan *et al.*, 2011; Wu *et al.*, 2011), some of which reported a positive effect (Xu *et al.*, 2007; Giese *et al.*, 2011). However, several scientists previously assumed that grazing may suppress N mineralization rates, and thus causes a reduction in N availability (Seagle *et al.*, 1992; Bardgett & Wardle, 2003; Wang *et al.*, 2010). Yet, few studies have been conducted in temperate grasslands, especially for sheep (*Ovis aries* L.) grazing systems in the Eurasian steppe (Xu *et al.*, 2007). Furthermore, such information has not been reported from long-term grazing experiments, despite how crucial they are in identifying the impact of increased grazing intensities on the availability of the soil N and the N cycles.

Within the framework of a 5-year grazing experiment (initiated in 2005) situated in the Inner Mongolian grasslands of China, we determined the relationship between available soil N (NO_3^- -N and NH_4^+ -N) and grazing intensity across a 2-year period (2009–2010). The main objectives of this study were to: 1) determine how grazing intensity affects soil N availability and 2) identify the main factors controlling soil N availability in grazed grasslands. An underlying hypothesis is that grazing should have a significant impact on soil N availability, but its effects might be modified by precipitation and temperature because of the limited water resources in the semiarid grasslands of Northern China.

Materials and Methods

Site description: This study was conducted in the Eurasian steppe. The experimental area is located in the Xilin River basin of Inner Mongolia, China, which is administered by the Inner Mongolia Grassland Research Station of the Chinese Academy of Sciences ($43^\circ 38'N$, $116^\circ 42'E$). The mean annual temperature (1981–2010) is 0.4°C , with the mean monthly temperature ranging from -21.4°C in January to 19.0°C in July. Annual precipitation levels are 355.0 mm, with about 80% occurring between May and September. The daily air temperature and precipitation during the grazing periods of 2009 to 2010 are shown in Fig. 1. *Leymus chinensis* (Trin.) Tzvel. and *Stipa grandis* P. Smirn. are the predominant plant species in the experimental area (Chen, 1988). The soils were classified as Calcic Chernozems (Wang & Cai, 1988), with a pH value of 7.8 (in a soil:water ratio of 1:2.5). The experimental area was used for free grazing prior to the onset of the experiment (Wang & Baoyin, 2005).

Grazing experiment design: The grazing experiment was initiated in 2005; experimental treatments were described in detail by Schönbach *et al.* (2009). In brief, the grazing experiment had 2 management systems (a traditional – free grazing versus a mixed system – grazing and haying-making alternated annually), and 7 levels of grazing intensity (GI = 0, 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 sheep/ha). The area of each experimental treatment was 2

ha. The experiment was representative of the different sheep densities of free-grazing situation in Inner Mongolian grassland. Each individual experimental treatment area was fenced to prevent sheep migration. Two-year-old female sheep were chosen for the experiment and treatments were continuously grazed from June 10 to September 10 during each year from 2005 onwards, which coincides with the growing period in the region. In our study, we used the traditional grazing system, and selected 5 levels of grazing intensity (viz., 0, 3.0, 4.5, 6.0, and 9.0 sheep/ha). Unfortunately, in 2010 the soil samples from within the 4.5 sheep/ha treatment were partly lost when deposited in refrigerator. Therefore, we had no data for this treatment for 2010.

Sampling and analysis: First, we established 6 sampling sub-plots ($3\text{m} \times 3\text{m}$) along a diagonal line within each treatment site. All sub-plots were located more than 10 m away from the fence to avoid edge effects. Before sampling, we removed vegetation and animal faeces out of soil sampling locations. In each subplot, three soil cores (diameter 3 cm and depth 10 cm) were taken at random. The samples were then combined and sieved through a 2 mm mesh, to obtain a composite sample for the measurement of soil inorganic N concentration (NO_3^- -N and NH_4^+ -N) and soil water content (SWC, %). Soil samples were taken at 30-day intervals from June to September in 2009, and at 10-day intervals from June to September in 2010. The increase in sampling to 10-day intervals in 2010 was to obtain more information about the impacts of grazing intensity on soil N availability. All soil samples were deposited in a refrigerator (-20°C). For soil inorganic N measurements (the methods of soil inorganic N measurements had been described in detail in Wang *et al.* (2006), 10-g of fresh soil subsamples were extracted with 50 ml of KCl solution (2.0 M), and then analyzed using a flow injection auto analyzer (FIAsar 5000 Analyzer; Foss Tecator, Hillerød, Denmark). Soil NO_3^- -N and NH_4^+ -N concentration was expressed using dry soil. Finally, the total inorganic N (In-N) of soil was equated to the concentration of NO_3^- -N plus NH_4^+ -N (Wang *et al.*, 2006). SWC was determined using 20 g of fresh soil subsamples dried at 105°C for 48 h to a constant weight.

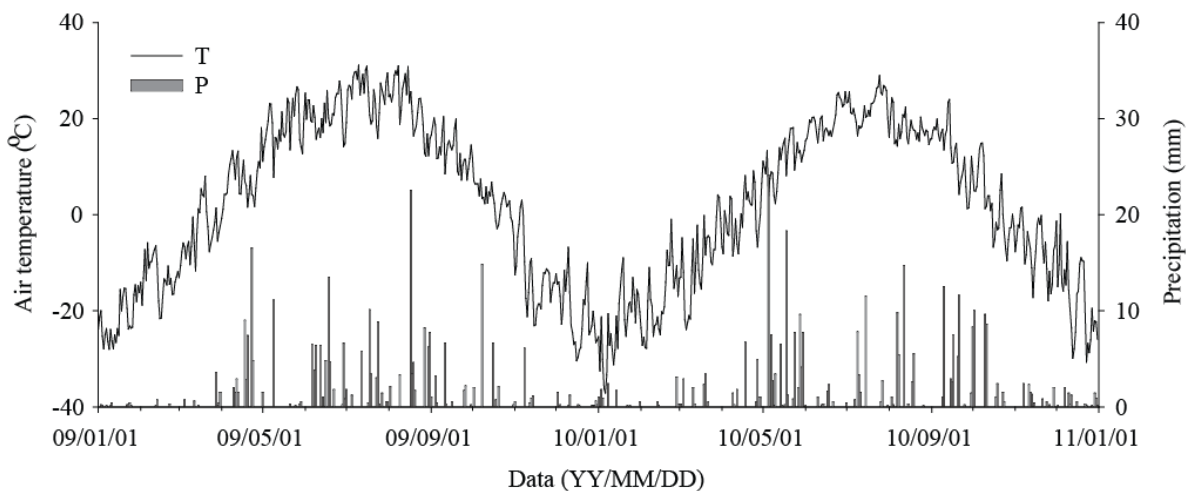


Fig. 1. Daily air temperature ($^\circ\text{C}$) and precipitation (mm) in 2009 and 2010.

Statistical analysis: We tested for block effects on all response variables (NO_3^- -N, NH_4^+ -N, In-N concentrations and SWC). Repeated-measure ANOVA were used to test the influence of grazing intensity on NO_3^- -N, NH_4^+ -N, In-N concentrations and SWC. To detect the effects of grazing intensity (GI), sampling date, and interactions between the two on soil NO_3^- -N, NH_4^+ -N and In-N concentrations, One-way ANOVAs were performed with a LSD of $\alpha = 0.05$. Annual relationships between GI, year, and interactions between the two on soil NO_3^- -N, NH_4^+ -N and In-N concentrations were explored using two-way ANOVAs. General Linear Mixed Effect Models were applied to investigate the impacts of SWC, air temperature and GI on soil NO_3^- -N, NH_4^+ -N and In-N concentrations across the 2 years of sampling. For data collected in 2009, curvilinear regressions were used to examine the relationships between soil NO_3^- -N, NH_4^+ -N and In-N concentrations with increasing GI. In 2010, curvilinear regressions could not be performed because the loss of samples for one of the grazing treatments meant that data for only 4 grazing intensities sampling gradients were not accurate enough for regressions. To explore underlying mechanisms linear regression was used to examine the relationship between soil NO_3^- -N, NH_4^+ -N and In-N concentrations with SWC. All analyses were conducted using SPSS statistical software (version 17.0, SPSS Inc., Chicago, IL, USA) and graphs were created using SigmaPlot (version 10.0, Systat Software, Inc.).

Results

Soil water content: Soil water content (SWC, %) was significantly lower within the moderate grazing treatment (i.e. 6.0 sheep/ha) (Fig. 2). In contrast, there were no significant differences in SWC between the control (0 sheep/ha), low (3.0 and 4.5 sheep/ha) and the high (9.0 sheep/ha) density grazing treatments.

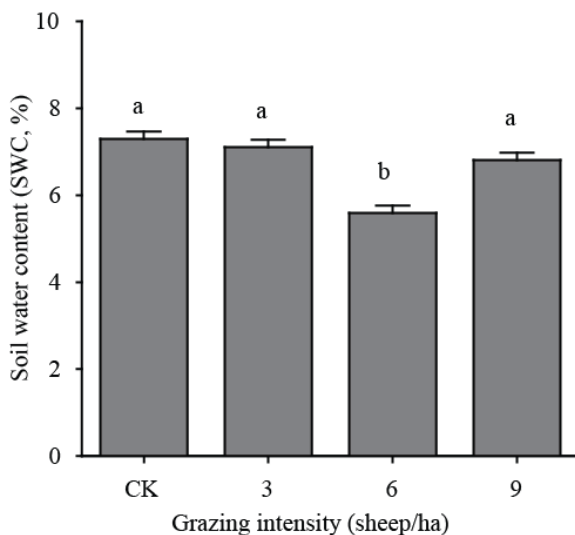


Fig. 2. Results of repeated-measure ANOVAs with respect to the effects of different grazing intensities (0, 3.0, 6.0, and 9.0 sheep/ha, respectively) on soil water content (SWC, %).

Soil NO_3^- -N and NH_4^+ -N concentrations: In 2009, soil NO_3^- -N and NH_4^+ -N concentrations were not significantly different across grazing treatments, except for one date in early summer (July 9; Fig. 3A, C). In 2010, soil NO_3^- -N differed across grazing treatments, except for one date in late spring (June 17; Fig. 3B). Similarly, soil NH_4^+ -N also differed in 2010 under the different grazing intensities, except for one date in late summer (August 14) (which was the period of the highest aboveground biomass; Fig. 3D). In summary, soil NO_3^- -N and NH_4^+ -N were both lower in 2009 than in 2010 (Fig. 3). Moreover, we found that soil NO_3^- -N had a curvilinear relationship to grazing intensity ($R^2 = 0.988$, $P = 0.006$; Fig. 4A). In other words, soil NO_3^- -N first increased with increasing grazing intensities, and then decreased at higher grazing intensities (i.e., 9 sheep/ha). Conversely, soil NH_4^+ -N did not show any apparent pattern with respect to increasing grazing intensity (Fig. 4B).

The results of repeated-measure ANOVAs indicated that soil NO_3^- -N was influenced by grazing intensity (GI), sample date (D), and $\text{GI} \times \text{D}$ in both 2009 and 2010 (Table 1). Soil NH_4^+ -N was not influenced by GI in 2009, but was significantly affected by D and the interaction between the two ($\text{GI} \times \text{D}$). In 2010, soil NH_4^+ -N was significantly influenced by GI, D, and $\text{GI} \times \text{D}$ (Table 1). When the variation due to sampling year (Y) was considered, soil NO_3^- -N was significantly influenced by GI, while soil NH_4^+ -N was not (Table 2).

Soil NO_3^- -N increased significantly with increasing SWC when SWC was $< 8\%$ ($R^2 = 0.160$, $P = 0.016$; Fig. 5A), then decreased significantly at higher SWC (i.e. SWC $> 8\%$; $R^2 = 0.373$, $P = 0.012$; Fig. 5A). Soil NH_4^+ -N did not increase significantly when SWC was $< 8\%$, but did when SWC was $> 8\%$ ($R^2 = 0.549$, $P = 0.001$; Fig. 5B). GI, SWC and air temperature all had a significant impact on soil NO_3^- -N (Table 3). Furthermore, SWC and temperature had a significant influence on NH_4^+ -N, but GI did not (Table 3).

Soil In-N concentration: Soil In-N concentration was lower in 2009 than in 2010 (Fig. 3, Table 2). In 2009, soil In-N was similar across all sampling dates, except for July 9th (Fig. 3E). In 2010, soil In-N varied with respect to grazing intensity, except for samples taken on September 23rd (Fig. 3F). Soil In-N increased between 0 – 6.0 sheep/ha, then decreased at the highest grazing intensity, which is highlighted by a weak curvilinear equation shown in Fig. 4C ($R^2 = 0.877$, $P = 0.062$).

The results of repeated measures ANOVA for 2009 showed that soil In-N was not affected by GI, but was significantly influenced by D and $\text{GI} \times \text{D}$ (Table 1). In 2010, soil In-N was significantly influenced by GI, D, and $\text{GI} \times \text{D}$ (Table 1). Soil In-N differed between Y but not between GI and $\text{GI} \times \text{Y}$ (Fig. 2, Table 2).

Soil in-N increased significantly with increasing SWC when soil moisture was $< 8\%$ ($R^2 = 0.182$, $P = 0.009$; Fig. 5C) and then decreased when SWC $> 8\%$ ($R^2 = 0.217$, $P = 0.070$; Fig. 5C). When examining the influence of GI, SWC and air temperature on soil In-N, only SWC and air temperature were significant (Table 3). When data from all grazing treatment samples were combined across both years, we found a significant relationship between In-N and air temperature when temperatures were $\geq 17^\circ\text{C}$, but not when they were $< 15^\circ\text{C}$ (Fig. 6).

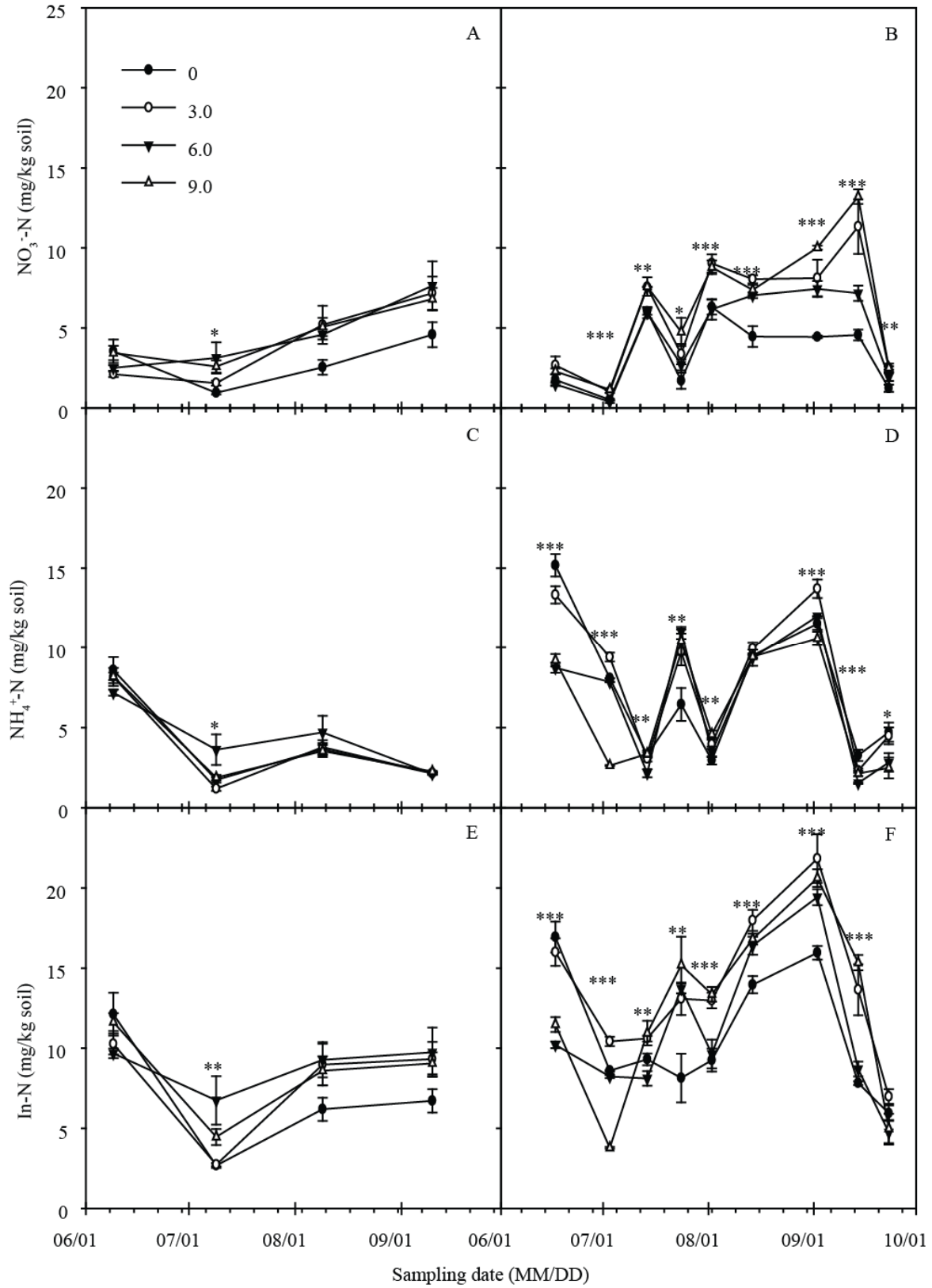


Fig. 3. Changes in NO₃⁻-N, NH₄⁺-N and total inorganic N concentrations (NO₃⁻-N plus NH₄⁺-N; In-N) (mg/kg soil) in soil depths of 0–10 cm with respect to increasing grazing intensities (0, 3.0, 6.0, and 9.0 sheep/ha, respectively) in 2009 and 2010. Error bars represent ±1 standard error of the mean (n = 6). Asterisks indicate any significant effect of grazing intensity treatment (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001).

Table 1. Results of repeated-measure ANOVAs with respect to the effects of grazing intensity (GI), date (D), and a combination of the two (GI×D) on NO₃⁻-N, NH₄⁺-N, and total inorganic N concentrations (NO₃⁻-N plus NH₄⁺-N; In-N) in 0–10 cm soil depths, with the significant of $\alpha = 0.05$. $P < 0.05$ was in bold.

	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
	NO ₃ ⁻ -N		NH ₄ ⁺ -N		In-N	
2009						
GI	4.53	0.007	1.32	0.290	2.78	0.158
D	53.69	<0.001	279.84	<0.001	311.73	<0.001
GI×D	2.51	0.024	2.88	0.008	24.76	0.032
2010						
GI	93.29	<0.001	26.46	<0.001	28.67	<0.001
D	363.36	<0.001	428.65	<0.001	311.73	<0.001
GI×D	15.62	<0.001	22.59	<0.001	27.76	<0.001

Table 2. Results of two-way ANOVAs with respect to the effects of grazing intensity (GI), Year (Y), and a combination of the two (GI × Y) on NO₃⁻-N, NH₄⁺-N, and In-N concentrations in 0–10 cm soil depths, with the significant of $\alpha = 0.05$. $P < 0.05$ was in bold.

	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
	NO ₃ ⁻ -N		NH ₄ ⁺ -N		In-N	
2009						
GI	7.39	<0.001	0.66	0.577	0.66	0.578
Y	7.25	0.008	39.58	<0.001	72.51	<0.001
GI×Y	1.64	0.179	1.14	0.332	0.89	0.448

Table 3. Results of mix model with the effects of soil water content (SWC), air temperature (T) and grazing intensity (GI) on NO₃⁻-N, NH₄⁺-N, and In-N concentrations in 0–10 cm soil depths, with the significant of $\alpha = 0.05$. $P < 0.05$ was in bold.

	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
	NO ₃ ⁻ -N		NH ₄ ⁺ -N		In-N	
2009						
SWC	12.04	<0.001	25.37	<0.001	1.98	<0.001
T	48.87	<0.001	66.17	<0.001	9.33	<0.001
GI	4.80	0.031	1.41	0.238	0.15	0.704

Discussion

Effects of grazing on SWC: Soil water content is modified by different land uses due to differences in vegetation cover and soil conditions from precipitation, temperature and evapotranspiration etc. Ungulate grazing alters soil thermal, hydraulic and mechanical properties. But disturbance of soil mechanical properties, interlinked with hydrological changes, often have detrimental effects on soil properties (water and air conductivity, retention). In the Inner Mongolian grasslands, where the warmer months coincide with the periods of greatest precipitation, primary productivity and vegetative cover could be strongly controlled by soil water availability. Sheep grazing influences standing biomass and the soil physic-character, which in turn affects SWC. Generally, SWC decreases with increasing grazing intensities (Wang & Ripley, 1997; Wu *et al.*, 2011). However, in our experiment, SWC was only significantly lower at the moderate grazing intensity (6.0 sheep/ha) (Fig. 2). Zhao *et al.* (2011) found that under sustainable sheep grazing treatments, SWC decreased when stocking rate was increased. In the same grazing management system, Shan *et al.* (2011) found the same

pattern of SWC and indicate that the highest grazing intensity (9.0 sheep/ha) used in these experiments were not sustainable within Inner Mongolian grasslands. However, the results from these studies should be interpreted with caution as they were only conducted over a three-month period. Our results suggest that there is not a linear relationship between decreasing SWC and increasing grazing intensity; indeed, SWC increased at the highest grazing intensity (9.0 sheep/ha) after it significantly decreased under 6.0 sheep/ha. This suggests that grazing >6.0 sheep/ha is unsustainable in the Inner Mongolian grasslands even though it continued for only three months every year. Using the same experimental plots as our study, He *et al.* (2011) found that soil physical attributes were changed at the 9.0 sheep/ha; sand content was higher and plant cover was lower under this grazing intensity than any other grazing treatment. Consequently, at the highest grazing intensity (9.0 sheep/ha), plant water consumption and soil surface evapotranspiration was lower than under the moderate grazing treatment (6.0 sheep/ha), resulting in greater soil moisture retention below 5cm (Chen *et al.*, 2004). In this study, SWC at the soil surface (0–10cm) was lower within the moderate grazing treatment.

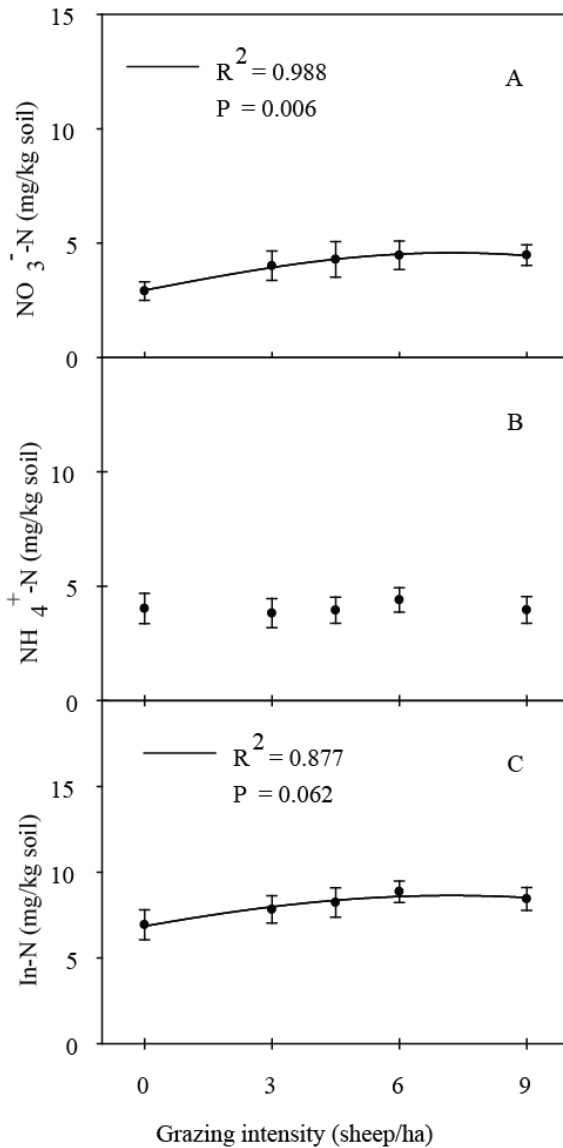


Fig. 4. Relationships of soil $\text{NO}_3^- \text{-N}$, $\text{NH}_4^+ \text{-N}$ and In-N concentrations with grazing intensities in 2009.

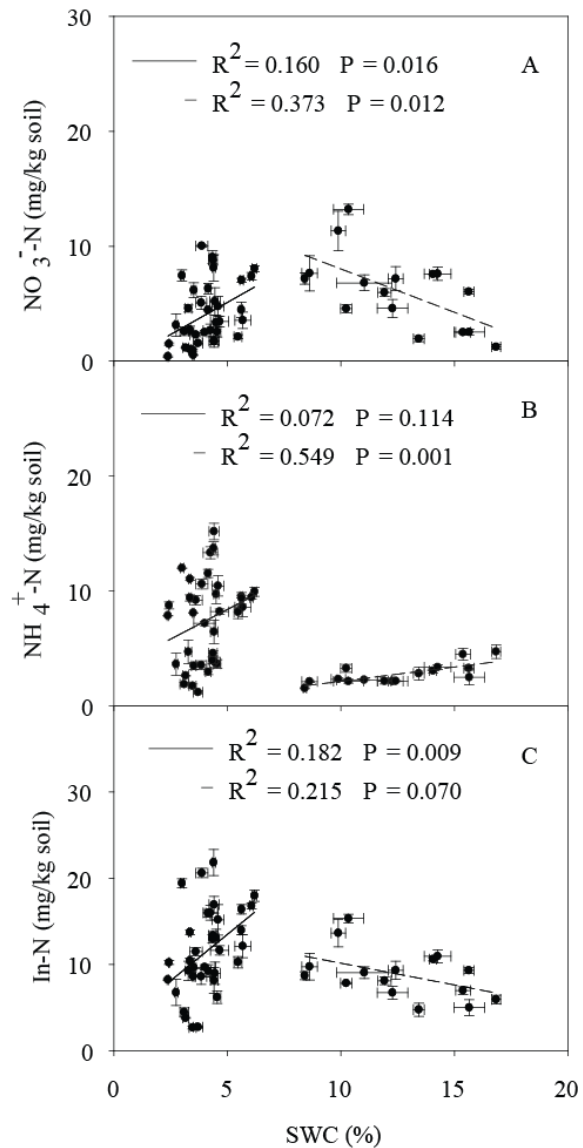


Fig. 5. Relationships of soil $\text{NO}_3^- \text{-N}$, $\text{NH}_4^+ \text{-N}$ and In-N concentrations with SWC (%).

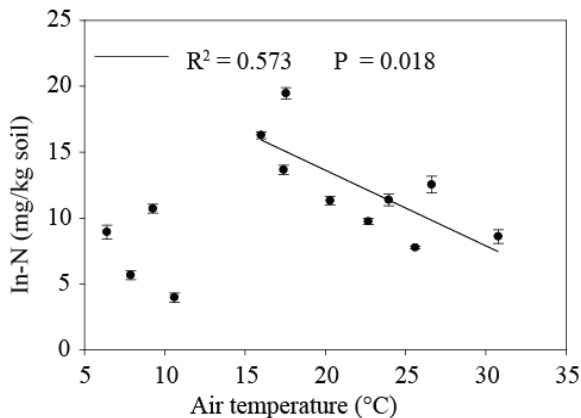


Fig. 6. The relationship between In-N concentrations and air temperature ($^{\circ}\text{C}$).

Effects of grazing on soil N availability: Increasing grazing intensity increased $\text{NO}_3^- \text{-N}$ accumulation in the upper 0–10 cm of soil (Fig. 4A). However, soil $\text{NH}_4^+ \text{-N}$ did not differ across grazing treatments (Fig. 4B). Soil In-N availability is the total concentration of $\text{NO}_3^- \text{-N}$ plus $\text{NH}_4^+ \text{-N}$. Grazing stimulated soil In-N availability at low and moderate grazing intensities (Fig. 4C), but then decreased under the highest grazing intensity (Fig. 4C). Grazing has different effects on soil $\text{NH}_4^+ \text{-N}$ and $\text{NO}_3^- \text{-N}$ due to their different transformation mechanisms. Using the same experimental treatment, Wu *et al.* (2011) found that the recovery ratio of ^{15}N in the $\text{NO}_3^- \text{-N}$ pool displayed a significant positive correlation with increasing grazing intensity, which may be explained by a reduction in SWC. Under certain circumstances (relative low SWC), soil $\text{NH}_4^+ \text{-N}$ can be used as an energy source by nitrifying bacteria, creating $\text{NO}_3^- \text{-N}$ (Schimel & Bennett, 2004). At lower SWC

levels, a reduction in N_2O reductase activity is expected, which in turn reduces N_2 emissions (Xu *et al.*, 2008). Moreover, grassland ecosystems may have developed unique functional roles among grazers, plants, and soil resources (such as N) after thousands of years of animal-plant-soil co-evolution (Milchunas *et al.*, 1988). Within Inner Mongolian grasslands soil NO_3^- -N may be the dominant source for plant uptake, with soil NH_4^+ -N being favored by microbes (Wu *et al.*, 2011). The uptake of N by plants from the soil causes a decline in soil N availability. In our grazing experiment, the above ground biomass of plants significantly decreased with increasing grazing intensity (Wan *et al.*, 2011). Therefore, high soil NO_3^- -N concentrations at high grazing intensities may be caused by a lower uptake of NO_3^- -N by plants. Grazing favors nitrate accumulation in the soil (Fig. 4A), which has a negative influence on the N cycles within grassland ecosystems (Wang *et al.*, 2010; Wu *et al.*, 2011).

Furthermore, grazing may influence soil N mineralization rates and N availability as a result of changes in N allocation, soil moisture, and temperature (Shan *et al.*, 2011). In our experimental system, net soil N mineralization rates diminished under increasing stocking rates in the 2008 and 2009 growing season (Shan *et al.*, 2011). This is probably due to the associated changes in SWC, soil temperature and above plant biomass under increasing grazing intensities. Furthermore, increasing grazing intensity led to a significant reduction in the number of plants, above ground plant biomass, amount of plant dead material, and area of plant cover that, in combination, protect the soil microenvironment (Li *et al.*, 2000; Schönbach *et al.*, 2011), since, reductions in these parameters significantly increase the area of bare soil (Schönbach *et al.*, 2011). In comparison to grazed plots, plants within ungrazed plots should achieve better growth, produce more root exudates, and develop a wider ramified root system (Gao *et al.*, 2008). In general, grazed grasslands have lower soil organic carbon and total soil N concentrations than those that are ungrazed (He *et al.*, 2011; Paz-Ferreiro *et al.*, 2011). Darrouzet-Nardi and Bowman (2011) suggested that the quantity of soil organic matter might be an important determinant of inorganic N because the concentration of soil organic matter was very important for soil N mineralization. Consequently, grazing management can directly and indirectly influence soil N availability.

Seasonal dynamics of the available soil N pool: In this study, the available soil N pool (i.e., soil inorganic N concentrations) showed significant seasonal dynamics (Table 1, Fig. 2, and Fig. 6), which may be directly or indirectly influenced by SWC (Fig. 5), temperature (Fig. 6), or the competitive effect of plant and microbe N uptake (Kahmen *et al.*, 2008). Xu *et al.* (2007) reported that the Inner Mongolia steppe generally maintains a lower level of N availability (2.6% total soil N). The increased uptake of available soil N by plants at higher temperatures combined with net soil N mineralization results in the production of more available N at higher

SWC (Wang *et al.*, 2006). Furthermore, higher SWC may increase soil enzyme activities in semiarid ecosystems. Generally, soil N availability is directly derived from N mineralization; therefore, the relationship of soil N transformation with temperature and SWC is complicated. SWC and dry-wet cycles have a stronger impact on soil N transformations in semiarid and arid ecosystems by controlling soil microbial activity and plant uptake capacity (Van Gestel *et al.*, 1993; Butterly *et al.*, 2011). Wang *et al.* (2006) reported that soil temperature and SWC are major controlling factors of N mineralization rates at our experimental site. Moreover, the input of different C:N ratios to substrates from plant aboveground senescence, root decomposition, and root excretion might cause variation in net N immobilization under high SWC conditions. Hence, labile C with high C:N ratios, in combination with suitable temperatures and SWC, may rapidly stimulate microbial growth and therefore increase microbial N biomass through the consumption of inorganic N (Luizão *et al.*, 1992).

Furthermore, the availability of degradable soil organic C (DOC) may partially explain seasonal patterns of soil N availability (Butterly *et al.*, 2011; Wu *et al.*, 2011). Soil DOC availability may influence both the consumption and production of soil NO_3^- -N. This is because soil DOC may change the competitive consequences of microbial NH_4^+ -N utilization and N immobilization capacity (Booth *et al.*, 2005). Austin *et al.* (2006) found that the majority of grassland species preferentially take up NO_3^- -N in the Patagonian steppe. Therefore, the species composition of the grass and microbial community may explain the seasonal and inter-annual differences of available soil N.

Grazing vs. rainfall and temperature: The effect of grazing on the available soil N pool varied across years (Table 2), due to the influence of climatic factors (Table 3, i.e., precipitation and temperature). Climatic factors actually had a larger impact on the dynamics of soil N availability than grazing (Tables 1, 2, and 3). Hence, low precipitation levels (183.3 mm, May to September) and frequencies in 2009 may explain the lower inorganic N availability compared to 2010, when precipitation levels were higher (215.0 mm, from May to September). This is only a difference of 30 mm, however, this is actually a substantial amount of the yearly precipitation in this region (approx. 10%). To some extent, SWC was directly influenced by precipitation and then modified by grazing. Some studies have demonstrated that changes in SWC, influenced by changes in the level and temporal distribution of rainfall, have a stronger impact on the available soil N pool than grazing (Xu *et al.*, 2007; Giese *et al.*, 2011). Similar results have also been reported in the semiarid grasslands of North-America (Biondini *et al.*, 1998) and the South African savanna (Stock *et al.*, 2010). Overall, grazing had less influence on the dynamics of soil N availability than SWC and temperature (Table 3). Soil water loss by plants should be reduced under higher grazing intensities due to the

removal of above-ground vegetation. Moreover, soil water loss by surface diffusion and evapotranspiration should be increased under higher grazing intensities due to the increase in bare soil (Schönbach *et al.*, 2011).

Abiotic factors also influence the seasonal dynamics of N availability (Figs. 5 and 6). The soil nitrogen mineralization experiment showed that soil temperature and SWC were significantly related with seasonal soil inorganic N concentrations. Soil temperature was directly influenced by air temperature (seasonal phenomena). SWC also was modified by rainfall. The climate within the inner Mongolian grasslands is typified by wet, warm summers and dry, cold winter, spring and autumn. Therefore, the seasonal influence of climate on soil temperature and SWC may directly affect available soil N. For example, the N pools within grasslands determine the relationship between biotic productivity and N availability (Biondini *et al.*, 1998). Seasonal dynamics in N availability, therefore, may have a significant influence on climatic factors, plant species composition and primary productivity dynamics (Augustine & McNaughton, 2004), with possible feedback mechanisms altering N cycles and C storage.

Conclusions

Grazing intensity is one of several important abiotic/biotic factors controlling N availability within the grasslands of the Inner Mongolian Steppe. Available soil N content is potential very important for sustainable plant growth. Sheep grazing influences available soil N due to trampling, decreases in above/belowground organic C input and root growth, and increased soil erosion. Our results suggest that available soil N in semiarid grasslands is primarily regulated by a combination of SWC (rainfall), temperature and grazing intensity. The collective effect of global warming, through changes in precipitation patterns and temperature, and the continual increase in grazing intensity make it important to determine sustainable N availability and N cycles to implement reasonable grazing intensities for sustainable grazing management in Inner Mongolian typical grassland.

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References

- Augustine, D. and S. McNaughton. 2004. Temporal asynchrony in soil nutrient dynamics and plant production in a semiarid ecosystem. *Ecosystems*, 7: 829-840.
- Austin, A., O. Sala and R. Jackson. 2006. Inhibition of nitrification alters carbon turnover in the Patagonian steppe. *Ecosystems*, 9: 1257-1265.
- Bardgett, R.D. and D.A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, 84: 2258-2268.
- Biondini, M.E., B.D. Patton and P.E. Nyren. 1998. Grazing intensity and ecosystem processes in a northern mixed-grass prairie, USA. *Ecol. Appl.*, 8: 469-479.
- Booth, M.S., J.M. Stark and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecol. Monogr.*, 75: 139-157.
- Burke, I.C., W.K. Lauenroth, M.A. Vinton, P.B. Hook, R.H. Kelly, H.E. Epstein, M.R. Aguiar, M.D. Robles, M.O. Aguilera, K.L. Murphy and R.A. Gill. 1998. Plant-soil Interactions in temperate grasslands. *Biogeochemistry*, 42: 121-143.
- Butterly, C., A. McNeill, J. Baldock and P. Marschner. 2011. Rapid changes in carbon and phosphorus after rewetting of dry soil. *Biol. Fertility Soils*, 47: 41-50.
- Chander, K., S. Goyal, M.C. Mundra and K.K. Kapoor. 1997. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. *Biol. Fertility Soils*, 24: 306-310.
- Chen, Y.J., M. Hong, S.L. Li and C.L. Qiao. 2004. Soil water regime under different plant coverage in Hunshandark sand land. *J. Arid Land Resour. Environ.*, 18: 68-73.
- Chen, Z.Z. (1988) Topography and climate of Xilin River Basin. In: Inner Mongolia Grassland Ecosystem Research Station AS eds. *Research on Grassland Ecosystem*. Science Press, Beijing. pp. 13-22.
- Darrouzet-Nardi, A. and W. Bowman. 2011. Hot spots of inorganic nitrogen availability in an Alpine-Subalpine ecosystem, Colorado Front Range. *Ecosystems*: 1-16.
- Davidson, E.A., P.A. Matson, P.M. Vitousek, R. Riley, K. Dunkin, G. Garcia-Mendez and J.M. Maass. 1993. Processes regulating soil emissions of NO and N₂O in a seasonally dry tropical forest. *Ecology*, 74: 130-139.
- Gao, Y.Z., M. Giese, S. Lin, B. Sattelmacher, Y. Zhao and H. Brueck. 2008. Belowground net primary productivity and biomass allocation of a grassland in Inner Mongolia is affected by grazing intensity. *Plant Soil*, 307: 41-50.
- Giese, M., Y.Z. Gao, S. Lin and H. Brueck. 2011. Nitrogen availability in a grazed semi-arid grassland is dominated by seasonal rainfall. *Plant Soil*, 340: 157-167.
- Harpole, W.S. and D. Tilman. 2007. Grassland species loss resulting from reduced niche dimension. *Nature*, 446: 791-793.
- He, N.P., Y.H. Zhang, Q. Yu, Q.S. Chen, Q.M. Pan, G.M. Zhang and X.G. Han. 2011. Grazing intensity impacts soil carbon and nitrogen storage of continental steppe. *Ecosphere*, 2: art8.
- Henry, H.A.L. and R.L. Jefferies. 2003. Plant amino acid uptake, soluble N turnover and microbial N capture in soils of a grazed Arctic salt marsh. *J. Ecol.*, 91: 627-636.
- Jarvis, S.C. and D. Barraclough. 1991. Variation in mineral nitrogen under grazed grassland swards. *Plant Soil*, 138: 177-188.
- Jiang, Y., X.I. Bi, J.H. Huang and Y.F. Bai. 2011. Patterns and drivers of vegetation degradation in Xilin River Basin, Inner Mongolia, China. *Chinese J. Plant Ecol.*, 34: 1132-1141.

- Kahmen, A., W. Wanek and N. Buchmann. 2008. Foliar $\delta^{15}\text{N}$ values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia*, 156: 861-870.
- Lavado, R.S., J.O. Sierra and P.N. Hashimoto. 1996. Impact of grazing on soil nutrients in a Pampean grassland. *J. Range Manage.*, 49: 452-457.
- Li, S.G., Y. Harazono, T. Oikawa, H.L. Zhao, Z.Y. He and X.L. Chang. 2000. Grassland desertification by grazing and the resulting micrometeorological changes in Inner Mongolia. *Agric. For. Meteorol.*, 102: 125-137.
- Luizão, R.C.C., T.A. Bonde and T. Rosswall. 1992. Seasonal variation of soil microbial biomass--The effects of clearfelling a tropical rainforest and establishment of pasture in the central Amazon. *Soil Biol. Biochem.*, 24: 805-813.
- Luo, Y.Q., B. Su, W.S. Currie, J.S. Dukes, A. Finzi, U. Hartwig, B. Hungate, R.E. Mc Murtrie, R. Oren, W.J. Parton, D.E. Pataki, M.R. Shaw, D.R. Zak and C.B. Field. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience*, 54: 731-739.
- Milchunas, D.G., O.E. Sala and W.K. Lauenroth. 1988. A Generalized Model of the Effects of Grazing by Large Herbivores on Grassland Community Structure. *Am. Nat.*, 132: 87-106.
- Pan, Q.M., Y.F. Bai, J.G. Wu and X.G. Han. 2011. Hierarchical plant responses and diversity loss after nitrogen addition: testing three functionally-based hypotheses in the Inner Mongolia grassland. *PLoS One*, 6: e20078.
- Paz-Ferreiro, J., C. Trasar-Cepeda, M. Leirós, S. Seoane and F. Gil-Sotres. 2011. Intra-annual variation in biochemical properties and the biochemical equilibrium of different grassland soils under contrasting management and climate. *Biol. Fertility Soils*, 47: 1-13.
- Rao, L.E., D.R. Parker, A. Bytnerowicz and E.B. Allen. 2009. Nitrogen mineralization across an atmospheric nitrogen deposition gradient in Southern California deserts. *J. Arid Environ.*, 73: 920-930.
- Schimel, J.P. and Bennett, J., 2004. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, 85: 591-602.
- Schönbach, P., H.W. Wan, A. Schiborra, M. Gierus, Y.F. Bai, K. Müller, T. Glindemann, C. Wang, A. Susenbeth and F. Taube. 2009. Short-term management and stocking rate effects of grazing sheep on herbage quality and productivity of Inner Mongolia steppe. *Crop and Pasture Science*, 60: 963-974.
- Schönbach, P., H.W. Wan, M. Gierus, Y.F. Bai, K. Müller, L.J. Lin, A. Susenbeth and F. Taube. 2011. Grassland responses to grazing: effects of grazing intensity and management system in an Inner Mongolian steppe ecosystem. *Plant Soil*, 340: 103-115.
- Seagle, S.W., S.J. McNaughton and R.W. Ruess. 1992. Simulated effects of grazing on soil-nitrogen and mineralization in contrasting serengeti grasslands. *Ecology*, 73: 1105-1123.
- Shan, Y.M., D.M. Chen, X.X. Guan, S.X. Zheng, H.J. Chen, M.J. Wang and Y.F. Bai. 2011. Seasonally dependent impacts of grazing on soil nitrogen mineralization and linkages to ecosystem functioning in Inner Mongolia grassland. *Soil Biol. Biochem.*, 43: 1943-1954.
- Steffens, M., A. Kölbl, K.U. Totsche and I. Kögel-Knabner. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). *Geoderma*, 143: 63-72.
- Stevens, C.J., N.B. Dise and D.J. Gowing. 2009. Regional trends in soil acidification and exchangeable metal concentrations in relation to acid deposition rates. *Environ. Pollut.*, 157: 313-319.
- Stock, W., W. Bond and C. van de Vijver. 2010. Herbivore and nutrient control of lawn and bunch grass distributions in a southern African savanna. *Plant Ecol.*, 206: 15-27.
- Teague, W.R., S.L. Dowhower, S.A. Baker, N. Haile, P.B. DeLaune and D.M. Conover. 2011. Grazing management impacts on vegetation, soil biota and soil chemical, physical and hydrological properties in tall grass prairie. *Agric. Ecosyst. Environ.*, 141: 310-322.
- Tilman, D. and H. Olf. 1991. An experimental-study of the effects of pH and nitrogen on grassland vegetation. *Acta Oecologica-International J. Ecol.*, 12: 427-441.
- Van Gestel, M., R. Merckx and K. Vlassak. 1993. Microbial biomass responses to soil drying and rewetting: The fate of fast- and slow-growing microorganisms in soils from different climates. *Soil Biol. Biochem.*, 25: 109-123.
- Verhagen, F.J.M., P.E.J. Hageman, J.W. Woldendorp and H.J. Laanbroek. 1994. Competition for ammonium between nitrifying bacteria and plant-roots in soil in pots - effects of grazing by flagellates and fertilization. *Soil Biol. Biochem.*, 26: 89-96.
- Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. *Am. Nat.*, 119: 553-572.
- Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger and G.D. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Appl.*, 7: 737-750.
- Wan, H.W., Y.F. Bai, P. Schönbach, M. Gierus and F. Taube. 2011. Effects of grazing management system on plant community structure and functioning in a semiarid steppe: scaling from species to community. *Plant Soil*, 340: 215-226.
- Wang, C.H., S.Q. Wan, X.R. Xing, L. Zhang and X.G. Han. 2006. Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biol. Biochem.*, 38: 1101-1110.
- Wang, C.H., X.G. Han and X.R. Xing. 2010. Effects of grazing exclusion on soil net nitrogen mineralization and nitrogen availability in a temperate steppe in northern China. *J. Arid Environ.*, 74: 1287-1293.
- Wang, J. and T.G.T. Baoyin. 2005. The study on characteristics of biomass composition of natural leymus chinensis steppe on a deteriorated series in Neimongol. *Acta Scientiarum Naturalium Universitatis Neimongol*, 36: 155-160.
- Wang, J.W. and Y.C. Cai. 1988. Studies on genesis, types and characteristics of the soils of the Xilin River Basin. In: Inner Mongolia Grassland Ecosystem Research Station AS eds. *Research on Grassland Ecosystem*. Science Press, Beijing. pp. 23-83.
- Wang, R.Z. and E.A. Ripley. 1997. Effects of grazing on a Leymus chinensis grassland on the Songnen plain of north-eastern China. *Journal of Arid Environments*, 36: 307-318.
- Wolf, B., X.H. Zheng, N. Brüggemann, W.W. Chen, M. Dannenmann, X.G. Han, M.A. Sutton, H.H. Wu, Z.S. Yao and K. Butterbach-Bahl. 2010. Grazing-induced reduction of natural nitrous oxide release from continental steppe. *Nature*, 464: 881-884.
- Wu, H.H., M. Dannenmann, N. Fanselow, B. Wolf, Z.S. Yao, X. Wu, N. Brüggemann, X.H. Zheng, X.G. Han, K. Dittert and K. Butterbach-Bahl. 2011. Feedback of grazing on gross rates of N mineralization and inorganic N partitioning in steppe soils of Inner Mongolia. *Plant Soil*, 340: 127-139.
- Xu, Y.Q., L.H. Li, Q.B. Wang, Q.S. Chen and W.X. Cheng. 2007. The pattern between nitrogen mineralization and grazing intensities in an Inner Mongolian typical steppe. *Plant Soil*, 300: 289-300.

- Xu, Y.Q., S.Q. Wan, W.X. Cheng and L.H. Li. 2008. Impacts of grazing intensity on denitrification and N₂O production in a semi-arid grassland ecosystem. *Biogeochemistry*, 88: 103-115.
- Yuan, Z.Y., L.H. Li, X.G. Han, S.P. Chen, Z.W. Wang, Q.S. Chen and W.M. Bai. 2006. Nitrogen response efficiency increased monotonically with decreasing soil resource availability: a case study from a semiarid grassland in northern China. *Oecologia*, 148: 564-572.
- Zhang, X.M., W. Liu, Y.F. Bai, G.M. Zhang and X.G. Han. 2011. Nitrogen deposition mediates the effects and importance of chance in changing biodiversity. *Mol. Ecol.*, 20: 429-438.
- Zhang, Y.H., J.C. Feng, F. Isbell, X.T. Lü and X.G. Han. 2015. Productivity depends more on the rate than the frequency of N addition in a temperate grassland. *Sci. Rep.*, 5: 12558.
- Zhang, Y.H., N.P. He, G.M. Zhang, J.H. Huang, Q.B. Wang, Q.M. Pan and X.G. Han. 2013. Ammonia emissions from soil under sheep grazing in Inner Mongolian grasslands of China. *J. Arid Land*, 5: 155-165.
- Zhang, Y.H., X.T. Lü, F. Isbell, C. Stevens, X. Han, N.P. He, G.M. Zhang, Q. Yu, J.H. Huang and X.G. Han. 2014. Rapid plant species loss at high rates and at low frequency of N addition in temperate steppe. *Global Change Biol.*, 20: 3520-3529.
- Zhao, Y., S. Peth, A. Reszkowska, L. Gan, J. Krümmelbein, X. Peng and R. Horn. 2011. Response of soil moisture and temperature to grazing intensity in a *Leymus chinensis* steppe, Inner Mongolia. *Plant Soil*, 340: 89-102.

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