

Impacts of grazing intensity on denitrification and N₂O production in a semi-arid grassland ecosystem

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Abstract N₂O production from denitrification in soils contributes to the enhanced greenhouse effect and the destruction of the stratospheric ozone. Ungulate grazing affects denitrification and the production of N₂O. The short-term effect of grazing on denitrification and N₂O production has been examined in several grassland ecosystems. However, the effects of long-term grazing have rarely been studied. We measured denitrification and N₂O production during the 2005 and 2006 growing seasons in a long-term (17 years) experiment that had five grazing intensities (GI; 0.00, 1.33, 2.67, 4.00 and 5.33 sheep ha⁻¹). We found that denitrification and N₂O production rates were seasonally variable during the measurement period, with higher values observed in summer and lower values found in spring and autumn. The grazed treatments resulted in decreased denitrification and N₂O production, primarily due to

the reduced soil nitrate concentration and organic N content under the long-term grazing. This supported our hypothesis that long-term over-grazing suppresses denitrification and N₂O production. Although significant differences in denitrification and N₂O production were not found between the four GI, there was a general trend that cumulative denitrification and N₂O production decreased as grazing intensity increased, especially in 2006. Lower N losses via denitrification and N₂O production in the grazed plots, to some extent, may contribute to the mitigation of greenhouse gas emission and help to preserve soil N and ameliorate the negative impacts of grazing on plant growth, productivity, and ecological restoration processes in the temperate steppe in northern China.

Keywords Denitrification · N₂O production · Grazing intensity · Temperate steppe

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Introduction

Nitrogen (N) has long been recognized as the most limiting nutrient for plant growth and net primary productivity in terrestrial ecosystems. Nitrous oxide (N₂O) emissions from denitrification and other sources cause the destruction of the ozone layer in the stratosphere (Cicerone 1987). N₂O is also a potent greenhouse gas which contributes to global climate

change (Finlayson-Pitts and Pitts 2000). Denitrification is the main process responsible for N losses as N_2O and N_2 from natural ecosystems (Oenema et al. 1998). As a microbe-mediated process, denitrification is affected by temporal fluctuations of environmental factors as well as natural and/or anthropogenic perturbations. Denitrification occurs in semi-arid, arid and desert ecosystems (Peterjohn and Schlesinger 1990), and it is not necessarily lower in these water-limited ecosystems than in other ecosystems (Peterjohn 1991; Groffman et al. 1993). Frequent drying-wetting cycles in arid ecosystems may account for the high denitrification rates (Peterjohn and Schlesinger 1990), since freshly wetted soils often have high C and N availability and that is linked to high denitrification rates (Peterjohn and Schlesinger 1991). Soil moisture, as influenced by rainfall and evaporation rates, is a key factor in regulating the spatial and temporal patterns of denitrification in various terrestrial ecosystems (Skiba et al. 1993; Pinay et al. 2007). In addition, with its impact on almost all physical, chemical, and biological activities, temperature could also affect temporal variability in denitrification. Furthermore, organic carbon (C) is used as an energy source in denitrification process (Koops et al. 1997), and therefore, the rate of denitrification is often linked to C availability (Frank and Groffman 1998; Hamilton and Frank 2001). The magnitude and rate of denitrification is also controlled by the availability of N substrate. e.g., soil nitrate and organic N.

Grazing, as a common land-use type in grassland ecosystems, can profoundly impact denitrification in these grassland ecosystems (Frank and Groffman 1998; Frank et al. 2000; Le Roux et al. 2006). Denitrification is often stimulated by grazing because animal excreta enhance soil carbon availability (Saggar et al. 2004). Ungulate trampling also intensify soil compaction and generate anaerobic conditions that are favorable for denitrification, particularly when soil water content is high (Menneer et al. 2005). Furthermore, ungulates reduce plant N uptake by defoliation and increase soil nitrate concentration, which often results in accelerated gaseous N emissions (Ruz-Jerez et al. 1994; Luo et al. 1999). Consequently, denitrification often increases in response to grazing in grassland ecosystems. However, long-term over-grazing may cause ecosystem N depletion due to obsessive N removal and soil

destruction. If N removed from long-term over-grazing is greater than the amount returned from animal excreta, soil nitrate N concentrations may decline, which may suppress the potential for gaseous N emissions. We hypothesize that long-term over-grazing will result in extra losses of nitrate, and thus reduce denitrification and N_2O production.

The temperate steppe in northern China is one of the regional vegetation types across the vast area of the Eurasian Continent and has been affected by grazing over thousands of years. However, rapid population increases coupled with poor management over the late half of the twentieth century have left the grasslands of the Inner Mongolia faced with severe degradation and desertification (Li et al. 2000; Kang et al. 2007). Hence, since the beginning of twenty first century, the Chinese government has put great effort to restore the temperate grassland by periodical exclusion of grazing. Understanding the quantitative relationship between grazing intensity and N losses in these grassland ecosystems is critical for adaptive management and restoration. In comparison with the well studied stimulating impacts of short-term grazing on denitrification and N_2O production in grassland ecosystems (De Klein and van Lotestijn 1994; Menneer et al. 2005), the potential carry-over suppressing impacts of long-term grazing activities on denitrification and N_2O production are poorly characterized. This study examines potential carry-over suppressing effects of long-term grazing at varying levels of intensities on denitrification and N_2O production in the temperate steppe in northern China. Our specific objectives were: (1) to determine the seasonal patterns of denitrification and N_2O production, (2) to explore the effects of grazing intensity on denitrification and N_2O production, and (3) to investigate the underlying processes that regulate denitrification and N_2O production in soils affected by grazing in this temperate steppe.

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 (43°50' N, 116°34' E, and 1,100 m above the sea level). Mean annual precipitation is 350 mm, with most rain events occurring in July and August. Average annual air temperature is -4°C . Vegetation is characterized as typical steppe, dominated by *Artemisia frigida*, *Kochia prostrata* and *Potentilla acaulis*. The soil at the experimental site is a sandy loam (73% sand, 15% silt, and 9% clay) (Barger et al. 2004).

Grazing treatments started on May 20th and ended on October 5th every year between 1989 and 2005. 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 (GI) of 0, 1.33, 2.67, 4.00 and 5.33 sheep ha^{-1} , respectively. 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. We chose these GI based on the traditional grazing guideline for proper land-use (Biondini et al. 1998). The moderate grazing intensity (4.00 sheep ha^{-1}) represents about 50% removal of the aboveground biomass (AGB) in a year with an average precipitation. In local practice, the light grazing intensity (1.33 sheep ha^{-1}) represents about 22% removal of the AGB. More than 64% removal of the AGB means over-grazing. In order to investigate the natural recovering process from grazing impacts, all grazing treatments ceased since 2006.

Soil sampling and incubation

During the 2005 and 2006 growing seasons, the rate of denitrification was measured using the acetylene inhibition technique (Yoshinari et al. 1977), by incubating minimally disturbed soil cores in a closed system under field conditions as described by Ryden et al. (1987). We sampled periodically, approximately every 15 days in warm and wet months of July and August in 2005 or every 30 days in other months. Within each replicated plot, seven 2×2 m quadrats were randomly demarcated and enclosed at the first sampling date in 2005 and used for later repeated soil sampling. Accordingly, seven paired PVC tubes, 3.2 cm in diameter and 10 cm in length, were driven into the top 10 cm soil layer for soil

sampling in the seven quadrates of each plot. Each of the seven pairs was divided and placed separately into two 1.2 l glass jars. Each jar, containing seven soil cores from each plot, was sealed with a tin lid which was fitted with a rubber gasket and incorporated with a rubber septum stopper for gas sampling from the headspace. To ensure a gas-tight closure, the circumambiences of rubber septum stoppers were coated with petroleum jelly and the edges of the lids were sealed with parafilm (plastic packaging). The paired jars were then divided into two groups. In one group, a 60-cm^3 air was drawn out from each jar at 0 h, and immediately, the same volume of acetylene was injected to produce a concentration of $\sim 10\%$ (v/v) in the remaining air space. In another group, no air was drawn out, and no acetylene was added. All the jars were placed together in order in a hole (at a depth of 10 cm) of slightly larger dimensions in the ground of an aisle between two experimental plots. These jars were covered with grass to minimize temperature differences between the soils inside the jars and the undisturbed soils during the incubation. Gas samples (10 ml) were withdrawn with gas-tight syringes after incubation. All the gas samples drawn out at 0 and 24 h were analyzed with a gas chromatograph (GC) within 24 h.

Measurement and calculation of N_2O production

N_2O concentrations of the gas samples were measured with an ECD detector in a GC (Hewlett Packard 5890 Series II). This GC was equipped with a 2-m stainless steel column and a detector whose temperatures were set to 55 and 330°C , respectively. Carrier gas was N_2 with a flow rate of 30 ml min^{-1} . Each gas sample analysis was completed within 4.05 min. A standard concentration of N_2O in N_2 gas was used to calibrate the chromatograph for N_2O concentrations ($\mu\text{l l}^{-1}$). The calculation method of N_2O mass was similar to those reported by Luo et al. (1999). Briefly, given a gas density of N_2O at 20°C of $1.83 \times 10^{-6}\text{ g N}_2\text{O } \mu\text{l}^{-1}$, the mass concentration of N_2O (g l^{-1}) in the headspace of the incubation jars could be calculated. Knowing the headspace volume, and considering the dissolution of N_2O in solution by using a Bunsen coefficient of 0.632 at 20°C (Tiedje 1982), the mass of N_2O was calculated using the following equation:

$$\text{N}_2\text{O}-\text{N}(\text{g}) = 1.83 \times 10^{-6} (\text{gN}_2\text{O}\mu\text{l}^{-1}) \times \text{N}_2\text{O}(\mu\text{l}\text{l}^{-1}) \\ \times [V_{\text{headspace}}(\text{l}) + V_{\text{soil solution}}(\text{l}) \times 0.632]$$

Results from samples incubated with 10% acetylene corresponded to total denitrification. N_2O production was estimated directly from samples incubated without acetylene. The differences in N_2O mass concentrations between 0 and 24 h were the daily production rates. Cumulative denitrification or N_2O production was derived by summing the amount over all the sampling intervals during the growing season. The amount at each sampling interval was calculated by multiplying the daily rate, estimated as the mean value of the two measurements spanning the interval, by the number of days of this interval (this estimation was using the method reported by Frank and Groffman (1998)).

Measurements of soil and environmental factors

After the headspace of each jar was sampled in the field, the soil cores contained in jars were transported to the laboratory for further analysis. The incubated soils in each jar without adding acetylene were thoroughly mixed and sieved through a 2 mm mesh. A 10-g sieved fresh soil subsample was extracted with 50 ml 2 M KCl on a rotary shaker for 1 h. Soil extracts were filtered and the filtered solutions were analyzed by the flow injection autoanalyser to determine soil nitrate concentrations.

Soil moisture was determined gravimetrically by drying fresh soil subsamples at 105°C for 24 h. Soil temperature at 10 cm depth in each replicate plot was measured in a 28-day pre-experiment. No significant differences in daily mean soil temperature were found between treatment plots. Accordingly, soil temperature only at one site was measured during the experimental period.

The air-dried soils were used for measuring pH (water:soil = 2.5:1), organic C content (H_2SO_4 - $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation method) and organic N content (Kjeldahl digestion method). Soil bulk density was determined using a core method. Peak plant AGB was determined by clipping and oven-drying plants at 70°C for 48 h, and then weighing dry materials.

Statistical analysis

The differences in denitrification and N_2O production rates between different GI for each growing season were analyzed with repeated measures ANOVA using a generalized linear model. Grazing intensity and season (sampling date) were the main effects. For the comparison of the differences in denitrification and N_2O production between different GI at each sampling time or at the annual time scale, one-way ANOVA was carried out. Duncan multiple range test was used to determine significance of differences.

Pearson correlation and stepwise multiple linear regression analyses were conducted to look for relationships of variations in denitrification and N_2O production across a seasonal range and a grazing intensity gradient with the relevant controlling factors. A level of 0.05 was used as a standard of significance for all analyses of variance, correlation and regression. All statistical analyses were performed using SPSS version 13.0 software package.

Results

Environmental conditions, plant biomass, and soil properties

In comparison with the 50-year average (350 mm) of mean annual precipitation, both year 2005 and 2006 had lower annual precipitation. However, year 2005 (annual precipitation 166 mm) was much drier than year 2006 (annual precipitation 304 mm). Soil temperature ranged from 10.1 to 27.5°C and peaked in summer (Fig. 1a). Gravimetric soil moistures were very low with peak values less than 16% and showed similar seasonal patterns under different GI, i.e., peaked in summer (Fig. 1b). However, due to the limited sampling dates, mean soil moisture was not necessarily higher in 2006 than in 2005. For example, the highest soil moisture observed on July 4 of 2005 was caused by a rainfall of 9.6 mm in the previous day. Grazing treatments marginally impacted soil moistures in 2005 ($P = 0.054$, repeated measures ANOVA) but had no effect in 2006 ($P = 0.462$). When averaged over the growing season, mean soil moistures in all the four grazed treatments in 2005 were significantly lower than that in the ungrazed

treatment ($P < 0.05$). No significant differences in mean soil moisture in 2006 were detected between all five grazing treatments irrespective of the slightly lower soil moistures in the four grazed treatments than in the ungrazed treatment.

Soil nitrate N concentrations showed different seasonal patterns compared to soil temperature and soil moisture (Fig. 1). In addition, GI significantly impacted soil nitrate N concentrations in both 2005 ($P < 0.001$, repeated measures ANOVA) and 2006 ($P < 0.05$). Annual mean soil nitrate N concentrations in the four grazed treatments were significantly lower than that in the ungrazed treatment in both 2005 ($P < 0.001$) and 2006 ($P < 0.05$). However, there were no differences in soil nitrate N

concentrations between the four grazed treatments in either year ($P > 0.05$; Fig. 1c).

Long-term grazing caused general declines in AGB, soil organic C and N with increasing grazing intensity even though the differences between all five treatments were not statistically significant ($P > 0.05$; Table 1). By contrast, soil bulk density in the four grazed treatments was markedly higher than that in the ungrazed treatment ($P < 0.05$).

Dynamics of denitrification and N_2O production

There were strong seasonal fluctuations in both denitrification and N_2O production in 2005 and 2006 ($P < 0.001$; Table 2). Relatively high denitrification and N_2O production rates were observed during the summer season. Denitrification rates ranged from 0.33 to 6.21 (averaged 2.50) $ng\ N_2O-N\ g^{-1}\ dry\ soil\ d^{-1}$, equivalent to 0.48–7.64 $g\ N_2O-N\ ha^{-1}\ d^{-1}$. N_2O production rates ranged from 0.42 to 11.28 (averaged 2.88) $ng\ N\ g^{-1}\ dry\ soil\ d^{-1}$, equivalent to 0.59–16.02 $g\ N\ ha^{-1}\ d^{-1}$ (Fig. 2).

Results of repeated measures of ANOVA revealed that GI had no significant effects ($P > 0.05$) on denitrification in both years. However, significant impacts of grazing on N_2O production were observed in 2005 ($P < 0.01$; Table 2). Nevertheless, denitrification and N_2O production rates tended to be higher in the ungrazed treatment than in the four grazed treatments (Fig. 2). In 2005, the ungrazed treatment had significantly higher N_2O production than the four grazed treatments, mainly in rainy and warm July ($P < 0.05$ for July 4; $P < 0.01$ for July 18, Fig. 2). In 2006, the ungrazed treatment had significantly higher denitrification rates than the highest grazing intensity of 5.33 sheep ha^{-1} ($P < 0.05$). Whereas there were no significant differences in denitrification and N_2O production rates between the four grazed treatments ($P > 0.05$; Fig. 2).

Across all sampling dates and treatments (i.e., the averages of each treatment were used multiplied by the number of sampling dates), denitrification rates showed significant positive correlations with N_2O production rates in both 2005 ($r^2 = 0.384$, $P < 0.001$, $n = 40$) and 2006 ($r^2 = 0.934$, $P < 0.001$, $n = 30$). There was a strong seasonal variability in N_2O production:denitrification ratios in 2005 ($P < 0.001$), but not in 2006 ($P > 0.05$; Table 2). Across the two growing seasons, the ratios ranged

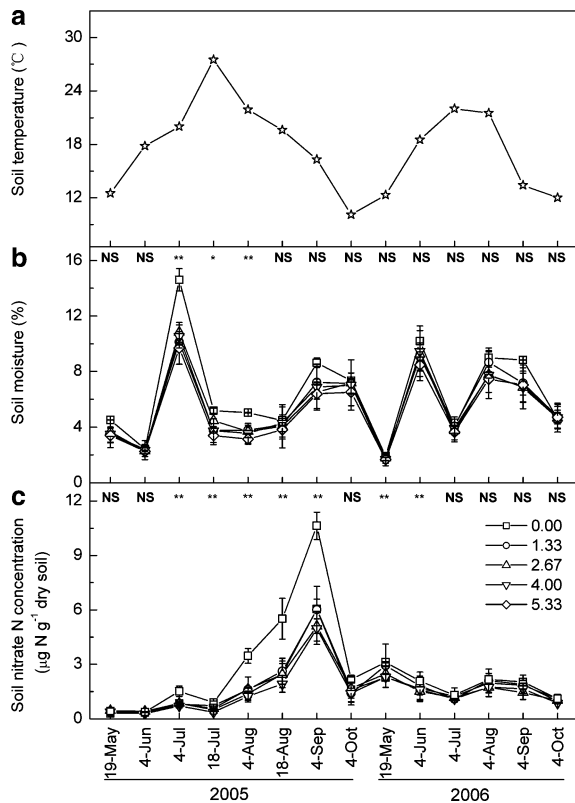


Fig. 1 Soil temperature ($^{\circ}C$, **a**), percentage gravimetric soil moisture ($g\ H_2O\ 100\ g^{-1}\ dry\ soil$, **b**), and soil nitrate N concentration ($\mu g\ N\ g^{-1}\ dry\ soil\ d^{-1}$, **c**) in the top 10 cm soil layer during the 2005 and 2006 growing seasons. Each point is the mean from three replicated plots. Error bars represent ± 1 SE. * indicates $P < 0.05$; ** indicates $P < 0.01$; NS indicates being not significant ($P > 0.05$). 0.00, 1.33, 2.67, 4.00 and 5.33 (sheep ha^{-1}) denote that 0, 4, 8, 12 and 16 sheep grazed rotationally in three replicated 1-ha plots, respectively.

Table 1 Peak plant AGB and soil properties under different GI in 2006

GI (sheep ha ⁻¹)	0.00	1.33	2.67	4.00	5.33
AGB (g m ⁻²)	132.54 (15.11)	112.65 (9.24)	109.03 (3.02)	113.98 (15.77)	105.57 (8.09)
Soil bulk density (g cm ⁻³)	1.23 (0.05) ^b	1.35 (0.02) ^a	1.36 (0.04) ^a	1.33 (0.04) ^a	1.40 (0.03) ^a
Soil organic C (g C kg ⁻¹)	17.19 (0.71)	15.92 (1.20)	14.91 (3.52)	14.92 (4.52)	13.73 (2.50)
Soil organic N (g N kg ⁻¹)	0.82 (0.01)	0.76 (0.09)	0.73 (0.10)	0.73 (0.19)	0.69 (0.13)

Values in parenthesis are standard errors. Numbers within rows followed by different letters in superscript are significantly different at $P < 0.05$ level

Table 2 Result of F -test based on repeated measures ANOVA for the effects of GI on denitrification rates, N₂O production rates, and N₂O production:denitrification ratios during the 2005 and 2006 growing seasons.

	GI		Season		GI × Season	
	F	P	F	P	F	P
Denitrification (2005)	1.169	0.381	106.141	0.000	1.470	0.212
Denitrification (2006)	1.604	0.248	225.103	0.000	1.641	0.135
N ₂ O production (2005)	11.261	0.001	99.587	0.000	1.328	0.281
N ₂ O production (2006)	0.870	0.515	295.405	0.000	0.963	0.533
N ₂ O:denitrification ratio (2005)	2.321	0.128	15.759	0.009	1.584	0.170
N ₂ O:denitrification ratio (2006)	0.401	0.804	1.922	0.225	0.561	0.899

from 0.40 to 4.82. N₂O production:denitrification >1 was found, primarily in May and July of 2005 and in July of 2006 (Fig. 2, lowest panels). However, no effects of GI on the ratios were observed in either year ($P > 0.05$; Table 2).

There were inter-annual variations in cumulative denitrification and N₂O production between the 2 years. Cumulative denitrification ($P < 0.001$) and N₂O production ($P < 0.01$) in 2006 were significantly greater than those in 2005. Cumulative denitrification and N₂O production generally decreased with increasing grazing intensity (Fig. 3). This trend was significant for denitrification and N₂O production in 2006 ($r^2 = 0.845$, $P < 0.05$; $r^2 = 0.880$, $P < 0.05$, $n = 5$, respectively). In 2005, cumulative N₂O production was significantly lower in the four grazed treatments than in the ungrazed treatment. In 2006, significant differences in cumulative denitrification were found between the ungrazed treatment and the highest grazing intensity of 5.33 sheep ha⁻¹ (Fig. 3).

The annual mean value of N₂O production:denitrification ratios range was higher in 2005 (1.33) than in 2006 (0.87), especially in the ungrazed treatment and the grazing intensity of 2.67 sheep ha⁻¹ (Fig. 3, lowest panels). GI significantly affected the mean

ratios in 2005 ($P < 0.001$), but not in 2006 ($P > 0.05$, Fig. 3). In 2005, the GI of 1.33, 4.00, and 5.33 sheep ha⁻¹ had lower N₂O production:denitrification ratios than the ungrazed treatment and the grazing intensity of 2.67 sheep ha⁻¹.

The main regulators for denitrification and N₂O production

Using the average values from the three replicated ungrazed plots, results from correlation analysis indicated that, in 2005, soil moisture explained 71.6% of the seasonal variability in denitrification ($P < 0.05$, $n = 8$), and soil temperature explained 54.5% of the seasonal variability in N₂O production ($P < 0.05$, $n = 8$). When stepwise multiple regressions were applied, both soil water content and temperature were significant independent variables for denitrification ($P < 0.05$ was the criteria to be included), while only soil temperature was a significant independent variables for N₂O production ($P < 0.05$). In 2006, denitrification rates only showed a marginal positive relationship ($r^2 = 0.65$, $P = 0.053$, $n = 6$) with soil temperature, and none of the three parameters exhibited any significant correlation with either denitrification or N₂O

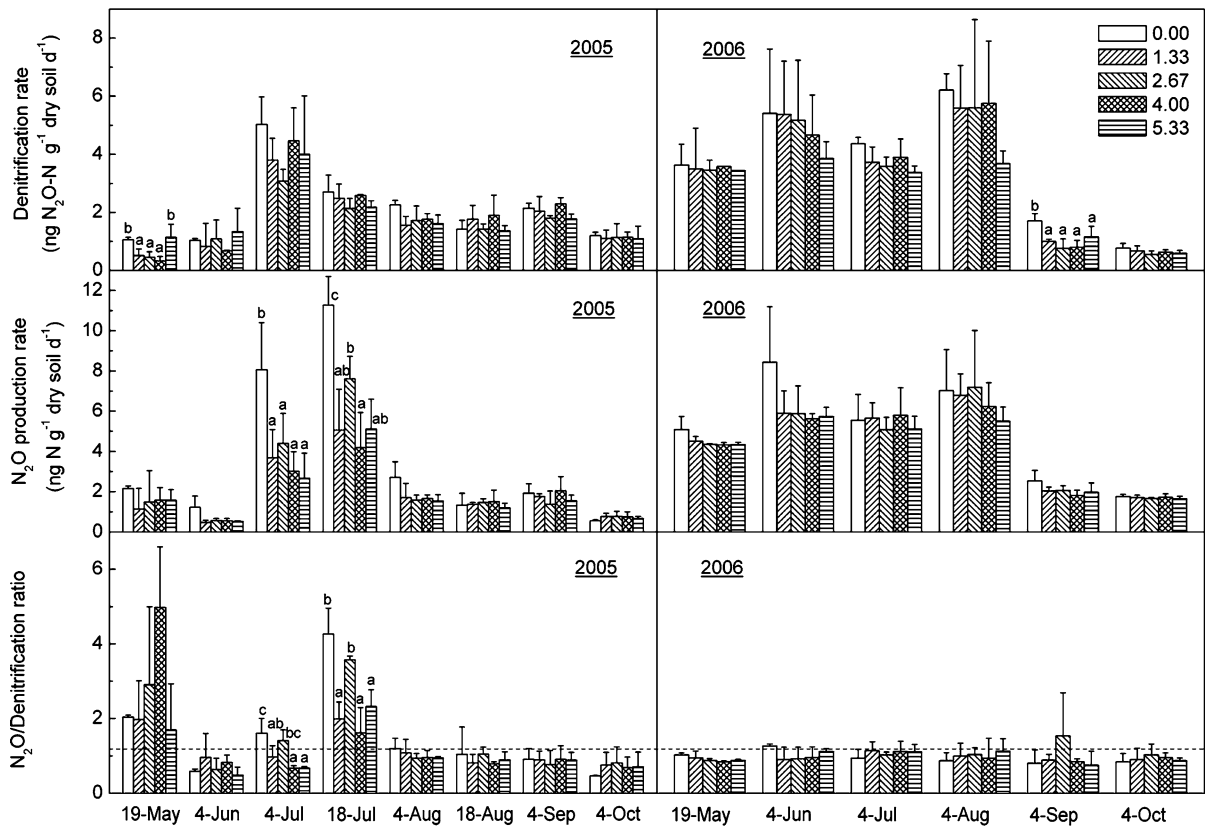


Fig. 2 Rates of denitrification and N_2O production (ng N g^{-1} dry soil d^{-1}), and N_2O production:denitrification ratios under different GI during the 2005 and 2006 growing seasons. Each column is the mean from three replicated plots.

Error bars represent ± 1 SE. Treatments with different letters are significantly different at $P < 0.05$ level. See Fig. 1 for grazing intensity abbreviations

production rates ($P > 0.05$). Across the 14 sampling dates over the two growing seasons, there was a positive linear relationship between N_2O production and soil temperature only (Fig. 4). Across the two growing seasons, the N_2O production:denitrification ratios in the ungrazed plots also showed a positive linear correlation with soil temperature ($r^2 = 0.32$, $P < 0.05$, $n = 14$).

In order to examine whether denitrification and N_2O production are related to the biotic and abiotic factors across a grazing intensity gradient, we plotted variations in cumulative denitrification and N_2O production across the 15 plots in 2005 and 2006 against annual mean soil moisture and nitrate N concentrations, soil organic N, soil bulk density, and total plant AGB. In 2005, none of the above five parameters showed any relationship with cumulative

denitrification ($P > 0.05$), whereas soil moisture and soil nitrate N concentrations explained 53.2% ($P < 0.01$) and 62.7% ($P < 0.001$) of the variability in cumulative N_2O production across the 15 plots, respectively. In addition, soil bulk density showed a marginal negative relationship ($r^2 = 0.23$, $P = 0.073$) with cumulative N_2O production in 2005. When stepwise multiple regressions were applied, only soil nitrate N concentration was a significant variable ($P < 0.05$). In 2006, soil nitrate N concentration and soil bulk density explained 50.1% ($P < 0.01$) and 44.3% ($P < 0.01$) of the variability in denitrification across the 15 plots, respectively. Soil bulk density displayed a significantly negative relationship ($r^2 = 0.52$, $P < 0.01$) with the cumulative N_2O production, whereas both soil moisture ($r^2 = 0.25$, $P = 0.055$) and soil organic N ($r^2 =$

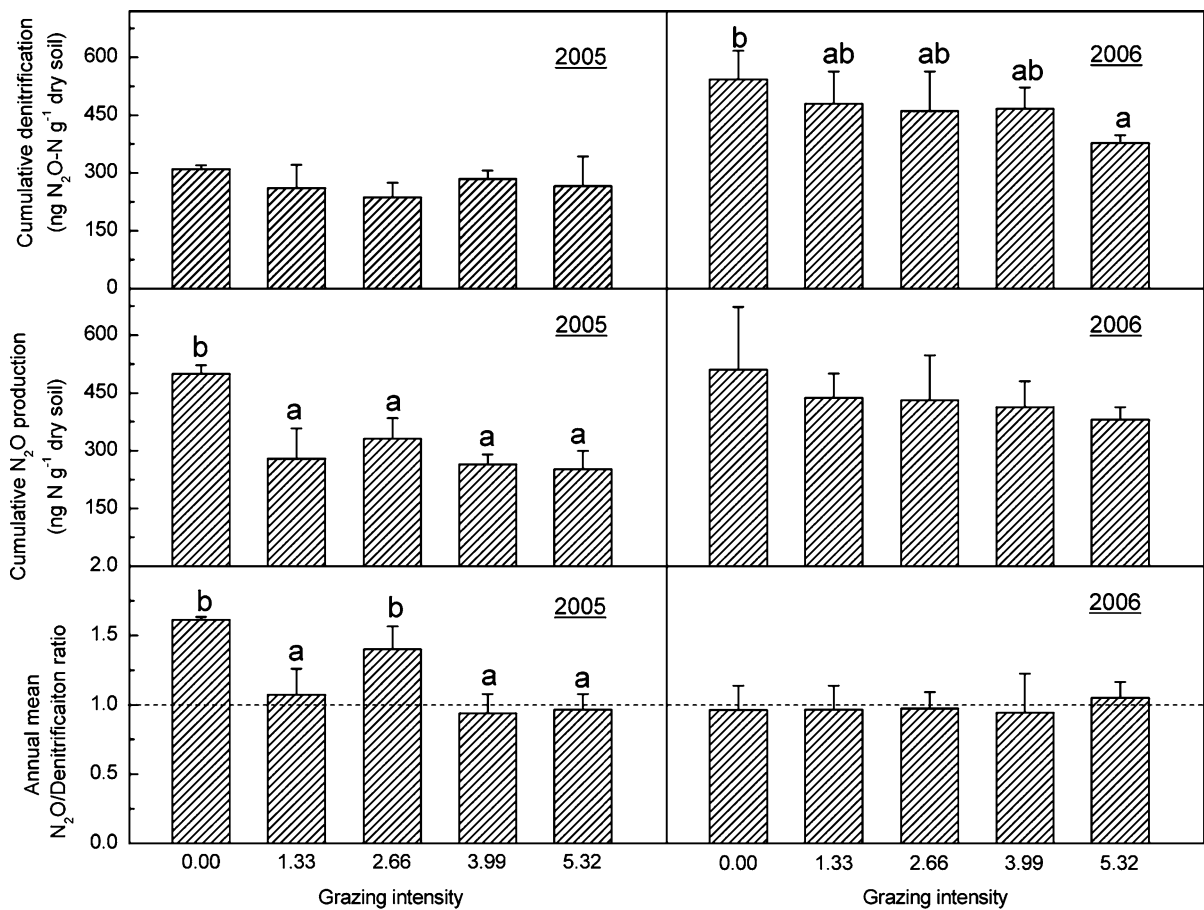


Fig. 3 Cumulative denitrification and N₂O production (ng N g⁻¹ dry soil), and annual mean N₂O production:denitrification ratios under different GI during the 2005 and 2006 growing seasons. Each column is the mean from three

replicated plots. Error bars represent ± 1 SE. Treatments with different letters are significantly different at $P < 0.05$ level. See Fig. 1 for grazing intensity abbreviations

0.27, $P = 0.051$) were marginally correlated with the cumulative N₂O production. When stepwise multiple regression analyses were conducted, both soil nitrate N concentration ($P < 0.05$) and soil bulk density ($P < 0.05$) were significant variables for denitrification and N₂O production. Using all data points over the two-year period, soil organic N and soil bulk density explained 26.7% and 66.7% of the variability in denitrification (Fig. 5). In addition, soil moisture ($r^2 = 0.13$, $P = 0.053$) and total plant AGB ($r^2 = 0.12$, $P = 0.058$) showed marginal negative relationships with denitrification. When stepwise multiple regression analyses were conducted, only soil bulk density ($P < 0.001$) was a significant variable for denitrification, and both soil nitrate N concentration

($P < 0.05$) and soil bulk density ($P < 0.001$) were significant variables for N₂O production. Soil moisture, nitrate N concentration, organic N and bulk density explained 42.8%, 34.0%, 36.2% and 55.4% of the variability in N₂O production, respectively (Fig. 6).

Across the 15 plots, the annual mean N₂O production:denitrification ratios showed significantly positive linear correlations with soil moisture ($r^2 = 0.53$, $P < 0.01$), nitrate N concentration ($r^2 = 0.58$, $P < 0.01$), and soil organic N content ($r^2 = 0.29$, $P < 0.05$) in 2005. Results of stepwise multiple regressions showed that only soil nitrate N concentration was a significant variable in the analysis ($P < 0.05$). By contrast, no such significant relationship was observed in 2006 ($P > 0.05$).

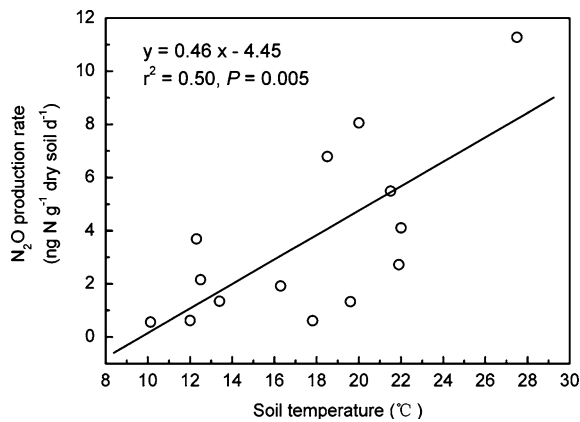


Fig. 4 Correlations of the temporal variations in N_2O production rate (ng N g^{-1} dry soil d^{-1}) with soil temperature ($^{\circ}\text{C}$) in the ungrazed treatment across the 14 sampling dates over the 2005 and 2006 growing seasons. Each point is the mean from three replicated plots. See Fig. 1 for grazing intensity abbreviations. $n = 14$

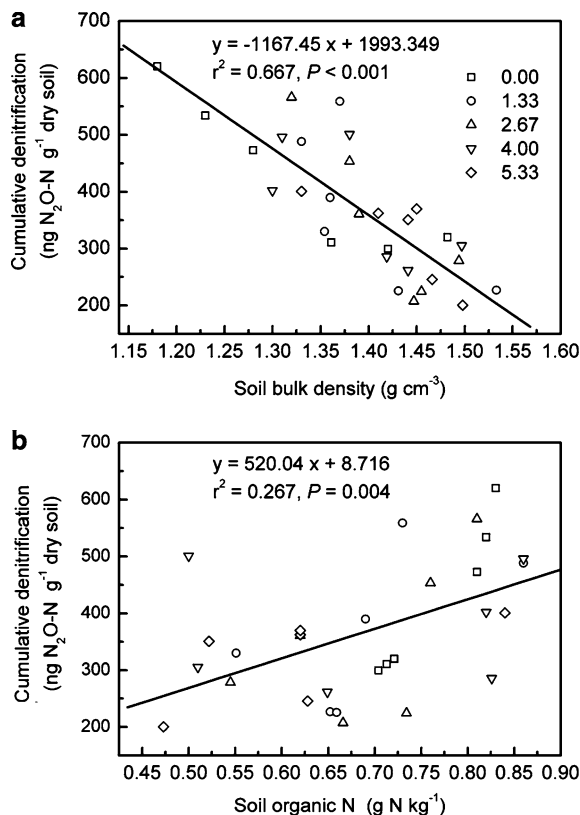


Fig. 5 Correlations of the variations in cumulative denitrification ($\text{ng N}_2\text{O-N g}^{-1}$ dry soil) across the 15 plots over the 2005 and 2006 growing seasons against soil bulk density (g N cm^{-3} , **a**) and soil organic N content (g N kg^{-1} , **b**). See Fig. 1 for grazing intensity abbreviations. $n = 30$

Discussion

Effects of grazing intensity

Stimulating effects of grazing on denitrification have been found in some grassland ecosystems (Frank and Groffman 1998; Frank et al. 2000; Le Roux et al. 2003). No effect of grazing has also been observed in other grassland ecosystems (Groffman et al. 1993). Our results show that long-term grazing reduces denitrification and N_2O production in this steppe grassland (Figs. 2 and 3), which is consistent with a previous study that reported decreased N_2O production in grazed treatment (Wang et al. 2005). Therefore, we accept our hypothesis that long-term grazing inhibits denitrification and N_2O production in this steppe ecosystem.

Enhanced denitrification and N_2O production by grazing reported previously were often ascribed to the anaerobic soil conditions caused by animal trampling and soil compaction (Menneer et al. 2005). In our study, soil bulk density was significantly higher in the grazed plots than in the ungrazed plots (Table 1). However, soil compaction by grazing has the potential to reduce soil moisture in our study possibly by increasing soil surface runoff and decreasing infiltration of water in the soil. Relatively lower soil moisture in grazed plots might constrain denitrifier activities and inhibit denitrification and N_2O production in this semi-arid grassland. Long-term grazing reduced soil nitrate N concentrations and soil organic C and N contents (Table 1 and Fig. 1c; Wang et al. 2006). On the sandy soils in this semi-arid grassland, the ungulate-induced apparent reductions in soil nitrate N concentrations was a critical factor responsible for decreasing denitrification and N_2O production in response to grazing, because nitrate is the substrate for denitrification and N_2O production. However, nitrate concentration was only significantly correlated with cumulative denitrification rates for separate year, not across years, and thus the inter-annual variability might obscure the relationship. In a previous study from grassland on peat soils, the decreased supply of organic C substrates was primarily responsible for the lower N_2O production (Koops et al. 1997). In our study, lower soil organic C and N contents induced by long-term grazing (Table 1) might reduce the C and N availability and subsequently limit denitrification.

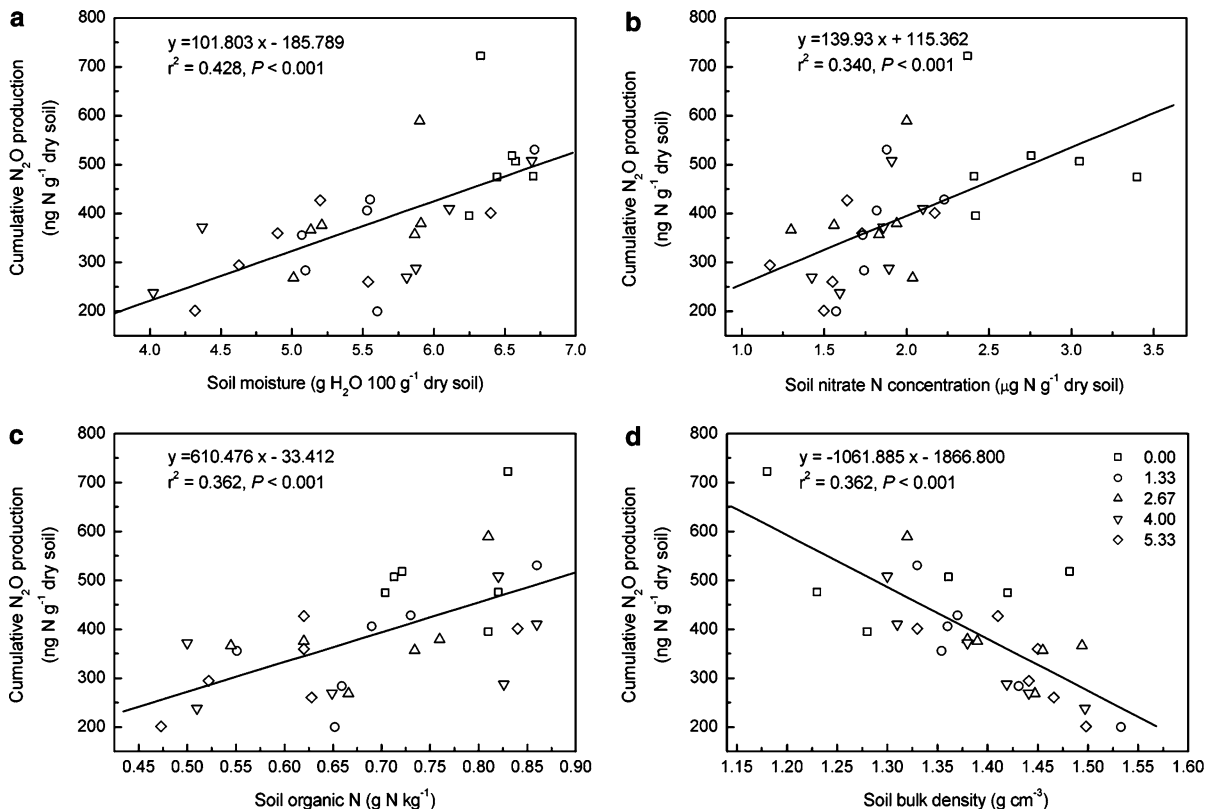


Fig. 6 Correlations of the variations in cumulative N_2O production (ng N g^{-1} dry soil) across the 15 plots over the 2005 and 2006 growing seasons against annual mean soil moisture ($\text{g H}_2\text{O } 100 \text{ g}^{-1}$ dry soil, **a**) and nitrate N

concentration ($\mu\text{g N g}^{-1}$ dry soil **b**), soil organic N (g N kg^{-1} , **c**), and soil bulk density (g cm^{-3} , **d**). See Fig. 1 for grazing intensity abbreviations. $n = 30$

Significant differences in denitrification and N_2O production were not found between the four grazed treatments, even though denitrification and N_2O production were generally lower in the grazed treatments than in the ungrazed treatment (Figs. 2 and 3). This result was probably due to the insignificant variations in such soil properties as nitrate N concentration, organic C and N content, and bulk density (Fig. 1 and Table 1), since there were close relationships of denitrification and N_2O production with these relevant factors across the seasonal range and grazing intensity gradient.

Seasonal patterns of denitrification and N_2O production

Denitrification rates from grasslands in the field are often very low ($<0.1 \text{ kg N ha}^{-1} \text{ d}^{-1}$) (Aulakh et al. 1992). The range of denitrification rates found in this

semi-arid grassland ecosystem was similar to that ($<10 \text{ g N ha}^{-1} \text{ d}^{-1}$) reported by Ryden (1983), but lower than those in other grasslands reported by most previous studies (Luo et al. 1999; Rudaz et al. 1999). The relatively low denitrification rates in the sandy soils in this semi-arid grassland might be due to a small portion of anaerobic microsites for biological denitrification or the occurrence of chemodenitrification (Wrage et al. 2001). When compared with the denitrification rates in desert ecosystems, our values were generally similar to those in arid environments of the Mojave Desert ($<32.26 \text{ g N ha}^{-1} \text{ d}^{-1}$, averaged $1.61 \pm 0.096 \text{ g N ha}^{-1} \text{ d}^{-1}$) (Billings et al. 2002) and the Chihuahuan Desert ($1.73 \text{ g N ha}^{-1} \text{ d}^{-1}$) (Peterjohn and Schlesinger 1991), while lower than those from wet soils under a canopy of a N fixing shrub in the Sonoran Desert ($11.6 \text{ g N ha}^{-1} \text{ h}^{-1}$) (Virginia et al. 1982). Extreme drying-wetting cycles (Peterjohn and Schlesinger 1990), high C and N availability after

rewetting the dry soils (Peterjohn and Schlesinger 1991), and the inputs to the amount of nitrogen stored in soil (Peterjohn and Schlesinger 1990) are the main causes to high denitrification rates in desert ecosystems.

Strong seasonal variations were observed in denitrification and N_2O production, which were characterized by a peak in summer and a low level of values in spring and autumn in this semi-arid grassland ecosystem (Fig. 2). The peak denitrification and N_2O production could have been attributed to high precipitation, high temperature and advanced plant development in summer (Ruz-Jerez et al. 1994; Pinay et al. 2007). Positive correlations of denitrification and N_2O production with soil temperature over the two growing seasons were observed in our study, consistent with previous findings (e.g., Magg and Vinther 1999; Chatskikh et al. 2005).

Soil moisture could also play an important role in regulating denitrification and N_2O production. High soil moisture can not only stimulate soil microbial activities, but also restrict oxygen diffusion and reduce oxygen concentration in soils, and thus promote denitrification under anaerobic conditions (De Klein and van Logtestijn 1994). We observed positive relationships of denitrification with soil moisture across temporal scale and of N_2O production with soil moisture across the 15 plots in the dry year 2005. These observations are in concert with previous studies (Skiba et al. 1993; Pinay et al. 2007). However, the soil moisture of the incubated soils was not different between years, which can't explain the higher annual denitrification and N_2O production in wetter year 2006 than the dry year 2005. In 2006, more frequent drying and re-wetting cycles might be a more important factor to enhance denitrification and N_2O production (Peterjohn and Schlesinger 1990, 1991). Because the drying and re-wetting process can result in a rapid increase in denitrifier activity, with an onset of anaerobic condition and a moisture-induced increase in availability of mineral N in rewetted soils (Venterink et al. 2002; Carrasco et al. 2004).

The dependence of denitrification and N_2O production on nitrate N concentration is a contentious issue. Although no correlations of temporal variations in denitrification and N_2O production rates with soil nitrate N concentrations were detected, we did find

positive relations of annual mean denitrification and N_2O production with soil nitrate N concentrations across different plots. Good correlations of denitrification and N_2O production with nitrate N concentrations have also been reported previously (Poe et al. 2003). However, Lowrance et al. (2001) found no such correlations. Soil nitrate sensitivities of denitrification and N_2O production are complex, because the role nitrate concentration plays is much dependent on the conditions of soil water content and oxygen. High water content and nitrate N concentration support high denitrification rates, and in that case, oxygen diffusion and C availability were the principal regulators. However, when water content is low, nitrate N diffusion is restricted, and denitrification and N_2O production might be subsequently controlled by the availability of nitrate N substrate (Estavillo et al. 1994; Strong and Fillery 2002). In our study, the average inorganic N concentration accounted for less than 2.6% of total N, and Inner Mongolia steppe generally maintains a relatively low level of N availability (Xu et al. 2007). Therefore, the low water content and low availability of nitrate substrate inhibited the denitrification and N_2O production in this semi-arid grassland.

The variations in annual gaseous N production could be attributed to the differences in climatic and environmental conditions, especially rain events. This underlines the importance of measurement time when estimating seasonal or annual gaseous N production. Our annual estimations were derived by monthly periodic measurements. Although more frequent sampling was necessary to estimate reliable annual gaseous N production (Billings et al. 2002), our estimations could give an indication for the comparison of the differences in annual denitrification and N_2O production between different GI.

N_2O can be emitted via both denitrification and nitrification. A ratio of N_2O production:denitrification >1 implies that production of N_2O through nitrification must have occurred, and that nitrification is the major source of N_2O (Koops et al. 1997). In our study, mean ratios of N_2O production:denitrification were generally lower than 1 (Figs. 2 and 3, lowest panels), indicating N_2O production through denitrification. High N supply and aerobic conditions contribute to nitrification. The generally lower N_2O production:denitrification ratios in 2005 in the grazed plots than in the ungrazed plots (Fig. 3) suggest that

long-term grazing might have suppressed nitrification in dry year due to N limitation.

In summary, substantial variations in denitrification and N₂O production across the seasonal range and grazing intensity gradient in this temperate steppe could have been explained by changes in soil temperature, soil moisture, soil bulk density and soil nitrate N concentrations. Long-term grazing resulted in reductions in soil nitrate N concentrations, which in turn reduce denitrification and N₂O production rates. Lower denitrification and N₂O production may be conducive to the mitigation of greenhouse effect and contribute to preserving soil N content and availability in the grazed plots. However, a back-of-the-envelope calculation showed that the N emission accounted for a little of the total N pool on an annual basis. As a result, the best management strategy for increasing N in the soil would be to increase N inputs through increased productivity. Due to the lower soil N content caused by the long-term grazing, restoration of the degraded grazed grassland may be slower in comparison with the ungrazed grassland, especially when lower plant growth and productivity in grazed plots are under consideration.

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References

- Aulakh MS, Doran JW, Mosier AR (1992) Soil denitrification—significant, measurement, and effect of management. *Adv Soil Sci* 18:1–57
- Barger NN, Ojima DS, Belnap J, Wang S, Wang Y, Chen Z (2004) Changes in plant functional groups, litter quality, and soil carbon and nitrogen mineralization with sheep grazing in an Inner Mongolian Grassland. *J Range Manage* 57:613–619
- Billings SA, Schaeffer SM, Evans RD (2002) Trace N gas losses and N mineralization in an intact Mojave Desert ecosystem with elevated CO₂. *Soil Biol Biochem* 34:1777–1784
- Biondini ME, Patton BD, Nyren PE (1998) Grazing intensity and ecosystem processes in a northern mixed-grass prairie, USA. *Ecol Appl* 8:469–479
- Carrasco D, Fernandez-Valiente E, Ariosa Y, Quesada A (2004) Measurement of coupled nitrification–denitrification in paddy fields affected by Terrazole, a nitrification inhibitor. *Biol Fertil Soils* 39:186–192
- Chatskikh D, Olesen JE, Berntsen J, Regina K, Yamulki S (2005) Simulation of effect of climate, soils and management on N₂O emission from grasslands. *Biogeochemistry* 76:395–419
- Cicerone RJ (1987) Changes in stratospheric ozone. *Science* 237:35–42
- De Klein CAM, van Logtestijn RSP (1994) Denitrification in the top soil of managed grasslands in the Netherlands in relation to the soil type and fertilizer level. *Plant Soil* 163:33–44
- Estavillo JM, Rodriguez M, Domingo M, Munoz-Rueda A, Gonzalez-Murua C (1994) Denitrification losses from a natural grassland in the Basque Country under organic and inorganic fertilization. *Plant Soil* 162:19–29
- Finlayson-Pitts BJ, Pitts NJ Jr (2000) Chemistry of the upper and lower atmosphere. Academic Press, San Diego, pp 969
- Frank DA, Groffman PM (1998) Denitrification in a semi-arid grazing ecosystem. *Oecologia* 117:564–569
- Frank DA, Groffman PM, David ER, Tracy BF (2000) Ungulates stimulation of nitrogen cycling and retention in Yellowstone Park grasslands. *Oecologia* 123:116–121
- Groffman PM, Rice CW, Tiedje JM (1993) Denitrification in a tallgrass prairie landscape. *Ecology* 74:855–862
- Hamilton EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82:2397–2402
- Kang L, Han XG, Zhang ZB, Sun OJ (2007) Grassland ecosystems in China: review of current knowledge and research advancement. *Philos Trans R Soc* 362:997–1008
- Koops JG, van Beusichem ML, Oenema O (1997) Nitrogen loss from grassland on peat soils through nitrous oxide production. *Plant Soil* 188:119–130
- Le Roux X, Bardy M, Loiseau P, Louault F (2003) Stimulation of soil nitrification and denitrification by grazing in grasslands: do changes in plant species composition matter? *Oecologia* 137:417–425
- Le Roux X, Abbadie L, Fritz H, Leriche H (2006) Modification of the savanna functioning by herbivores. In: Abbadie L, Gignoux J, Le Roux X, Lepage M (eds) *Lamto: structure. Functioning and dynamics of a Savanna ecosystem. Ecological studies*. Springer Verlag, New York, pp 158–169
- Li SG, Harazono Y, Oikawa T, Zhao HL, He ZY, Chang XL (2000) Grassland desertification by grazing and the resulting micrometeorological changes in Inner Mongolia. *Agric For Meteorol* 102:125–137
- Lowrance R, Robert K, Hubbard PK (2001) Denitrification from a swine lagoon overland flow treatment system at a pasture-riparian zone interface. *J Environ Qual* 30:617–624
- Luo J, Tillman RW, Ball PR (1999) Grazing effects on denitrification in a soil under pasture during two contrasting seasons. *Soil Biol Biochem* 31:903–912

- Magg MF, Vinther P (1999) Effect of temperature and water on gaseous emissions from soils treated with animal slurry. *Soil Sci Soc Am J* 63:858–865
- Menneer JC, Ledgard S, Mclay C, Silvester W (2005) Animal treading stimulates denitrification in soil under pasture. *Soil Biol Biochem* 37:1625–1629
- Oenema O, Boers PCM, Van Eerd MM, Fraters B, Van Der Meer HG, Roest CWJ, Schröder JJ, Willems WJ (1998) Leaching of nitrate from agriculture to groundwater: the effect of policies and measures in the Netherlands. *Environ Pollut* 102:471–478
- Peterjohn WT (1991) Denitrification: enzyme content and activity in desert soils. *Soil Biol Biochem* 23:845–855
- Peterjohn WT, Schlesinger WH (1990) Nitrogen loss from deserts in the southwestern United States. *Biogeochemistry* 10:67–79
- Peterjohn WT, Schlesinger WH (1991) Factors controlling denitrification in a Chihuahuan Desert ecosystem. *Soil Sci Soc Am J* 55:1694–1701
- Pinay G, Gumiero B1, Tabacchi E, Gimenez O, Tabacchi-Planty AM, Hefting MM, Burt TP, Black VA, Nilsson C, Iordache V, Bureau F, Vought L, Petts GE, Décamps H (2007) Patterns of denitrification rates in European alluvial soils under various hydrological regimes. *Freshw Biol* 52:252–266
- Poe AC, Piehler MF, Suzanne P, Thompson SP, Paerl HW (2003) Denitrification in a constructed wetland receiving agricultural runoff. *Wetlands* 23:817–826
- Rudaz AO, Wälti E, Kyburz G, Lehmann P, Fuhrer J (1999) Temporal variation in N₂O and N₂ fluxes from a permanent pasture in Switzerland in relationship to management, soil water content and soil temperature. *Agric Ecosyst Environ* 73:83–91
- Ruz-Jerez BE, White RE, Ball PR (1994) Long-term measurement of denitrification in three contrasting pastures grazed by sheep. *Soil Biol Biochem* 26:29–39
- Ryden JC (1983) Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. *J Soil Sci* 34:355–365
- Ryden JC, Skinner JH, Nixon DJ (1987) Soil core incubation system for the field measurement of denitrification using acetylene-inhibition. *Soil Biol Biochem* 19:753–757
- Saggar S, Andrew RM, Tate KR, Hedley CB, Rodda NJ, Townsend JA (2004) Modelling nitrous oxide emissions from New Zealand dairy grazed pastures. *Nutr Cycl Agroecosyst* 68:243–255
- Skiba U, Smith KA, Fowler D (1993) Nitrification and denitrification as sources of nitric oxide in a sandy loam soil. *Soil Biol Biochem* 25:1527–1536
- Strong DT, Fillery IRP (2002) Denitrification response to nitrate concentrations in sandy soils. *Soil Biol Biochem* 34:945–954
- Tiedje JM (1982) Denitrification. In: Page AL (ed) *Methods of soil analysis*, part 2, 2nd edn. Am Soc Agric, Madison, pp 1011–1026
- Venterink HO, Davidsson TE, Kiehl K, Leonardson L (2002) Impact of drying and re-wetting on N, P and K dynamics in a wetland soil. *Plant Soil* 243:119–130
- Virginia RA, Jarrell WM, Franco-Vizcaino E (1982) Direct measurement of denitrification in a *Prosopis* (mesquite) dominated Sonoran Desert ecosystem. *Oecologia* 53: 120–122
- Wang Y, Xue M, Zheng X, Ji B, Du R, Wang Y (2005) Effects of environmental factors on N₂O emission from and CH₄ uptake by the typical grasslands in the Inner Mongolia. *Chemosphere* 58:205–215
- Wang C, Wan S, Xing X, Zhang L, Han X (2006) Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biol Biochem* 38:1101–1110
- Wrage N, Velthof GL, van Beusichen ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol Biochem* 33:1723–1732
- Xu Y, Li L, Wang Q, Chen Q, Cheng W (2007) The pattern between nitrogen mineralization and grazing intensities in an Inner Mongolian typical steppe. *Plant Soil* 300: 289–300
- Yoshinari T, Hynes R, Knowles R (1977) Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. *Soil Biol Biochem* 9:177–183