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2016 Environ. Res. Lett. 11 054004

(http://iopscience.iop.org/1748-9326/11/5/054004)

Environmental Research Letters

LETTER

Grazing intensity and driving factors affect soil nitrous oxide fluxes during the growing seasons in the Hulunber meadow steppe of China

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Keywords: soil N_2O fluxes, meadow steppe, grazing intensity, driving factor, response and mechanism

Supplementary material for this article is available online

OPEN ACCESS

RECEIVED
9 December 2015

REVISED
12 April 2016

ACCEPTED FOR PUBLICATION
13 April 2016

PUBLISHED
26 April 2016

Abstract

In this study, the effects of cattle grazing intensity on soil nitrous oxide (N_2O) fluxes were examined in the Hulunber meadow steppe of north-eastern China. Six stocking-rate treatments (0, 0.23, 0.34, 0.46, 0.69, and 0.92 AU ha^{-1}) with three replicates were established, and observations were conducted from 2010 to 2014. Our results showed that substantial temporal fluctuations in N_2O flux occurred amongst the different grazing intensities, with peak N_2O fluxes after natural rainfall. Grazing had a long-term effect on the soil N_2O flux in the grasslands. After 4–5 years of grazing, the N_2O fluxes under increased levels of grazing intensity began to decrease significantly by 31.4%–60.2% in 2013 and 32.5%–50.5% in 2014 compared to the non-grazing treatment. We observed a significant negative linear relationship between the soil N_2O fluxes and grazing intensity for the five-year mean. The soil N_2O flux was significantly affected each year in all of the treatments. Over the five years, the temporal coefficient of variation (CVs) of the soil N_2O flux generally declined significantly with increasing grazing intensity. The soil N_2O emission rate was significantly positively correlated with soil moisture (SM), soil available phosphorus (SAP), soil NH_4^+ −N, soil NO_3^- −N, above-ground biomass (AGB), plant ground cover and height and was negatively correlated with total soil nitrogen (TN). Stepwise regressions showed that the N_2O flux was primarily explained by SM, plant height, TN, soil pH, and soil NH_4^+ −N. Using structural equation modelling, we show that grazing significantly directly influenced the plant community and the soil environment, which then influenced the soil N_2O fluxes. Our findings provide an important reference for better understanding of the mechanisms and identifying the pathways of grazing effects on soil N_2O emission rates, and the key drivers plant community and soil environment within the nitrogen cycle that are mostly likely to affect N_2O emissions in the Inner Mongolian meadow steppes.

1. Introduction

Nitrogen (N) is likely the most limiting nutrient for plant growth and net primary production (NPP) in terrestrial ecosystems; however, it can also be responsible for negative effects of environment. Nitrous oxide (N_2O) is the third most important greenhouse gas globally (de Klein *et al* 2014). N_2O is produced in soils mainly as a product of denitrification. Denitrification is a microbially mediated process that occurs under reducing conditions and is the main process responsible for N losses in the form of N_2O and N_2 (Oenema *et al* 2007). Although denitrification is generally associated with wetter areas, it has been shown to occur in dryer ecosystems such as deserts and arid and semi-arid grasslands. In such systems (Peterjohn 1991, Groffman *et al* 1993), denitrification rates are not necessarily lower where water is limiting,

and regular drying and rewetting may render both N and C increasingly available, leading to higher denitrification rates (Peterjohn 1991) (Peterjohn and Schlesinger 1991). Skiba *et al* (1993) showed that soil moisture (SM) is key to explaining the temporal patterns of denitrification across a range of ecosystems.

Grasslands are by far one of the most important biome types of all terrestrial ecosystems; they cover approximately one-fifth of the global land surface and account for a large portion of the terrestrial soil carbon and N pools. Grazing is the main form of human-related activity in these systems. Animal grazing removes herbage (Leriche *et al* 2001, Yan *et al* 2015), increases soil compaction by trampling (Oenema *et al* 2007, Han *et al* 2008, Houlbrooke *et al* 2008) and changes the quantity and quality of soil nutrients through the deposition of dung and urine (Han *et al* 2008, Yan *et al* 2014). The effect of selective grazing on spp composition and the impact this might have on quantity and quality of litter. Also, type of grazing management not just increasing stocking densities, i.e., intensive short term grazing with rest vs. continuous grazing at different intensities. All of these effects have been shown to influence denitrification and N_2O emissions (Jackson *et al* 2015). Furthermore, grazing also affects the rates of soil N cycling processes that are direct or indirect sources of N_2O (Davidson and Kanter 2013). de Klein *et al* (2014) indicated that the excreta deposited by grazing animals are the largest source of N_2O in grazed livestock systems (de Klein *et al* 2014). Therefore, understanding the relationships between grazing management and N cycling within the plant–soil–animal continuum and its many feedback loops and interactions is critical for the development of efficient and effective N_2O mitigation strategies for grazed livestock systems.

Previous studies on the effects of grazing on N_2O emissions have been somewhat inconclusive, with studies reporting increases in N_2O emissions by enhanced N cycling rates for most managed temperate grassland (Hyde *et al* 2006, Luo *et al* 2008); decreases in emissions driven by the grazing-induced reduction in soil organic matter and SM in arid and semi-arid grasslands, where N_2O was predominantly produced by microbial nitrification (Cookson *et al* 2006, Wolf *et al* 2010); or no effect (Groffman *et al* 1993). Such discrepancies suggest that the response of soil N_2O emissions to grazing may vary with grazing intensity, grazing history, climate and soil type.

The Hulunber grassland in north-eastern China comprises one of the largest areas of natural, temperate sub-humid meadow grasslands in the world, covering an area of approximately 10×10^6 km^2; it plays an important role in the ecological environment and socioeconomics of the region by supporting a diverse array of plant and animal species (Kang *et al* 2007). However, recently, overgrazing has resulted in degradation of 50% of the total available grassland area and has led to significant depletion of soil organic matter and biomass production in the Hulunber meadow steppe (Cui *et al* 2005, Wang *et al* 2008), significantly affecting N_2O fluxes (Dong *et al* 2000, Wolf *et al* 2010). Previous studies have focused on the effects of animal grazing on plant species diversity and productivity (Zhou *et al* 2006, Gao *et al* 2012, Yan *et al* 2015) and/or soil quality (Han *et al* 2008, Yan *et al* 2014), but relatively little research has been conducted on N_2O or other GHG emissions.

In this study, we report the effects of increasing grazing intensity on soil N_2O fluxes in the Hulunber meadow steppe, north-eastern China, during the June–October growing season over a 5-year period. The aims of the study were to (1) determine the seasonal and annual changes in soil N_2O fluxes in response to increasing grazing intensity; (2) establish the mechanisms underlying any changes by examining the relationships between soil N_2O fluxes and environmental, soil and biological factors; and (3) identify the pathways through which grazing affects soil N_2O fluxes and the key drivers and pathways within the N cycle that are mostly likely to affect N_2O emissions in this temperate meadow steppe.

2. Materials and methods

2.1. Study site

This study was conducted at the Hulunber Grassland Ecosystem Observation and Research Station located at Xiertala farm in the centre of the Hulunber meadow steppe (N 49°19′349″-49°20′173″, E 119°56′521″-119°57′854″) in the north-eastern region of Inner Mongolia, China. The elevation varies from 666 to 680 m. The climate zone is continental temperate semi-arid with an annual average of 110 frost-free days. The average annual precipitation ranges from 350 to 400 mm, of which approximately 80% falls between July and September. The mean annual air temperature in this area is −5 to −2 °C, with a mean monthly maximum of 36.2 °C in July and a minimum of −48.5 °C in January. The vegetation is characterised as a typical *Leymus chinensis* and forbs meadow steppe. The dominant species are *L. chinensis, Scutellaria baicalensis, Carex pediformis, Galium verum, Bupleurum scorzonerifolium* and *Filifolium sibiricum.* The husbandry and utilisation of Hulunber meadow steppe is particular comparing with other region in northern grassland of China. Grazing is only available from June to October in Hulunber grassland limited by the short growing season. There is a long history of hay cutting and a semi-intensive feeding system is developed.

2.2. Treatments

The grazing experiment was established in 2009 with 18 paddocks of 5 ha (300 × 167 m) each with six stocking densities (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92

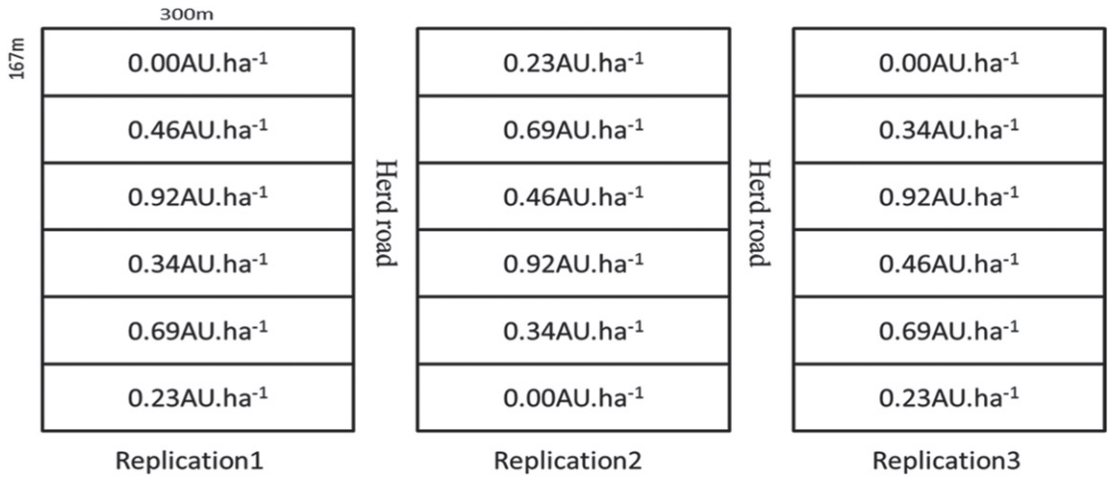

Figure 1. Experimental design and plot layout. (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU ha^{-1} where 1 AU = 500 kg of adult cattle). The stocking rates were achieved by using 0, 2, 3, 4, 6 or 8 young cows (250–300 kg) per plot.

AU ha^{-1}; where 1 AU = 500 kg of adult cattle). Each stocking rate was replicated three times in a randomised block design (figure 1). 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 to 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. In the summer of 2008, baseline measurements were taken prior to the implementation of the field treatments using a 50 m transect in each plot to characterise the vegetation and soil traits. Across all grazing intensities, the above-ground biomass (AGB) ranged between 800–850 kg ha^{-1}, the ground vegetation cover averaged 36%–42%, and the average pasture height was 7–9 cm. The soil type is chernozem or chestnut soil. The total soil nitrogen (TN) was 3.73–4.08 g kg^{-1}, and the organic carbon concentration of the surface soil was 36.4–39.5 g kg^{-1}; these values did not vary significantly between the different grazing intensity plots (Yan *et al* 2014).

2.3. Measurement of N_2O fluxes

The N_2O fluxes were measured using the opaque static chamber method (Yuesi and Yinghong 2003). The static chamber system consisted of a stainless steel frame (without a top and bottom, length × width × height = 50 cm × 50 cm × 10 cm) that was driven into the soil (installed prior to treatment initiation in August 2009) and a stainless steel chamber (without bottom, length × width × height = 50 cm × 50 cm × 50 cm) that was placed tightly in the base groove during the sampling period.The square box inserted directly into the meadow soil about 10 cm below the soil surface. And the cover was placed on top during sampling times and removed afterwards. A fan 10 cm in diameter was installed on the top wall of each chamber to make turbulence when chamber was closed. The external surface of each chamber was covered with white plastic foam to minimise the effect of direct radiative heating during sampling. Three replicate chambers were randomly established in each plot and used for simultaneous measurements of the N_2O flux. The headspace in each chamber was sampled at intervals of 0, 10, 20 and 30 min after the chamber was closed. The gas was transferred immediately into a pre-evacuated 50 ml air bag using a 60 ml plastic syringe (Hede Inc., Dalian, China). The headspace N_2O concentrations were sampled twice per month during the growing season (June–October) in 2010 and four times per month during the growing season from 2011 to 2014. All of the measurements were collected between 9 and 11 am. The N_2O concentrations of the gas samples (stored in specific air bags) were analysed within one week using gas chromatography (Agilent 7890A, Agilent Technologies Limited Co., US). The N_2O flux was calculated according to Zhang *et al* (2010).

2.4. Measurements of ancillary factors

2.4.1. Climate factors

Rainfall and temperature data were collected from an automatic meteorological station (MILOS 520, VAISALA, FINLAND) at 30 min intervals.

2.4.2. Soil factors

Each year, soil samples were obtained from ten points per plot (to a 10 cm depth) at the beginning of August. The samples from each plot were combined to form a composite sample and stored at 4 °C in a refrigerator. One part was kept fresh for the measurement of soil ammonium nitrogen and nitrate nitrogen (NH_4^+ −N and NO_3^- −N, respectively) using a flow injection autoanalyser (FIAstar 5000 Analyzer, Foss Tecator, Denmark) (Bao 2000). The remainder was air-dried and

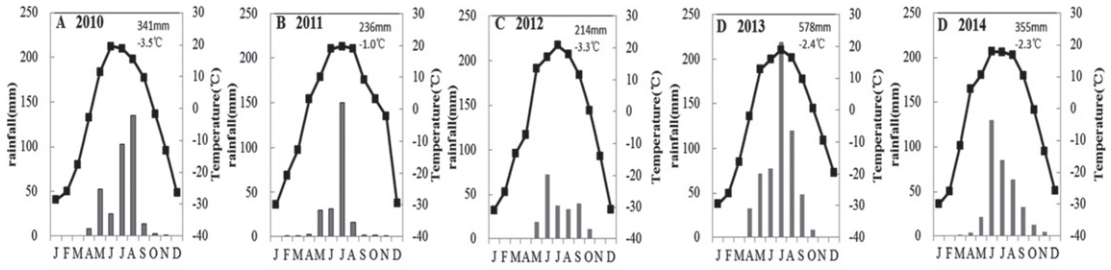

Figure 2. Monthly rainfall and temperature during 2010–2014 (A–E) for the grazing experimental site. Values shown in each panel are total annual rainfall and mean temperature.

ground to 2 mm for determination of the soil nutrients (Bao 2000). All of the results were expressed on a dry weight basis. The soil organic carbon content (TOC) was determined using the dichromate oxidation method. The TN was determined using semi-micro kjeldahl determination. The total soil phosphorus (TP) was determined using the molybdenum antimony resistance to colorimetric method, the total soil potassium (TK) was determined using the NaOH molten flame photometer method, the soil available nitrogen (SAN) was determined using alkali diffusion method, the soil available phosphorus (SAP) was determined using 0.5 mol L^{-1} sodium bicarbonate extraction and the soil available potassium (SAK) was determined using NH_4OAc extraction and flame photometry. The soil pH was measured using the electrode method. The soil bulk density (BD) was measured with the ring knife method, and the SM was measured the oven-drying method (Bao 2000).

2.4.3. Plant factors

Each year, five 1 m^2 quadrats were randomly located in each grazing plot in the peak biomass period (early August) such that above-ground net primary production (ANPP) could be estimated. A 50 × 50 cm point frame with 100 cross-hairs forming a grid was used to measure the ground cover in each quadrat; the plant height was measured using a multipoint method with a ruler and averaged. The forage within the quadrat was clipped at ground level, and the AGB was oven-dried for 48 h at 65 °C to constant weight. The below-ground biomass (BGB) samples for all three replications in each plot were collected in early August from 2010 to 2014. A soil pit was dug to a depth of 60 cm, and the root mass in a 30 × 30 cm cross-section column was extracted from depths of 0–10, 10–20, 20–30, 30–40, 40–50 and 50–60 cm and washed through a 1 mm sieve. Fine roots or segments were retained on 0.25 mm sieves. The screened materials were further washed to separate the roots from the soil. All of the roots were oven-dried at 80 °C for 12 h prior to weighing.

2.5. Statistical analyses and modelling

For each selected sampling date, the means and standard error (s.e.) of N_2O flux was calculated, and the plot values represent means ($n = 3$) ± s.e. One-way ANOVAs and least significant difference (LSD) tests were used to examine the effects of grazing treatments on seasonal and total emissions, with effects of $p < 0.05$ being significant. Chamber-based gas data were analysed using a general linear model-repeated measures analysis of variance (ANOVA; SPSS software, version 21.0) to assess the significance of the effects of grazing intensity, sampling date and their interactions on the N_2O flux and on the plant and soil factors. The data for all of the measured soil parameters across treatments and years are presented in table S1. Both linear and nonlinear regression models were used to detect the relationships between N_2O fluxes and key impact factors for all of the treatments combined.

A path analysis was used to better understand the interrelationships between the factors that affect the N_2O flux on the Hulunber meadow steppe. The path diagram was structured such that measurements of N_2O flux were the ultimate response variables. A concurrently measured biotic or abiotic variable was assumed to cause a variation in flux when the correlation was significant ($p < 0.05$).

3. Results

3.1. Seasonal changes in meteorological factors over the study period

Compared to the long-term average (350–400 mm), there were two relatively dry years (2011 and 2012) and one wet year (2013). However, the rainfall pattern was not consistent; for example, even in the dry years, most of the rainfall in 2011 occurred in July, whereas in 2012 the rainfall was more evenly distributed (figure 2). The temperature profiles were relatively consistent over the study period and did not differ significantly from the long-term averages.

3.2. Plant responses to grazing intensity

The AGB and the associated plant height significantly decreased with grazing pressure each year (table 1). AGB increased in all treatments in 2013 and 2014 compared to earlier years. In the first year of the study, there were no significant differences in BGB. However,

Table 1. AGB (above-ground biomass), BGB (below-ground biomass), litter biomass and canopy structure traits in grasslands under different grazing intensities.

		AGB (g m^{-2})	BGB (g m^{-2})	litter (g m^{-2})	coverage (%)	height (cm)
2010	G0.00	155 ± 9.3a	1631 ± 80.9a	24 ± 1.7a	70.6 ± 0.4a	18.1 ± 1.0a
	G0.23	122 ± 15.5ab	1446 ± 54.2a	18 ± 1.0bc	58.3 ± 2.6b	14.1 ± 0.8bc
	G0.34	97 ± 18.9bc	1194 ± 134.0a	19 ± 2.3b	45.6 ± 5.0c	14.3 ± 0.9b
	G0.46	102 ± 7.1bc	1907 ± 495.9a	17 ± 0.9bcd	34.6 ± 2.0d	11.6 ± 0.9cd
	G0.69	69 ± 11.6c	1481 ± 359.6a	15 ± 1.2cd	33.0 ± 3.4d	9.1 ± 1.0de
	G0.92	41 ± 8.9cd	1487 ± 179.2a	14 ± 0.1d	23.7 ± 1.8e	8.4 ± 0.6e
2011	G0.00	177 ± 6.7a	929 ± 178.6ab	83 ± 5.0a	71.8 ± 0.7a	22.2 ± 0.7a
	G0.23	136 ± 5.7b	1000 ± 152.4ab	48 ± 9.4b	62.9 ± 2.9b	15.7 ± 0.7b
	G0.34	129 ± 7.2b	871 ± 35.3ab	41 ± 1.6bc	53.2 ± 2.8c	15.8 ± 0.9b
	G0.46	113 ± 19.1b	1150 ± 146.5a	42 ± 6.2bc	50.9 ± 1.1c	11.6 ± 1.2c
	G0.69	62 ± 5.6c	885 ± 50.2ab	23 ± 8.4cd	42.0 ± 3.0d	6.4 ± 0.3d
	G0.92	49 ± 7.8c	742 ± 118.5b	14 ± 4.0d	36.4 ± 2.0d	5.7 ± 0.5d
2012	G0.00	162 ± 12.9a	1062 ± 172.7a	69 ± 8.2a	54.8 ± 10.8a	21.1 ± 1.8a
	G0.23	74 ± 5.7b	1279 ± 474.6a	45 ± 3.0b	40.7 ± 3.8ab	12.3 ± 1.4b
	G0.34	77 ± 1.1b	855 ± 134.9a	31 ± 9.2bc	37.0 ± 3.1b	10.0 ± 0.7bc
	G0.46	72 ± 8.0b	928 ± 88.8a	30 ± 7.1bc	39.3 ± 3.1ab	9.9 ± 1.5bc
	G0.69	34 ± 5.7c	766 ± 139.1a	15 ± 3.9c	31.3 ± 1.6b	4.3 ± 0.6d
	G0.92	18 ± 4.2c	784 ± 91.7a	13 ± 4.7c	26.5 ± 4.9b	2.9 ± 0.2d
2013	G0.00	271 ± 23.6a	1049 ± 93.8a	123 ± 14.5a	80.3 ± 1.8a	29.7 ± 3.5a
	G0.23	175 ± 9.9b	612 ± 45.4bc	72 ± 18.2b	74.0 ± 2.2ab	20.2 ± 1.0b
	G0.34	137 ± 20.3bc	810 ± 10.1b	27 ± 7.0cd	61.9 ± 0.6c	11.6 ± 0.4c
	G0.46	131 ± 24.2bcd	733 ± 32.8bc	56 ± 0.0bc	64.6 ± 6.2bc	11.5 ± 4.5c
	G0.69	89 ± 4.5cd	569 ± 37.9c	19 ± 0.0d	58.6 ± 2.3c	6.2 ± 0.9c
	G0.92	78 ± 18.3d	765 ± 145.5bc	33 ± 0.0cd	56.4 ± 4.5c	6.2 ± 2.2c
2014	G0.00	205 ± 21.9a	1083 ± 290.9a	181 ± 49.7a	64.8 ± 2.5ab	27.4 ± 1.8a
	G0.23	176 ± 20.9ab	951 ± 108.1a	52 ± 4.7b	68.6 ± 3.9a	21.4 ± 2.0b
	G0.34	137 ± 20.7bc	991 ± 111.6a	30 ± 4.7b	68.5 ± 1.6a	21.0 ± 2.4b
	G0.46	110 ± 16.5c	683 ± 53.8a	26 ± 3.4b	62.1 ± 7.2ab	16.7 ± 0.7bc
	G0.69	88 ± 21.0c	1092 ± 124.4a	25 ± 8.5b	55.7 ± 0.5bc	7.1 ± 0.3d
	G0.92	81 ± 15.7c	756 ± 38.8a	16 ± 1.7b	47.2 ± 3.3c	6.2 ± 0.7d

Notes: data were measured between 2010 and 2014 at sites of different grazing intensities: no grazing (G0.00), light grazing (G0.23, G0.34), intermediate grazing(G0.46), heavy grazing (G0.69 ,G0.92). Different superscript letters after means indicate significant difference between sites at $p < 0.05$. AGB, litter, community coverage and community height were sampled average from June to October in 2010, 2011, 2012, 2013 and 2014. BGB was sampled at peak biomass time in August in 2010, 2011, 2012, 2013 and 2014.

BGB tended to decline as the grazing intensity increased, and by the final year it decreased by nearly 50% in the G0.92 plots. There was a significant build-up of litter in the ungrazed plots during the experimental period. The amount of litter present tended to decrease with increasing grazing density.

3.3. Seasonal and annual responses of N_2O fluxes to grazing intensity

Prior to the start of the grazing season, there were no significant differences between the treatments ($p > 0.05$). Temporal fluctuations in the N_2O fluxes were observed throughout the experiment for all grazing intensities (figure 3). In 2013 and 2014, the fluxes of N_2O from the soil consistently showed that the ungrazed (G0.00) and G0.23 treatments had higher N_2O emission rates compared to the other grazing plots. The Hulunber soils acted as a source of soil N_2O in all years (figures 3(A)–(E)). Overall, we found that the soil was a net source of N_2O during the growing season. Our analysis further revealed that peak N_2O fluxes during the growing season usually occurred after effective rainfall.

The mean annual N_2O flux showed no significant differences between treatments in 2010 and 2011. However, the soil N_2O flux was substantially influenced by grazing intensity (figure 4(A)) from 2012 onwards. There were significantly ($p < 0.05$) greater

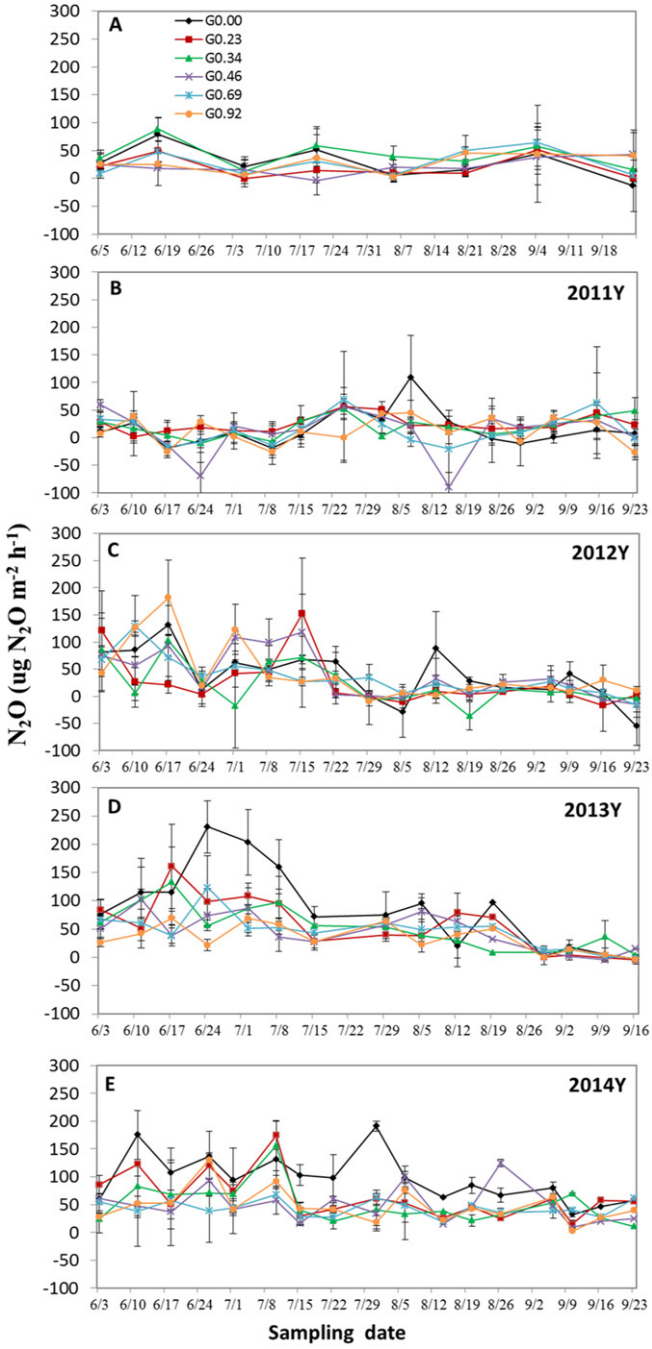

Figure 3. Seasonal variations of N_2O flux rates (mean ± s.e.) from June to October in 2010, 2011, 2012, 2013 and 2014 (A, B, C, D, E) under different grazing intensities (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU ha^{-1} where 1 AU = 500 kg of adult cattle).

fluxes from the ungrazed treatment than from the G0.92 treatment in 2013. In 2014, all of the grazing treatments had significantly ($p < 0.05$) lower emissions than the ungrazed plots. Relative to the ungrazed treatment, the N_2O fluxes under the different grazing intensities decreased by 31.4%–60.2% in 2013 and 32.5%–50.5% in 2014. The temporal coefficients of variation (CVs) of the soil N_2O flux declined significantly with increased grazing intensity, indicating a significant negative relation to grazing intensity ($R^2 = 0.789$, $p < 0.05$, figure 4(B)).

3.4. Effect of impact factor responses to grazing intensity on N_2O fluxes

The relationships between soil N_2O fluxes and impact factors (including meteorological, soil and vegetation factors) differed substantially (table 2). The soil parameters over the different grazing intensities for the five years of the experiment are shown in supplemental table 1. Across all of the treatments and years (figure 5), for meteorological factors, N_2O flux was shown to be significantly positively correlated with rainfall ($R^2 = 0.309$, $p < 0.001$), whereas significant negative

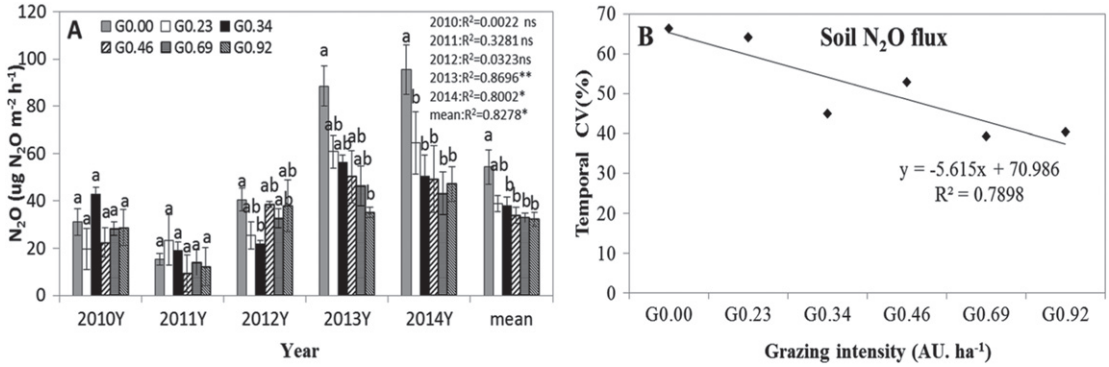

Figure 4. (A) Annual mean flux of soil N_2O under the different grazing intensities from 2010 to 2014. Bars represent the means of three replicate plots (±s.e.). Regression was estimated each year using a linear model with grazing intensity as a continuous predictor. Significant differences are reported as NS, $p > 0.05$; ∗, $p < 0.05$; and ∗∗, $p < 0.01$. Different letters indicate significant differences among the levels of grazing intensities (one-way ANOVA, $P < 0.05$). (B) Temporal variability (coefficient of variation (CV)) in soil N_2O fluxes under different grazing intensities.

correlations were observed between N_2O flux and air temperature ($R^2 = 0.360$, $p < 0.001$). There were significant positive linear relationships between soil N_2O flux and SM ($R^2 = 0.588$, $p < 0.001$), SAP ($R^2 = 0.206$, $p < 0.01$), soil NH_4^+−N ($R^2 = 0.384$, $p < 0.001$), AGB ($R^2 = 0.337$, $p < 0.001$), plant cover ($R^2 = 0.164$, $p < 0.001$), plant height ($R^2 = 0.314$, $p < 0.01$) and litter quantity ($R^2 = 0.393$, $p < 0.001$). In contrast, there were negative relationships between soil N_2O flux and TN ($R^2 = 0.148$, $p < 0.01$). No significant relationship was detected between soil N_2O flux and soil pH, soil bulk density, soil TP, soil total potash, soil AN, soil available potash and BGB (data not shown).

To test the relative effect of each factor (i.e., all biological and soil parameters and climate) on the N_2O flux, multiple regression tests were conducted. The results from these stepwise regressions showed that the N_2O flux was primarily explained by SM, NO_3^-−N, plant height, air temperature, soil AN and litter ($R^2 = 0.927$, $F = 48.81$, $p < 0.001$).

3.5. Pathways determining N_2O fluxes and grazing grassland ecosystem plant and soil factors

Most of the variables examined in this study were correlated with one another, making this data set well-suited for SEM analysis. The SEM models suggested that the grazing and meteorological factors had a significant and direct effect on the plant community and soil environment based on the significant standardized path coefficients for grazing and meteorological factors. For the SEM model, we found that the plant community was the most important pathway for determining soil N_2O emissions. Grazing and meteorological factors indirectly affected the soil N_2O emission via modification of the plant community. The soil environment was indirectly affected by changes in the plant community, whereas the soil nutrients and soil environment had no significant relationship with the soil N_2O emissions (figure 6).

4. Discussion

4.1. Responses of soil N_2O fluxes to grazing intensity

Understanding the effects of grazing on soil N_2O fluxes is important for predicting the effects of global climate change and human activities on N dynamics and for investigating management strategies to mitigate losses. Most N_2O emission studies are of short duration and therefore may not accurately measure the total N_2O evolved from either the N deposited by grazing animals or soils. Thus, the use of longer-term studies, such as that presented herein, provide a more representative estimate of the annual emission rate and allow for a more in-depth analysis of grazing treatment effects. In our study, the grazed steppe was a source of soil N_2O. The peak N_2O fluxes during the growing season usually occurred after natural rainfall, and the intensity of grazing substantially altered the soil N_2O fluxes. Somewhat curiously, our results demonstrate that grazing decreased rather than increased soil N_2O fluxes and showed that soil N_2O fluxes decreased with an increasing grazing rate. Significant negative linear relationships were found between the soil N_2O fluxes and grazing intensity for the 5-year average. This is consistent with the results of other grassland studies (Rey *et al* 2002, Wang and Fang 2009, Hou *et al* 2014). However, our results contradict the reports arguing that grazing increases the soil N_2O fluxes (Frank 2002). Several possible reasons could explain the differences in flux between our study and previous studies: variations in climate, soil, vegetation conditions and the underlying mechanism of the N_2O flux. Firstly, grazing changes the soil and environmental conditions that determine emissions of N_2O during the growing season. With the increasing stocking rate, the AGB, vegetation height and quantity of litter decreased at our sites. Vegetation height and litter are the determining factors in SM-holding capacity: SM declines more quickly at grazed sites with low vegetation than at sites with denser and taller vegetation. We also found a significant positive

Table 2. Pearson relationships between climate factor (rainfall and air temperature), soil factor (SM, SBD, pH, SOC, TN, TP, TK, C/N ratio, SAN, SAP, SAK, NH_4^+ −N, NO_3^- −N) biological factor (AGB, BGB, litter, plant cover and plant height), and the annual mean flux rates of N_2O under different grazing intensities.

Factor/grazing intensity		G0.00	G0.23	G0.34	G0.46	G0.69	G0.92
Climatic	Rainfall	0.463	0.494	0.754**	0.502	0.586*	0.162
	Temperature	−0.486	−0.548*	−0.859**	−0.491	−0.593*	−0.222
Soil	SM	0.588*	0.795**	0.685**	0.615*	0.549*	0.324
	SBD	0.267	0.081	−0.081	0.131	−0.114	0.134
	pH	0.070	0.044	0.377	0.264	−0.283	−0.066
	SOC	−0.096	−0.050	−0.218	−0.205	−0.589*	0.226
	TN	−0.315	−0.504	−0.422	−0.457	−0.556*	−0.086
	TP	0.055	−0.026	−0.465	−0.018	−0.258	0.114
	TK	−0.185	−0.213	−0.080	−0.271	−0.340	−0.611*
	C/N	0.416	0.549*	0.340	0.319	−0.021	0.173
	SAN	0.209	−0.304	−0.036	−0.446	−0.097	−0.041
	SAP	−0.235	0.086	0.321	0.242	0.304	0.375
	SAK	−0.017	−0.253	0.321	−0.357	−0.434	−0.303
	NH_4^+-N	0.415	0.655**	0.731**	0.562*	0.664**	0.088
	NO_3^--N	−0.031	0.660**	0.057	0.191	0.205	0.196
Biological	AGB	0.438	0.450	0.499	0.284	0.511	−0.065
	BGB	−0.071	−0.311	0.009	−0.519*	−0.245	−0.008
	Litter	0.683**	0.368	−0.268	0.185	−0.025	0.093
	Coverage	0.157	0.566*	0.537*	0.402	0.487	0.158
	Height	0.445	0.810**	0.351	0.459	−0.082	−0.120

Data from five experimental years were used for statistical analysis. Different treatments were analysed separately. There were three replicates under each grazing intensity each year for both the impact factor and N_2O flux data ($n = 15$). $^*p < 0.05$; $^{***}p < 0.01$.

correlation between vegetation height and SM, thereby stimulating the soil N_2O fluxes. Secondly, gas emissions from the soil to the atmosphere have a complicated delivery process. The N_2O fluxes were significantly correlated with soil structure; grazing can alter soil structure as a result of animal trampling, which increases soil bulk density and compaction, reduces the soil pore diameter and decreases water-filled pore space, which in turn restricts N_2O production rates from the soil. Thirdly, denitrification is the dominant process of N_2O production from these grazed pastures under humid climate conditions (Wrage *et al* 2001). The grazing enhancement of soil nitrification and denitrification on these pastures is primarily associated with the enhancement of N and C cycling and soil microorganism activity through animal excreta deposition and with the anaerobic conditions created by animal treading. The cattle trampling on soil is more likely to form the anaerobic zone. The denitrification potential of denitrifying bacteria in the soil is higher, and denitrification may continue into N_2 before N_2O emissions, which might result in a significant decrease in soil N_2O fluxes.

4.2. Responses of factors towards controlling N_2O fluxes to grazing intensity

In establishing empirical relationships between N_2O fluxes and meteorological, soil, vegetation and management conditions, temperature and precipitation are considered to be the most important factors for determining the spatial variations. We found that the N_2O flux was significantly positively correlated with rainfall, whereas significant negative correlations were observed between N_2O flux and air temperature. Our analysis showed that rainfall rather than air temperature is the critical climatic factor determining soil N_2O fluxes under the different grazing intensities. Our results are consistent with the conclusions of Du *et al* (2006), who reported a significant linear relationship between annual N_2O flux and the frequency of effective rainfall. Across all of the treatments and years, N_2O emissions were significantly positively correlated with SM, SAP and soil NH_4^+ −N. This indicates that N_2O fluxes from semi-arid ecosystems are mostly limited by SM and inorganic N content, which is consistent with previous studies (Mummey *et al* 1994, Holst *et al* 2007 Yao *et al* 2010). However, some contradictory results also have shown that the annual soil N_2O flux is negatively correlated with soil water (Lin *et al* 2009, Tenuta *et al* 2010). In these studies, the SM content affected N_2O emissions to the atmosphere, mainly indirectly by influencing the soil aeration, soil REDOX conditions, the activity of soil microorganisms and soil N_2O diffusion. In general, there is an optimum range of SM for N_2O emissions (Zheng *et al* 2003). In dry soils, nitrification is the

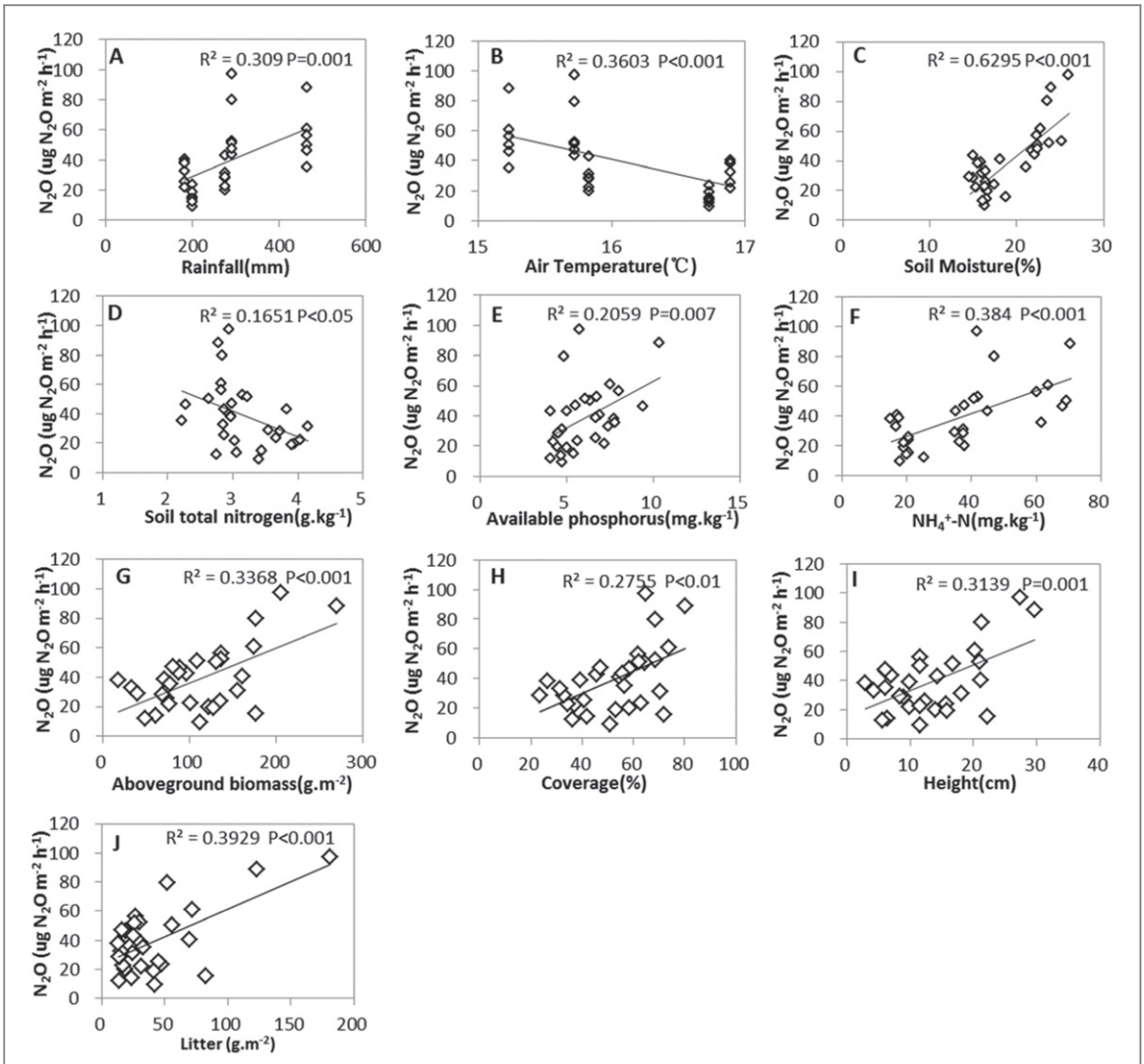

Figure 5. Relationships between the mean soil N_2O fluxes and meteorological factors (rainfall, air temperature), soil factors (soil moisture, soil total nitrogen, soil available phosphorus, and NH^{4+}-N) and vegetation factors (AGB, cover, height and litter) from all plots.

dominant process for N_2O production. However, in wetter soils, denitrification is the dominant process for N_2O production. With the increase in SM, the soil oxygen supply decreases and denitrifying bacteria gradually increase the proportion of N_2O emissions; thus, under the condition of high moisture content, the production of N_2O is not proportional to the soil water content (Mummey *et al* 1994). In our studies, we found that the N_2O flux increased rapidly when the SM was greater than 20%. When the SM was favourable, N and C availability became important (Corre *et al* 1996). Nitrous oxide emissions increased with soil NO_3^- and NO_4^+ concentrations, which is consistent with Tenuta *et al* (2010), who found that N_2O emissions from slurry treatments applied to hayed paddocks at the same site were positively correlated with the soil NO_3^- concentration (Tenuta *et al* 2010). In our studies, there were negative relationships between soil N_2O flux and TN. However, our results do not agree well with those of of Ri *et al* (2003) who noted that N_2O fluxes from the typical steppe ecosystems in Inner Mongolia generally decreased with decreasing SOC and TN contents (Ri *et al* 2003). These authors reported that the soils with higher total C and N contents typically emitted more N_2O than the grassland soils with lower total C and N contents. To specifically address the environmental conditions in the Inner Mongolian Hulunber meadow steppe, we also considered the effects of AGB, plant height and plant coverage on water capture, with denser and taller vegetation being a major controller of the potential water infiltration during plant growth periods. Sun and Li (2001) who noted that SM had the negative correlation with community evapotranspiration, which had positive correlation with community evaporation. And the studies also showed that the evaporation decreased with the increase of plant biomass, while transpiration showed the opposite trend (Sun and Li 2001). Therefore, plant biomass may increase evapotranspiration and it decrease evaporation of soil moisture from bare to near bare soil, i.e., evaporation is greater than evapotranspiration such

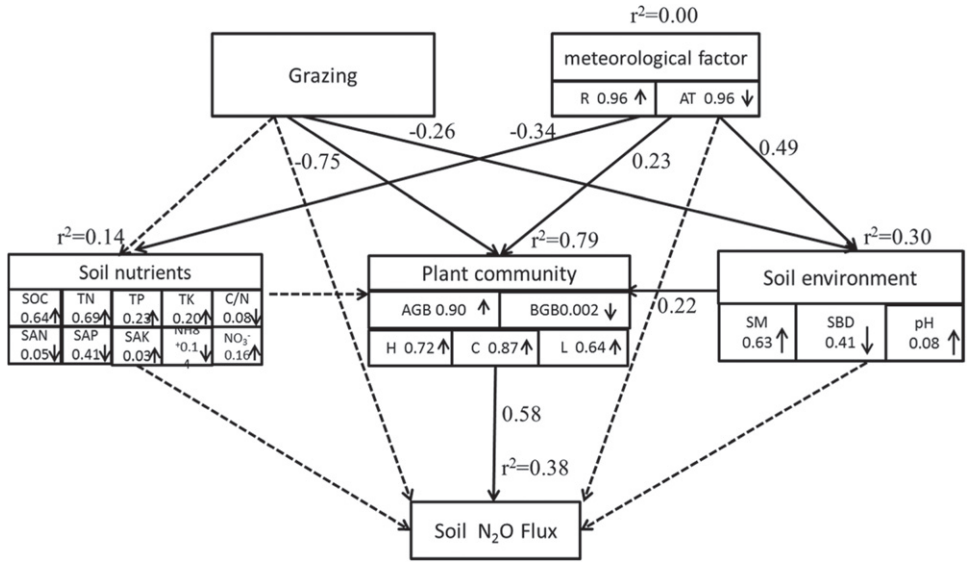

Figure 6. Final model results of structural equation modelling (SEM) analysis for the grazing and meteorological factor effects on soil N_2O emission or via pathways of plant community, soil environment, and soil nutrients. Square boxes denote variables included in the models. Plant community variables include AGB (above-ground biomass), C (coverage), H (height), BGB (below-ground biomass) and L (litter); soil environment variables include SBD (soil bulk density), soil pH and SM (soil moisture); soil nutrient variables include SOC (soil organic carbon), TN (total nitrogen), TP (total phosphorus), TK (total potassium), SAN (soil available nitrogen), SAP (soil available phosphorus), SAK (soil available potassium), C/N (Carbon to Nitrogen Ratio), soil NH_4^+ −N and NO_3^- −N. The symbols '↑' and '↓' indicate a significant increase or decrease, respectively, in the response of the variables to grazing. The plant community, soil nutrients, soil environment and meteorological factor are PC1 using principal component analysis (PCA). The value within each box indicates the mean response to grazing over different levels of intensity (i.e. 0, 0.23, 0.34, 0.46, 0.69 and 0.92 AU ha −1). Results of model fitting: (Chi-square = 0.306, P = 0.858, d.f. = 2, CMIN/df = 0.153, GFI = 0.999, AGFI = 0.988, RMR = 0.01,PGFI = 0.095, FI = 0.996, RFI = 0.968, FMIN = 0.003, RMSEA = 0). Solid arrows denote the directions and effects that were significant ($P < 0.05$); dashed arrows represent the directions and effects that were not significant ($P > 0.05$). r^2 values associated with response variables indicate the proportion of variation explained by relationships with other variables. Values associated with solid arrows represent standardized path coefficients.

that shading causes the soil to retain more moisture. In our study, we found that the soil N_2O emission rate was significantly positively correlated with AGB, plant cover, plant height and litter across all sites. Hence, our results showed that grazing can also affect plant physiological processes and resource allocation between shoots and roots and therefore alter soil N_2O emissions. Plant height and the amount of litter present are more important in regulating the seasonal dynamics of soil N_2O fluxes in this temperate meadow steppe. Therefore, based on the above results, this further confirmed the result of the stepwise regressions showing that SM, NO_3^- −N, plant height, air temperature, soil AN and litter played a major role in soil N_2O flux.

4.3. Key factors influencing the N_2O fluxes with grazing intensity

The SEM analysis revealed that grazing directly altered the plant community, which further influenced the soil N_2O emissions. In contrast, the soil environment and soil nutrients exerted indirect influences on the soil N_2O fluxes. There are, however, uncertainties with respect to the ecological linkages between soil N_2O fluxes and environmental factors because plant and soil factors are responsive to grazing. For example, it has been documented that the intra- and inter-annual variations in rainfall are key climatic factors controlling ANPP in semi-arid grasslands (Bai 1999). Intra- and inter-annual variations in climate factors have been shown to be the key climatic factors that control the fluctuations in soil N_2O fluxes. Therefore, the intra- and inter-annual variations in ABG may affect the strength of the linkages. Some previous studies found that the correlations were different between the annual soil N_2O flux and SM (Holst *et al* 2007; Lin *et al* 2009). Therefore, more research is needed to understand how grazing intensity affects the linkages between soil N_2O fluxes and environmental factors under intra- and inter-annual variations in climatic conditions. Our study provides new insights into the mechanisms and pathways of the effects of grazing on the soil N_2O emission rates in an Inner Mongolian meadow steppe.

5. Conclusions

This study to our knowledge has taken the lead in examining the effects of grazing on plant community, soil nutrients, soil environment and soil N_2O emissions in a Hulunber *L. chinensis* meadow steppe ecosystem. There were substantial temporal fluctuations in the N_2O flux in the growing seasons for different grazing intensities. The peak N_2O flux during

the growing season usually occurred after an natural rainfall. The soil N_2O flux of grasslands was substantially affected by different grazing intensities. Significant negative linear relationships were found between soil N_2O fluxes and grazing intensity over the period of the experiment.

Using SEM analysis, this study provides an important reference for better understanding the mechanisms and identifying the pathways of grazing effects on soil N_2O emission rates, and the key drivers factors plant community and soil environment within the N cycle that are mostly likely to affect N_2O emissions in the Inner Mongolian meadow steppe.

Acknowledgments

We are grateful to many colleagues with the Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences and the Hulunber Grassland Ecosystem Research Station for the assistance with field observations and sample collection. This work was funded by the following projects: the National Natural Science Foundation of China (41471093, 41201199), the International Science and Technology Cooperation Project (2012DFA31290), public sector projects in the Ministry of Agriculture (201303060), basic special funding from the central public welfare scientific research institutes (No. 402-11) and special funding for the modern agricultural technology system of the Chinese Ministry of Agriculture.

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