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during the growing seasons in the Hulunber meadow steppe of China

Grazing intensity and driving factors affect soil nitrous oxide fluxes

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#### 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  $0.92 \text{ AU ha}^{-1}$ ) with three replicates were established, and observations were conducted from 2010 to 2014. Our results showed that substantial temporal fluctuations in N<sub>2</sub>O flux occurred amongst the different grazing intensities, with peak N<sub>2</sub>O fluxes after natural rainfall. Grazing had a long-term effect on the soil N<sub>2</sub>O flux in the grasslands. After 4–5 years of grazing, the N<sub>2</sub>O 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<sub>2</sub>O flux generally declined significantly with increasing grazing intensity. The soil N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O fluxes. Our findings provide an important reference for better understanding of the mechanisms and identifying the pathways of grazing effects on soil N<sub>2</sub>O emission rates, and the key drivers plant community and soil environment within the nitrogen cycle that are mostly likely to affect N2O 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<sub>2</sub>O) is the third most important greenhouse gas globally (de Klein *et al* 2014). N<sub>2</sub>O 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 N2O emissions (Jackson et al 2015). Furthermore, grazing also affects the rates of soil N cycling processes that are direct or indirect sources of N<sub>2</sub>O (Davidson and Kanter 2013). de Klein et al (2014) indicated that the excreta deposited by grazing animals are the largest source of N<sub>2</sub>O 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 N2O 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  $10 \times 10^6$  km<sup>2</sup>; 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<sub>2</sub>O 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<sub>2</sub>O 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  $(300 \times 167 \text{ m})$  each with six stocking densities (0.00, 0.23, 0.34, 0.46, 0.69 and 0.92





AU ha<sup>-1</sup>; 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<sup>-1</sup>, 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  $3.73-4.08 \text{ g kg}^{-1}$ , and the organic carbon concentration of the surface soil was  $36.4-39.5 \text{ g kg}^{-1}$ ; these values did not vary significantly between the different grazing intensity plots (Yan et al 2014).

#### 2.3. Measurement of N<sub>2</sub>O fluxes

The N<sub>2</sub>O 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  $\times$ width  $\times$  height = 50 cm  $\times$  50 cm  $\times$  10 cm) that was driven into the soil (installed prior to treatment initiation in August 2009) and a stainless steel chamber (without bottom, length  $\times$  width  $\times$  height = 50 cm  $\times$  50 cm  $\times$  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 N2O 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<sub>2</sub>O 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 N2O 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<sub>2</sub>O 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, VAI-SALA, 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





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  $0.5 \text{ mol } \text{L}^{-1}$  sodium bicarbonate extraction and the soil available potassium (SAK) was determined using NH<sub>4</sub>OAc 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<sup>2</sup> 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  $\times$  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  $\times$  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<sub>2</sub>O flux was calculated, and

the plot values represent means  $(n = 3) \pm$  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 N2O 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<sub>2</sub>O 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<sub>2</sub>O flux on the Hulunber meadow steppe. The path diagram was structured such that measurements of N<sub>2</sub>O 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	$1631\pm80.9a$	$24\pm1.7a$	70.6±0.4a	18.1 ± 1.0a
	G0.23	$122\pm15.5ab$	$1446\pm54.2a$	$18\pm1.0bc$	$58.3\pm2.6b$	$14.1 \pm 0.8 bc$
	G0.34	$97\pm18.9bc$	$1194\pm134.0a$	$19\pm2.3b$	$45.6\pm5.0c$	$14.3\pm0.9b$
	G0.46	$102\pm7.1bc$	$1907\pm495.9a$	$17 \pm 0.9 bcd$	$34.6\pm2.0d$	$11.6\pm0.9$ cd
	G0.69	$69 \pm 11.6c$	$1481\pm359.6a$	$15\pm1.2cd$	$33.0\pm3.4d$	$9.1 \pm 1.0 de$
	G0.92	$41 \pm 8.9$ cd	$1487\pm179.2a$	$14\pm0.1d$	$23.7\pm1.8e$	$8.4\pm0.6e$
2011	G0.00	177 ± 6.7a	929±178.6ab	83±5.0a	71.8±0.7a	$22.2\pm0.7a$
	G0.23	$136\pm5.7b$	$1000 \pm 152.4 ab$	$48\pm9.4b$	$62.9\pm2.9b$	$15.7\pm0.7b$
	G0.34	$129\pm7.2b$	$871 \pm 35.3 ab$	$41 \pm 1.6 bc$	$53.2\pm2.8c$	$15.8\pm0.9b$
	G0.46	$113 \pm 19.1b$	$1150\pm146.5a$	$42\pm 6.2bc$	$50.9 \pm 1.1c$	$11.6 \pm 1.2c$
	G0.69	$62\pm5.6c$	$885\pm 50.2ab$	$23\pm 8.4cd$	$42.0\pm3.0d$	$6.4\pm0.3d$
	G0.92	$49\pm7.8c$	$742\pm118.5b$	$14\pm4.0d$	$36.4\pm2.0d$	$5.7\pm0.5d$
2012	G0.00	$162 \pm 12.9a$	$1062 \pm 172.7a$	$69 \pm 8.2a$	$54.8 \pm 10.8a$	$21.1 \pm 1.8a$
2012	G0.23	$74 \pm 5.7b$	$1279 \pm 474.6a$	$45 \pm 3.0b$	$40.7 \pm 3.8ab$	$12.3 \pm 1.4b$
	G0.34	$77 \pm 1.1b$	$855 \pm 134.9a$	$31 \pm 9.2$ bc	$37.0 \pm 3.1b$	$10.0 \pm 0.7$ bc
	G0.46	$72 \pm 8.0b$	$928 \pm 88.8a$	$30 \pm 7.1 bc$	39.3 ± 3.1ab	$9.9 \pm 1.5 bc$
	G0.69	$34 \pm 5.7c$	$766 \pm 139.1a$	$15 \pm 3.9c$	$31.3 \pm 1.6b$	$4.3 \pm 0.6d$
	G0.92	$18 \pm 4.2c$	$784\pm91.7a$	$13 \pm 4.7c$	$26.5\pm4.9b$	$2.9\pm0.2d$
2013	G0.00	$271\pm23.6a$	1049±93.8a	123 ± 14.5a	$80.3 \pm 1.8a$	29.7 ± 3.5a
	G0.23	$175 \pm 9.9 \mathrm{b}$	$612\pm45.4bc$	$72\pm18.2b$	$74.0\pm2.2ab$	$20.2\pm1.0b$
	G0.34	$137 \pm 20.3 bc$	$810\pm10.1b$	$27 \pm 7.0$ cd	$61.9\pm0.6c$	$11.6\pm0.4c$
	G0.46	$131 \pm 24.2 bcd$	$733 \pm 32.8 bc$	$56 \pm 0.0 bc$	$64.6 \pm 6.2 bc$	$11.5 \pm 4.5c$
	G0.69	$89 \pm 4.5 cd$	$569\pm37.9c$	$19\pm0.0d$	$58.6 \pm 2.3c$	$6.2\pm0.9c$
	G0.92	$78\pm18.3d$	765 ± 145.5bc	$33\pm0.0cd$	$56.4\pm4.5c$	$6.2\pm2.2c$
2014	G0.00	$205\pm21.9a$	$1083 \pm 290.9a$	$181\pm49.7a$	64.8±2.5ab	$27.4 \pm 1.8a$
	G0.23	$176\pm20.9ab$	$951 \pm 108.1a$	$52\pm4.7b$	$68.6 \pm 3.9a$	$21.4\pm2.0b$
	G0.34	$137 \pm 20.7 bc$	991±111.6a	$30 \pm 4.7 b$	$68.5 \pm 1.6a$	$21.0\pm2.4b$
	G0.46	$110 \pm 16.5c$	$683\pm53.8a$	$26\pm3.4b$	$62.1\pm7.2ab$	$16.7 \pm 0.7 bc$
	G0.69	$88\pm21.0c$	$1092\pm124.4a$	$25\pm 8.5b$	$55.7\pm0.5bc$	$7.1\pm0.3d$
	G0.92	$81 \pm 15.7c$	$756\pm38.8a$	$16 \pm 1.7 b$	$47.2 \pm 3.3c$	$6.2\pm0.7$ d

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 buildup 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 $\rm N_2O$ 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<sub>2</sub>O fluxes were observed throughout the experiment for all grazing intensities (figure 3). In 2013 and 2014, the

fluxes of N<sub>2</sub>O from the soil consistently showed that the ungrazed (G0.00) and G0.23 treatments had higher N<sub>2</sub>O emission rates compared to the other grazing plots. The Hulunber soils acted as a source of soil N<sub>2</sub>O in all years (figures 3(A)–(E)). Overall, we found that the soil was a net source of N<sub>2</sub>O during the growing season. Our analysis further revealed that peak N<sub>2</sub>O fluxes during the growing season usually occurred after effective rainfall.

The mean annual N<sub>2</sub>O flux showed no significant differences between treatments in 2010 and 2011. However, the soil N<sub>2</sub>O flux was substantially influenced by grazing intensity (figure 4(A)) from 2012 onwards. There were significantly (p < 0.05) greater







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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O flux was shown to be significantly positively correlated with rainfall ( $R^2 = 0.309$ , p < 0.001), whereas significant negative







correlations were observed between N<sub>2</sub>O flux and air temperature (R<sup>2</sup> = 0.360, *p* < 0.001). There were significant positive linear relationships between soil N<sub>2</sub>O flux and SM (R<sup>2</sup> = 0.588, *p* < 0.001), SAP (R<sup>2</sup> = 0.206, *p* < 0.01), soil NH<sub>4</sub><sup>+</sup> -N (R<sup>2</sup> = 0.384, *p* < 0.001), AGB (R<sup>2</sup> = 0.337, *p* < 0.001), plant cover (R<sup>2</sup> = 0.164, *p* < 0.001), plant height (R<sup>2</sup> = 0.314, *p* < 0.01) and litter quantity (R<sup>2</sup> = 0.393, *p* < 0.001). In contrast, there were negative relationships between soil N<sub>2</sub>O flux and TN (R<sup>2</sup> = 0.148, *p* < 0.01). No significant relationship was detected between soil N<sub>2</sub>O 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<sub>2</sub>O flux, multiple regression tests were conducted. The results from these stepwise regressions showed that the N<sub>2</sub>O flux was primarily explained by SM, NO<sub>3</sub><sup>-</sup> -N, plant height, air temperature, soil AN and litter ( $R^2 = 0.927$ , F = 48.81, p < 0.001).

## 3.5. Pathways determining N<sub>2</sub>O 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 wellsuited 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 N2O emissions. Grazing and meteorological factors indirectly affected the soil N2O 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<sub>2</sub>O fluxes to grazing intensity Understanding the effects of grazing on soil N<sub>2</sub>O 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<sub>2</sub>O emission studies are of short duration and therefore may not accurately measure the total N<sub>2</sub>O 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<sub>2</sub>O. The peak N<sub>2</sub>O fluxes during the growing season usually occurred after natural rainfall, and the intensity of grazing substantially altered the soil N2O fluxes. Somewhat curiously, our results demonstrate that grazing decreased rather than increased soil N2O fluxes and showed that soil N2O fluxes decreased with an increasing grazing rate. Significant negative linear relationships were found between the soil N2O 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 N2O 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<sub>2</sub>O flux. Firstly, grazing changes the soil and environmental conditions that determine emissions of N<sub>2</sub>O 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 Temperature	0.463 -0.486	$0.494 \\ -0.548^{*}$	$0.754^{**}$ $-0.859^{**}$	$0.502 \\ -0.491$	$0.586^{*}$ -0.593 <sup>*</sup>	0.162 -0.222
Soil	SM SBD pH SOC TN TP TK C/N	$\begin{array}{c} 0.588^{*} \\ 0.267 \\ 0.070 \\ -0.096 \\ -0.315 \\ 0.055 \\ -0.185 \\ 0.416 \\ 0.416 \\ \end{array}$	$0.795^{**}$ 0.081 0.044 -0.050 -0.504 -0.213 $0.549^{*}$	$\begin{array}{c} 0.685^{**} \\ -0.081 \\ 0.377 \\ -0.218 \\ -0.422 \\ -0.465 \\ -0.080 \\ 0.340 \\ 0.340 \end{array}$	$\begin{array}{c} 0.615^{*} \\ 0.131 \\ 0.264 \\ -0.205 \\ -0.457 \\ -0.018 \\ -0.271 \\ 0.319 \\ 0.416 \end{array}$	$\begin{array}{c} 0.549^{*} \\ -0.114 \\ -0.283 \\ -0.589^{*} \\ -0.556^{*} \\ -0.258 \\ -0.340 \\ -0.021 \\ -0.021 \\ -0.021 \end{array}$	0.324 0.134 -0.066 0.226 -0.086 0.114 $-0.611^*$ 0.173
	SAN SAP SAK NH4 <sup>+</sup> -N NO3 <sup>-</sup> -N	$\begin{array}{c} 0.209 \\ -0.235 \\ -0.017 \\ 0.415 \\ -0.031 \end{array}$	-0.304 0.086 -0.253 $0.655^{**}$ $0.660^{**}$	-0.036 0.321 0.321 0.731** 0.057	-0.446 0.242 -0.357 0.562* 0.191	-0.097 0.304 -0.434 0.664** 0.205	-0.041 0.375 -0.303 0.088 0.196
Biological	AGB BGB Litter Coverage Height	0.438 -0.071 $0.683^{**}$ 0.157 0.445	$0.450 \\ -0.311 \\ 0.368 \\ 0.566^* \\ 0.810^{**}$	0.499 0.009 -0.268 $0.537^{*}$ 0.351	$0.284 \\ -0.519^* \\ 0.185 \\ 0.402 \\ 0.459$	0.511 -0.245 -0.025 0.487 -0.082	-0.065 -0.008 0.093 0.158 -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<sub>2</sub>O flux data (n = 15). \*p < 0.05; \*\*\*p < 0.01.

correlation between vegetation height and SM, thereby stimulating the soil N2O fluxes. Secondly, gas emissions from the soil to the atmosphere have a complicated delivery process. The N<sub>2</sub>O 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 N2O production rates from the soil. Thirdly, denitrification is the dominant process of N<sub>2</sub>O 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<sub>2</sub> before N<sub>2</sub>O emissions, which might result in a significant decrease in soil N2O fluxes.

### 4.2. Responses of factors towards controlling N<sub>2</sub>O 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<sub>2</sub>O flux was significantly positively correlated with rainfall, whereas significant negative correlations were observed between N<sub>2</sub>O flux and air temperature. Our analysis showed that rainfall rather than air temperature is the critical climatic factor determining soil N<sub>2</sub>O 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<sub>2</sub>O flux and the frequency of effective rainfall. Across all of the treatments and years, N<sub>2</sub>O emissions were significantly positively correlated with SM, SAP and soil  $NH_4^+$  – N. This indicates that N2O 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<sub>2</sub>O flux is negatively correlated with soil water (Lin et al 2009, Tenuta et al 2010). In these studies, the SM content affected N<sub>2</sub>O emissions to the atmosphere, mainly indirectly by influencing the soil aeration, soil REDOX conditions, the activity of soil microorganisms and soil N2O diffusion. In general, there is an optimum range of SM for N<sub>2</sub>O emissions (Zheng et al 2003). In dry soils, nitrification is the





dominant process for N<sub>2</sub>O production. However, in wetter soils, denitrification is the dominant process for N<sub>2</sub>O production. With the increase in SM, the soil oxygen supply decreases and denitrifying bacteria gradually increase the proportion of N<sub>2</sub>O emissions; thus, under the condition of high moisture content, the production of N<sub>2</sub>O is not proportional to the soil water content (Mummey et al 1994). In our studies, we found that the N2O 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<sub>2</sub>O emissions from slurry treatments applied to haved 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<sub>2</sub>O flux and TN. However, our results do not agree well with those of of Ri et al (2003) who noted that N<sub>2</sub>O 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<sub>2</sub>O 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 SBD (soil bulk density), soil pH and SM (soil moisture); soil nutrient variables include SGC (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<sub>4</sub><sup>+</sup> – N and NO<sub>3</sub><sup>-</sup> – 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 = 0.996, RFI = 0.996, RFI = 0.908, RMIN = 0.003, RMSEA = 0). Solid arrows denote the directions and effects that were not significant (*P* < 0.05); r<sup>2</sup> 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<sub>2</sub>O 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<sub>2</sub>O emissions. Plant height and the amount of litter present are more important in regulating the seasonal dynamics of soil N<sub>2</sub>O 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<sub>3</sub><sup>-</sup> – N, plant height, air temperature, soil AN and litter played a major role in soil N<sub>2</sub>O flux.

## 4.3. Key factors influencing the N<sub>2</sub>O 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). Intraand inter-annual variations in climate factors have been shown to be the key climatic factors that control the fluctuations in soil N<sub>2</sub>O 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<sub>2</sub>O 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 N2O 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<sub>2</sub>O 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.

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