Response of N₂O emission to water and nitrogen addition in temperate typical steppe soil in Inner Mongolia, China

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ARTICLE INFO

Article history:

Received 6 July 2014

Received in revised form 7 November 2014

Accepted 20 January 2015

Keywords:

Nitrous oxide
Nitrogen deposition
Precipitation
Temperate steppe
China

ABSTRACT

The Chinese steppe is undergoing a drastic increase in nitrogen (N) deposition, and the precipitation in this region is predicted to increase. However, the response of soil N₂O emissions to the coupling changes of precipitation and N deposition in grassland ecosystem has been seldom discussed. A manipulative field experiment was conducted to investigate the individual and interactive effects of precipitation increase and N deposition on soil N₂O efflux in semi-arid temperate grassland in Inner Mongolia during the growing seasons of 2010 and 2011. The treatments included four N addition levels [20 g N m⁻² y⁻¹ (N20), 10 g N m⁻² y⁻¹ (N10), 5 g N m⁻² y⁻¹ (N5), and a zero-N control (CK)] and two water application levels [natural precipitation for dry (D) and 15% increase of long-term mean annual precipitation for wet (W) treatments]. Results indicated that N and water addition both significantly increased soil N₂O effluxes ($P < 0.01$). The maximum N₂O emissions were observed within 2–3 days after N addition to all treatments, and the N₂O effluxes in W treatments were generally higher than in D treatments for the same N input level. For the treatments without N inputs, the N₂O emission peak of WCK was 9.8% higher than DCK in 2010. The effect of water addition on N₂O emission was more evident when more N fertilizer was applied. For the high N input treatments, the maximum N₂O emission of WN20 treatment was 222.6% higher than DN20 in 2010. The changes in N and water availabilities accounted for 91.3% (2010) and 75.6% (2011) of the N₂O cumulative efflux variation among different treatments ($P < 0.01$). The N₂O effluxes were significantly affected by the interactive effect between N and water in 2010 and 2011 ($P < 0.05$). Significant interannual variability in the cumulative N₂O emissions was observed, the cumulative N₂O emissions in 2011 were significantly lower than those in 2010 even though the summer of 2011 experienced higher rainfall ($P < 0.01$). Temperature also significantly influenced soil N₂O emission apart from the effects of water and N. The temperature change accounted for 41.3% (W) to 47.2% (D) of the temporal variations in N₂O emission during the relatively dry 2010. The combined changes in soil moisture, NH₄⁺-N, and temperature accounted for 43.1% (D) to 54.5% (W) of the temporal variations in N₂O emission in the relatively wet year of 2011.

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1. Introduction

Nitrous oxide (N₂O) is an important greenhouse gas that contributes to global warming and ozone destruction in the stratosphere (IPCC, 2007; Wuebbles, 2009). Many biotic and abiotic factors, especially the availability of N and water, intensively affect N₂O (Weier et al., 1993; Weitz et al., 2001; Dobbie and Smith, 2003). Anthropogenic N deposition has drastically increased globally since the Industrial Revolution

Abbreviations: D, dry treatments; W, wet treatments; WFPS, soil water-filled pore space.

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<http://dx.doi.org/10.1016/j.still.2015.01.008>

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because of fertilizer application and fossil fuel combustion, and it is estimated to double by 2030 (Matson et al., 2002; Galloway et al., 2008). Data reported by Liu et al. (2013) indicated that the total annual N bulk deposition rates increased significantly over time in China ($P < 0.001$), with an average annual increase of $0.41 \text{ kg N ha}^{-1}$. The increasing anthropogenic N deposition affects N balance in terrestrial ecosystems (Kugler et al., 2008; Jiang et al., 2010; Li et al., 2012), and accordingly, the production and emission of N_2O were inevitably influenced by the variations in the availability of ammonium and nitrate in the soil (Weier et al., 1993; Rafique et al., 2011).

In addition, soil moisture also an important factor that strongly influences the turnover, transference and emission of N_2O (Mummey et al., 1994; Jacinthe et al., 2000; Pihlatie et al., 2004; Jungkunst et al., 2008). Thirteen climate models from the Program for Climate Model Diagnosis and Inter comparison in the IPCC Fourth Assessment Report predicted that the rainfall in China will increase (IPCC, 2007), and the precipitation in North China will increase by 12–18% under the SRES A2 scenario (Jiang et al., 2008).

Temperate grassland is the largest terrestrial ecosystem in China, covering an area of approximately $3 \times 10^8 \text{ ha}$ and accounting for 40% of the national land area (Wang et al., 2005). The annual mean N_2O emission from grasslands in China was $76.5 \pm 12.8 \text{ Gg N}$, approximately 57% of which was from the temperate grassland soil. N_2O emission from grassland soil is an important part of the Chinese budget and even the global N_2O budget (Zhang et al., 2010). The N_2O emission in the temperate grassland in China is sensitive to the changes in rainfall and N deposition because of the arid or semi-arid climate and the N nutrient deficiency in this region. Numerous field studies have discussed the influence of soil moisture on N_2O efflux in Chinese temperate grassland (e.g., Dong et al., 2000; Chen et al., 2000; Du et al., 2006; Liu et al., 2010), and some studies have explored the relationship between N addition and N_2O efflux in this area (Zhang and Han, 2008; Peng et al., 2011). However, their conclusions regarding the effects of N addition on N_2O efflux varied because of the comprehensive effects of other climatic and environmental conditions, especially rainfall (Xu et al., 2008; Barton et al., 2008; Smith et al., 2012; Cantarel et al., 2011). For example, Peng et al. (2011) pointed out that the pulse of N_2O efflux observed after N fertilizer application was smaller than those obtained in other studies (Dobbie and Smith, 2003; Jones et al., 2007). This phenomenon could have been caused by the drought soil condition in the study area. Thus, the relationship between soil water and N should be considered when we predict the N_2O effluxes from the temperate grassland soils under global change conditions. However, little information so far has been provided on the interactive effects of water and N on soil N_2O emission in Chinese temperate grasslands. Thus, an in situ N and water addition experiment was conducted in a semi-arid temperate typical steppe in Inner Mongolia, China. The objectives of this study are as follows: (1) to evaluate quantitatively the seasonal and interannual change in the N_2O emission from temperate grassland soils in China based on the hypothesis of increased precipitation and N deposition; and (2) to probe the interaction of soil moisture and N on N_2O efflux.

Table 2

The time and amounts of water input.

Amounts of water addition (mm)	Dates of water addition	
	2010	2011
11.2	2010/06/24	2011/06/28
9.7	2010/07/21	2011/07/17
9.7	2010/08/03	2011/08/03
7.6	2010/08/16	2011/08/17
7.6	2010/08/29	2011/08/30
6.5	2010/09/09	2011/09/10

2. Materials and methods

2.1. Site description

The experiment site is located at a *Leymus chinensis* steppe ($43^\circ 26'$ to $44^\circ 39' \text{N}$, $115^\circ 32'$ to $117^\circ 12' \text{E}$, 1265 m a.s.l.) in the Baiinxile Pasture, Inner Mongolia, China. The climate is a semi-arid temperate climate with a mean annual temperature of -0.4°C , ranging from -21.41°C in January to 18.53°C in July. The mean annual precipitation is about 350–450 mm (70% falling between July and September). The soil is classified as chestnut soil in Chinese classification or Calcic Orthic Aridisol in soil taxonomy classification with a texture comprised of 60% sand, 21% clay, and 19% silt, other main soil properties are shown in Table 1. The experiment site was used for grazing before enclosure, and the grazing intensity is about 2.25 sheep per hectare.

2.2. Experimental design

The experiments were performed from 20 May 2010 to 20 October 2011. A randomized block design was used to prepare three replicates of eight treatments to examine the effects of water and N addition on N_2O emission. The area of each plot was $8 \text{ m} \times 8 \text{ m}$. We added N in late June and early August every year as NH_4NO_3 based on the current N deposition level and its predicted change in the next 30 years (Galloway and Cowling, 2002). The dose of N addition in the manipulative experiments ranged from 50 to $200 \text{ kg N ha}^{-1} \text{ y}^{-1}$. The simulated studies applied large doses of N over a short period, with the assumption that this method effectively mimics small doses of N over a long period (Dise and Stevens, 2005). For example, assuming that N is not lost by different means, such as seepage, immobilization in deep soil layers, or volatilization, in a N addition experiment of $100 \text{ kg N ha}^{-1} \text{ y}^{-1}$ over 5 years. The cumulative N is 500 kg N ha^{-1} or approximately 25 years of N deposition at a rate of $22.6 \text{ kg N ha}^{-1} \text{ y}^{-1}$, which was the average annual bulk deposition of N in China in 2010 (Dise and Stevens, 2005; Liu et al., 2013). The annual water addition was based on the hypothesis that annual precipitation increased by 15% (12–18% predicted by IPCC-AR4 models), amounting to 51.68 mm (approximately 15% of the long-term mean annual precipitation of 344.5 mm). Based on the assumption that the increased precipitation all fell in the rainy season (from June to September), added water was applied during the four months according to the rainfall proportion of each month versus that of the four months (Table 2).

Table 1

Soil physiochemical properties of the sampling site.

Soil depth (cm)	Organic C (g kg^{-1})	Total N (g kg^{-1})	C/N	pH	Soil bulk density (g cm^{-3})
0–10	16.520 ± 1.049	1.426 ± 0.272	11.6338	7.03	1.284 ± 0.012
10–20	14.047 ± 1.412	1.354 ± 0.277	10.37445	7.09	1.336 ± 0.033
20–30	13.831 ± 1.652	1.206 ± 0.289	11.52583	7.23	1.347 ± 0.030

The experiment included 8 treatments randomly assigned to 24 plots: (1) control, without N and without water addition, DCK; (2) plus N at a low rate of $5 \text{ g N m}^{-2} \text{ y}^{-1}$ without water, DN5; (3) plus N at a medium rate of $10 \text{ g N m}^{-2} \text{ y}^{-1}$ without water, DN10; (4) plus N at a high rate of $20 \text{ g N m}^{-2} \text{ y}^{-1}$ without water addition, DN20; (5) plus water without N, WCK; (6) plus N at a low rate of $5 \text{ g N m}^{-2} \text{ y}^{-1}$ and water, WN5; (7) plus N at a medium rate of $10 \text{ g N m}^{-2} \text{ y}^{-1}$ and water, WN10; and (8) plus N at a high rate of $20 \text{ g N m}^{-2} \text{ y}^{-1}$ and water, WN20. The fertilizers were split into halves and applied in late June and early August each year (24 June and 1 August in 2010, and 28 June and 3 August in 2011). For the treatments with water addition (WCK, WN5, WN10, and WN20), water was manually applied from a water tank through a hose and dispersed evenly into the experimental plots (flow rate: 10 L/min).

2.3. Sample collection

N_2O gas was collected using a static closed opaque chamber, which was made of 8 mm-thick black acrylic material with a reflective tinfoil surface covering on each side, and the inner dimensions of the chamber were 50 cm (length) $\times$ 40 cm (width) $\times$ 30 cm (height) (Dong et al., 2000; Qi et al., 2007; Peng et al., 2011). Samples were collected before and on the 3rd, 7th, 11th, 15th, 25th day after N-addition from all plots and twice in the other months during the two year experimental period. Gas samples, about 200 mL each time, were extracted from the chamber at 0, 7, 14, and 21 min after closing the chamber and placed into the polyethylene-coated aluminum bags for N_2O gas concentration analysis.

The concentration of N_2O was analyzed using a gas chromatograph (Hewlett-Packard 6890) equipped with an electron capture detector. During gas sampling, the internal temperature of the chamber, air temperature and soil temperature were recorded simultaneously with temperature sensor, mechanical ventilated thermometer and digital thermo detector respectively. During gas sampling, soil samples at 0–10 cm, 10–20 cm, and 20–30 cm depths were collected to analyze the soil water contents, and those at 0–10 cm depths were collected to analyze the soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents. The soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents were measured by an automated flow injection analyzer (Bran+Lubbe, Germany). The soil water content was determined by using the oven-drying method at 105°C for 24 h and was converted to WFPS combined with bulk density and porosity as Eq. (1):

$$\text{WFPS} = \frac{\text{soil water contents} \times \text{bulk density}}{100} \times \frac{100}{(1 - \text{bulk density/particle density})} \quad (1)$$

2.4. Data analysis

The N_2O efflux was calculated using the method by Dong et al. (2000) and Qi et al. (2006), as follows:

$$F_{\text{N}_2\text{O}} = \frac{\Delta m}{\Delta t} D \frac{V}{A} = h_c D \frac{\Delta m}{\Delta t} \quad (2)$$

where, $F_{\text{N}_2\text{O}}$ refers to N_2O efflux ($\mu\text{g m}^{-2} \text{ h}^{-1}$); V is the volume of the sampling chamber (m^3); A is the land area covered by the chamber (m^2); D is gas density of the chamber (mol m^{-3}); $D = n/v = P/RT$, n is the molar mass of N_2O (g mol^{-1}), v is the gas volume (m^3), P is the air pressure (Pa), T is the temperature inside the chamber (K), and R is the gas constant ($\text{J mol}^{-1} \text{ K}^{-1}$); $\Delta m/\Delta t$ denotes the linear slope of the concentration change over the measurement period; h_c represents the height of the sampling chamber (m).

For each sampling, means and standard deviations of N_2O fluxes were calculated, and the plot values represented means ($n=3$) $\pm$ standard error (SE). The cumulative N_2O fluxes were calculated by interpolating the N_2O fluxes measured between sampling periods, using Matlab 7.0. The statistical analyses, such as repeated two-way analysis of variance, independent samples t test, and Pearson's correlation, were conducted by SPSS 17.0. Graphs were prepared using Origin 8.0.

3. Results

3.1. Temporal dynamics of soil N_2O effluxes

Significant seasonal variations ($P < 0.01$) in N_2O efflux were observed in different water- and N-treated soils in 2010 (Table 3). Nitrogen and water addition significantly influenced N_2O effluxes ($P < 0.01$) and the temporal variation of N_2O effluxes ($P < 0.05$). The peak N_2O effluxes were observed 2–3 days after N addition. After the first N and water addition on 24 June 2010, the peak effluxes of N_2O ranged from $28.77 \pm 2.56 \mu\text{g m}^{-2} \text{ h}^{-1}$ (DCK) to $162.55 \pm 96.90 \mu\text{g m}^{-2} \text{ h}^{-1}$ (WN20), which was the highest N_2O emission flux for all the treatments during the two-year experimental period. The

Table 3
Soil N_2O fluxes ($\mu\text{g h}^{-1} \text{ m}^{-2}$) from the experimental plots under different N and water treatments in 2010.

Date	Soil N_2O fluxes ($\mu\text{g h}^{-1} \text{ m}^{-2}$)							
	DCK	DN5	DN10	DN20	WCK	WN5	WN10	WN20
2010/5/20	4.58 $\pm$ 7.64							
2010/6/24	1.58 $\pm$ 2.61ab ^a	6.30 $\pm$ 0.20a	0.46 $\pm$ 3.19ab	5.95 $\pm$ 1.30a	7.46 $\pm$ 0.75b	3.25 $\pm$ 2.67a	0.72 $\pm$ 1.83ab	0.98 $\pm$ 3.10a
2010/6/26	28.77 $\pm$ 2.56a	34.79 $\pm$ 8.95a	47.51 $\pm$ 9.73a	50.38 $\pm$ 9.48a	31.59 $\pm$ 5.29a	36.23 $\pm$ 3.02a	76.04 $\pm$ 16.22ab	162.55 $\pm$ 96.90b
2010/7/1	5.94 $\pm$ 0.40ab	−0.18 $\pm$ 6.50ab	1.57 $\pm$ 1.01a	2.34 $\pm$ 4.30a	17.96 $\pm$ 5.69c	−0.14 $\pm$ 1.99a	16.12 $\pm$ 4.13bc	35.59 $\pm$ 6.39d
2010/7/5	1.65 $\pm$ 4.32a	5.77 $\pm$ 1.50ab	22.84 $\pm$ 4.83bc	31.71 $\pm$ 2.14c	17.82 $\pm$ 2.06bc	21.79 $\pm$ 3.95bc	32.86 $\pm$ 3.69 cd	54.69 $\pm$ 18.28cd
2010/7/9	−0.64 $\pm$ 0.96a	12.73 $\pm$ 0.65b	25.26 $\pm$ 0.65c	38.23 $\pm$ 5.10d	−0.34 $\pm$ 6.09a	17.56 $\pm$ 0.61bc	28.38 $\pm$ 7.88bc	46.76 $\pm$ 7.75d
2010/7/19	1.96 $\pm$ 1.97a	7.09 $\pm$ 1.50a	23.52 $\pm$ 1.09b	33.36 $\pm$ 10.27b	2.70 $\pm$ 0.84a	7.96 $\pm$ 0.83a	26.95 $\pm$ 3.60b	34.32 $\pm$ 11.43b
2010/8/5	1.32 $\pm$ 0.40a	15.35 $\pm$ 1.75bc	30.28 $\pm$ 9.89c	40.78 $\pm$ 8.13d	3.09 $\pm$ 4.85ab	17.68 $\pm$ 1.83bc	31.14 $\pm$ 6.05c	44.31 $\pm$ 10.01d
2010/8/9	3.56 $\pm$ 3.32a	12.62 $\pm$ 2.08b	21.72 $\pm$ 2.10 cd	25.79 $\pm$ 2.82cd	3.73 $\pm$ 2.31a	13.98 $\pm$ 0.38b	20.12 $\pm$ 3.54bc	27.22 $\pm$ 3.39d
2010/8/12	8.10 $\pm$ 6.94a	11.10 $\pm$ 13.85bc	20.63 $\pm$ 4.03bc	36.71 $\pm$ 2.53c	7.55 $\pm$ 1.31ab	15.36 $\pm$ 2.70ab	24.86 $\pm$ 5.44c	33.57 $\pm$ 0.19c
2010/8/16	1.52 $\pm$ 1.16a	5.25 $\pm$ 0.92ab	10.36 $\pm$ 0.50ab	17.70 $\pm$ 17.02ab	1.93 $\pm$ 3.68a	14.23 $\pm$ 3.00ab	20.84 $\pm$ 6.03b	29.09 $\pm$ 5.98c
2010/8/29	1.36 $\pm$ 0.67a	6.41 $\pm$ 1.03ab	16.42 $\pm$ 3.30c	20.10 $\pm$ 0.42c	0.29 $\pm$ 3.52d	14.48 $\pm$ 0.13bc	21.10 $\pm$ 2.51c	28.46 $\pm$ 2.46c
2010/9/9	2.41 $\pm$ 0.07ab	4.37 $\pm$ 1.36ab	16.03 $\pm$ 0.27abc	20.60 $\pm$ 6.04bc	0.50 $\pm$ 7.94a	4.83 $\pm$ 0.27ab	15.06 $\pm$ 3.04abc	26.15 $\pm$ 20.26c
2010/9/19	3.24 $\pm$ 1.44a	4.19 $\pm$ 1.47a	3.44 $\pm$ 1.88ab	2.76 $\pm$ 2.31ab	3.52 $\pm$ 0.09a	3.47 $\pm$ 0.50b	3.11 $\pm$ 0.17a	3.88 $\pm$ 0.83a
2010/10/5	1.30 $\pm$ 5.47a	−0.07 $\pm$ 0.88ab	2.44 $\pm$ 2.39b	−1.78 $\pm$ 0.81ab	1.02 $\pm$ 2.28b	0.83 $\pm$ 1.69ab	1.07 $\pm$ 1.94ab	3.05 $\pm$ 0.10b
2010/10/18	2.40 $\pm$ 0.13a	2.31 $\pm$ 2.77a	2.30 $\pm$ 2.99a	3.03 $\pm$ 1.65a	1.03 $\pm$ 3.89a	1.75 $\pm$ 3.36a	3.26 $\pm$ 2.28a	5.38 $\pm$ 1.82a

^a Different letters indicate significant difference between different treatments in the same sampling date ($P < 0.05$).

maximal effluxes increased as the N input rate increased. Relative to the N₂O emission in DCK treatment, the highest N₂O efflux in DN10, DN20, WN10 and WN20 treatments were increased for 65.1%, 75.1%, 164.3% and 465.0%, respectively ($P < 0.01$). The higher N₂O effluxes and obvious differences among treatments persisted until the middle of September, thereafter, no significant difference was observed between any two treatments.

Nitrogen and water addition in 2011 also significantly influenced N₂O efflux and its temporal variation ($P < 0.01$) (Table 4). However, the peak N₂O effluxes after N addition, ranging from $5.47 \pm 0.20 \mu\text{g m}^{-2} \text{h}^{-1}$ (DCK) to $77.51 \pm 36.36 \mu\text{g m}^{-2} \text{h}^{-1}$ (WN20), were smaller than those in 2010. Compared with the DCK treatment, DN20 and WN20 treatments showed significant increases ($P < 0.05$) in N₂O emission peaks, the increments were 432.7% for DN20 and 1317.0% for WN20. The N₂O effluxes began to decrease from 10 September 2011, and the effluxes varied between $-0.28 \pm 3.19 \mu\text{g m}^{-2} \text{h}^{-1}$ (DN5 and DN10) and $0.18 \pm 1.90 \mu\text{g m}^{-2} \text{h}^{-1}$ (WCK) on 20 October 2011, showing insignificant differences between any two treatments.

Water addition showed significant influence on N₂O effluxes ($P < 0.01$) in both 2010 and 2011 (Tables 3 and 4). The N₂O effluxes in W treatments were generally higher than in D treatments for the same N input level. The effect of water addition on N₂O emission was more evident when more N fertilizer was applied. In 2010, relative to the DCK treatment, the N₂O emission peak of WCK has increased for 9.8%. For the high N input treatments, the increment of N₂O emission peaks from DN20 to WN20 was 222.6%. In 2011, the N₂O emission peak of WCK treatment was 24.3% higher than DCK treatment. For high N input treatments, the N₂O emission peak of WN20 was 166.0% higher than DN20 treatment.

The cumulative N₂O fluxes were calculated for the different treatments in 2010 (Fig. 1a) and 2011 (Fig. 1b). In 2010, the cumulative N₂O fluxes in different treatments were between $0.066 \text{ kg N ha}^{-1}$ (DCK) and $0.534 \text{ kg N ha}^{-1}$ (WN20). Relative to the DCK treatment, the increments of cumulative N₂O fluxes of other treatments were between 47.4% (WCK) and 701.5% (WN20). Fig. 1b shows that the cumulative N₂O fluxes of the growing season, varying from $0.052 \text{ kg N ha}^{-1}$ (DCK) to $0.316 \text{ kg N ha}^{-1}$ (WN20) in 2011, and relative to the DCK treatment, the increments of cumulative N₂O fluxes of other treatments were between 45.8% (WCK) and 501.5% (WN20), respectively. The cumulative N₂O

Fig. 1. Cumulative N₂O fluxes under different water and N treatments in the growing seasons of 2010 (a) and 2011 (b). Bars with different letters denote treatments that differ significantly at $P < 0.05$.

emissions were significantly smaller than those of corresponding treatments in 2010 ($P < 0.01$). Compared with 2010, the cumulative N₂O fluxes in 2011 were decreased in the range of 21.0% (DCK)–60.8% (DN10).

The results of MANOVA (LSD test was used) showed that both water and N addition significantly increased the cumulative N₂O fluxes over the growing season ($P < 0.01$) (Table 5), and 91.3% and 75.6% of these cumulative N₂O emission variations could be explained by the changes in water and N addition respectively in 2010 and 2011 ($R^2 = 0.913$ and $R^2 = 0.756$, respectively, $P < 0.01$). In addition, two-way ANOVA showed that the N₂O effluxes were insignificantly affected by the interactive effect between N and water in 2010 and 2011 ($P > 0.05$), but the interaction of water and N was significant when we analyzed the experimental data of two years as a whole ($P < 0.05$).

3.2. Variations in environmental factors

The WFPS of the soil in the D and W treatments in 0–10 cm soil layers are shown in Fig. 2. Distinct differences in WFPS were

Table 4
Soil N₂O fluxes ($\mu\text{g h}^{-1} \text{m}^{-2}$) from the experimental plots under different N and water treatments in 2011.

Date	Soil N ₂ O fluxes ($\mu\text{g h}^{-1} \text{m}^{-2}$)							
	DCK	DN5	DN10	DN20	WCK	WN5	WN10	WN20
2011/5/12	$-0.26 \pm 1.03a^a$	$-0.14 \pm 2.12a$	$-0.33 \pm 1.82a$	$0.16 \pm 1.03a$	$0.05 \pm 2.25a$	$-0.50 \pm 0.80a$	$-0.08 \pm 0.67a$	$0.11 \pm 0.86a$
2011/6/20	$4.26 \pm 1.63a$	$4.78 \pm 1.64a$	$4.73 \pm 0.09a$	$4.57 \pm 0.12a$	$4.35 \pm 0.98a$	$6.50 \pm 0.72ab$	$7.56 \pm 0.09b$	$7.76 \pm 1.38b$
2011/6/28	$3.40 \pm 1.53a$	$3.71 \pm 0.33a$	$3.57 \pm 3.67a$	$3.60 \pm 0.62a$	$3.11 \pm 5.06a$	$5.57 \pm 0.40a$	$6.14 \pm 0.09a$	$6.84 \pm 2.14a$
2011/7/2	$5.47 \pm 0.20a$	$12.20 \pm 4.61a$	$14.02 \pm 0.14a$	$29.14 \pm 7.93a$	$6.80 \pm 1.59a$	$17.81 \pm 2.95a$	$24.57 \pm 5.57a$	$77.51 \pm 36.36b$
2011/7/6	$3.93 \pm 0.29a$	$4.02 \pm 2.48a$	$6.90 \pm 3.34a$	$11.98 \pm 2.83a$	$4.95 \pm 2.61a$	$4.88 \pm 0.21a$	$9.48 \pm 2.73a$	$34.98 \pm 18.19b$
2011/7/9	$5.00 \pm 0.20a$	$12.98 \pm 5.59ab$	$14.43 \pm 0.23a$	$26.14 \pm 11.12b$	$6.66 \pm 1.07ab$	$14.49 \pm 0.26ab$	$15.59 \pm 0.24ab$	$41.94 \pm 18.77c$
2011/7/14	$2.76 \pm 0.06a$	$7.10 \pm 0.85a$	$9.01 \pm 2.41a$	$12.30 \pm 5.69ab$	$3.55 \pm 1.14a$	$8.67 \pm 0.07a$	$10.83 \pm 1.26ab$	$20.75 \pm 9.52b$
2011/7/23	$4.70 \pm 1.07a$	$8.60 \pm 1.32a$	$10.37 \pm 1.39a$	$13.04 \pm 2.43a$	$5.29 \pm 0.67a$	$9.20 \pm 1.71a$	$13.55 \pm 3.34ab$	$23.15 \pm 8.76b$
2011/8/3	$1.32 \pm 0.62a$	$2.09 \pm 2.14ab$	$2.40 \pm 1.32ab$	$3.72 \pm 0.74ab$	$1.12 \pm 0.07a$	$3.10 \pm 1.78ab$	$3.42 \pm 1.59ab$	$5.78 \pm 2.57b$
2011/8/5	$2.62 \pm 0.01a$	$4.62 \pm 1.21a$	$4.47 \pm 1.72a$	$9.31 \pm 3.57a$	$3.89 \pm 0.52a$	$5.19 \pm 0.29a$	$7.73 \pm 0.00a$	$18.59 \pm 6.63b$
2011/8/8	$3.72 \pm 1.03a$	$5.82 \pm 0.56ab$	$7.84 \pm 2.68ab$	$13.05 \pm 7.31b$	$4.43 \pm 1.56ab$	$8.48 \pm 1.86a$	$12.47 \pm 0.00b$	$23.53 \pm 4.74c$
2011/8/10	$0.19 \pm 0.08a$	$1.90 \pm 0.50ab$	$3.77 \pm 0.12bc$	$5.00 \pm 0.02bc$	$3.29 \pm 2.22bc$	$5.84 \pm 1.02ce$	$8.88 \pm 1.32e$	$16.15 \pm 2.56f$
2011/8/14	$6.41 \pm 4.70a$	$11.05 \pm 0.00ab$	$14.25 \pm 0.98ab$	$17.28 \pm 4.44ab$	$17.68 \pm 7.33abc$	$20.60 \pm 14.13abc$	$23.75 \pm 2.34bc$	$32.61 \pm 2.48c$
2011/8/18	$1.71 \pm 2.44a$	$2.69 \pm 0.21ab$	$3.01 \pm 0.27ab$	$4.26 \pm 0.70bc$	$4.20 \pm 0.12bc$	$5.41 \pm 0.03cd$	$6.85 \pm 0.39d$	$9.21 \pm 0.71e$
2011/8/20	$3.19 \pm 0.58a$	$5.53 \pm 0.58a$	$6.57 \pm 1.49a$	$7.37 \pm 5.02a$	$4.98 \pm 1.03a$	$6.91 \pm 0.37a$	$8.77 \pm 0.05a$	$18.35 \pm 7.92b$
2011/8/28	$0.47 \pm 0.13a$	$1.13 \pm 0.03a$	$3.74 \pm 1.06b$	$4.28 \pm 0.19b$	$1.92 \pm 0.18ac$	$3.16 \pm 1.36bc$	$7.06 \pm 0.86d$	$8.92 \pm 2.81e$
2011/9/10	$3.03 \pm 0.58a$	$4.67 \pm 0.32ab$	$6.61 \pm 1.98ab$	$9.17 \pm 1.61b$	$3.24 \pm 1.09a$	$4.95 \pm 0.64ab$	$8.10 \pm 3.17ab$	$16.32 \pm 4.22c$
2011/9/20	$-0.36 \pm 0.28a$	$0.25 \pm 1.47ab$	$0.92 \pm 0.04ab$	$1.67 \pm 0.30abc$	$0.98 \pm 0.10ab$	$2.53 \pm 1.38bc$	$3.22 \pm 1.22c$	$5.04 \pm 1.09d$
2011/10/20	$-0.12 \pm 2.52a$	$-0.28 \pm 3.19a$	$-0.29 \pm 5.34a$	$-0.27 \pm 2.20a$	$0.18 \pm 1.90a$	$0.06 \pm 0.73a$	$0.13 \pm 4.28a$	$0.17 \pm 2.10a$

^a Different letters indicate significant difference between different treatments in the same sampling date ($P < 0.05$).

Table 5Multivariate analysis of variance for water and N addition on the cumulative N_2O emission.

Statistical period	Water addition (W)		N addition (N)		W $\times$ N		Year		R^2
	F	P	F	P	F	P	F	P	
2010	10.248	0.006	51.235	0.000	1.482	0.257	–	–	0.913
2011	10.490	0.005	11.049	0.000	1.963	0.160	–	–	0.756
2010 and 2011	20.721	0.000	55.321	0.000	254.484	0.029	55.816	0.000	0.897

observed between 2010 and 2011. The weather was dry in June and July of 2010, and the WFPS remained low. After August, the WFPS increased following the increase in precipitation. By contrast, the precipitation was concentrated in July 2011, the weather became relatively dry from late August, and the WFPS decreased and remained low until October. The WFPS between D and W treatments revealed no significant differences on most observed dates. The differences reached a significant level ($P < 0.05$) for a few samplings shortly after water addition. However, the significant difference only lasted 3–4 days.

The soil NO_3^- -N and NH_4^+ -N contents in the 0–10 cm soil layer are shown in Fig. 3. After N addition, the soil NO_3^- -N and NH_4^+ -N contents in N addition treatments were significantly higher than those in the control treatments (DCK and WCK) from late June to late August in 2010. Similar results were observed in 2011. However, the soil NO_3^- -N and NH_4^+ -N contents after the two N additions were significantly lower in 2011 than those in 2010 until September ($P < 0.05$). The statistical analyses indicated that water addition also significantly increased the soil NO_3^- -N and NH_4^+ -N contents ($P < 0.05$) in 2010, whereas in 2011 the soil NO_3^- -N and NH_4^+ -N contents in water addition treatments were smaller ($P < 0.05$) than in the treatments without water addition.

3.3. Main influence factors on N_2 effluxes under water and N addition

The Pearson correlations among N_2O effluxes and soil water, temperature, and N conditions were analyzed for the D and W treatments in the two-year experimental period (Table 6).

Correlation analysis showed that N_2O effluxes were negatively correlated with soil water contents and positively correlated with

temperatures in 2010. The N_2O fluxes were also significantly correlated with soil NO_3^- -N and NH_4^+ -N contents ($P < 0.05$). The correlation between N_2O effluxes and environmental factors in 2011 was more complex. Unlike in 2010, the WFPS was significantly positively correlated with N_2O effluxes. In addition, N_2O effluxes were extremely significantly correlated with soil NH_4^+ -N contents ($P < 0.01$), but the correlations between N_2O fluxes and NO_3^- -N were insignificant ($P > 0.05$).

4. Discussion

4.1. Effects of water and N addition on the seasonal variations in soil N_2 effluxes

Similar temporal variations in N_2O effluxes were observed in 2010 and 2011 (Tables 3 and 4), the peaks of N_2O effluxes were observed 2–3 days after N addition. Both water and N additions significantly increased the peak effluxes in different treatments ($P < 0.01$), and the interactive effect between water and N addition affected the peak effluxes among the different treatments ($P < 0.05$). The functions of N and water addition in improving soil N_2O effluxes are described as follows: (1) N input provides sufficient substrate for the N_2O generation processes; (2) the drying–rewetting transformation after water addition allows the release of several organic and inorganic substances into the soil, which provides sufficient substrates and energy for nitrification and denitrification (Mummey et al., 1994; Bollmann and Conrad, 1998; Beare et al., 2009); and (3) the variations in soil NO_3^- and NH_4^+ contents suggest that water addition facilitates the immediate transfer of fertilizers to mineral N from surface soil to the 0–10 cm

Fig. 2. Water-filled pore spaces (WFPS) in 0–10 cm soil layers during the observation period. D indicates the means of DCK, DN5, DN10, and DN20 treatments and W indicates the means of WCK, WN5, WN10, and WN20 treatments. The columns in the top of the figure indicate the amount of added water in W treatments and the columns in the bottom of the figure indicate the amount of natural precipitation.

Fig. 3. Variation of mean soil nitrate and ammonium N content ($n = 3$) with different treatments at 0–10 cm soil layers in the observed period. Arrows indicate the dates of N application.

soil layer, which also provides ample substrate for nitrification and denitrification (Goldberg et al., 2010; Kim et al., 2010).

Short bursts of N_2O emissions after fertilization were also observed in previous studies (Dobbie and Smith, 2003; Jones et al., 2007); however, the highest effluxes observed in our study was in the lower range relative to the peak values of N_2O obtained by some other N addition experiments in temperate grasslands abroad (ranged from 100 to $7500 \mu\text{g m}^{-2} \text{h}^{-1} \text{N}_2\text{O-N}$) (e.g., Kim et al., 2010; Pinto et al., 2004; Van Beek et al., 2011). In this study, the highest peak efflux among different treatments was only $162.55 \mu\text{g m}^{-2} \text{h}^{-1}$ observed in WN20 treatments on 26 June 2010;

similar low N_2O emission peaks (below $150 \mu\text{g m}^{-2} \text{h}^{-1}$) have been obtained previously in the fertilization experiments of temperate semi-arid grassland (Peng et al., 2011). The drought climatic condition in these regions could account for the low N_2O effluxes peaks. (Zhang and Han, 2008; Peng et al., 2011). In late June 2010, when the peaks of N_2O effluxes were observed, the WFPS was nearly the lowest during the whole growing season; the highest N_2O effluxes under drought soil water condition (WFPS < 30%) were also observed in previous studies both in fertilized (Zhang and Han, 2008; Peng et al., 2011) and native grasslands (Du et al., 2006; Yao et al., 2010) in Inner Mongolia. The processes of N_2O

Table 6Pearson correlation between N₂O flux and main environmental factors.

Treatments	WFPS	T_a^a	T_s^b			NH ₄ ⁺ -N ^c	NO ₃ ⁻ -N ^c
	0–10 cm		0 cm	5 cm	10 cm	0–10 cm	0–10 cm
D-2010	-0.365**	0.371**	0.506**	0.454**	0.448**	0.386**	0.344**
D-2011	0.416**	0.110	0.080	0.297**	0.334**	0.341**	0.066
W-2010	-0.291**	0.359**	0.371**	0.408**	0.413**	0.278**	0.287*
W-2011	0.243*	0.049	0.004	0.227*	0.265*	0.363**	0.060

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

^a Air temperature (°C).^b Soil temperature (°C).^c Quality contents in dry soil.

production were generally considered depressed in such drought-affected soil conditions, whereas Kim et al. (2010) and Cantarel et al. (2011) found that greater N₂O emissions occurred under drought conditions in the European temperate grasslands. The high soil temperature could contribute to the high N₂O emission rates during the drought period, both in the current study and in the previous studies with similar results; the N₂O effluxes during the drought period showed positive correlation with soil temperature (Peng et al., 2011; Kim et al., 2010). Furthermore, the N uptake of plants would be reduced during the drought period, and the N threshold for N₂O generation would be relieved, causing higher N₂O effluxes (Cantarel et al., 2011).

4.2. Effects of water and N addition on the cumulative N₂O efflux and its interannual variations

The N and water addition in this study increased the cumulative N₂O fluxes both in 2010 and 2011, significantly ($P < 0.01$). Relative to the treatment without N nor water addition (DCK), the cumulative N₂O efflux for WN20 treatment increased by 701.5% in 2010 and 501.5% in 2011, respectively. However, in 2010, the low N input (DN5 and WN5) did not arouse a significant increase in the cumulative N₂O effluxes ($P > 0.05$), and in 2011, the cumulative N₂O effluxes in low and medium N input treatments (DN5, DN10, WN5, and WN10) did not significantly increase either. There existed a threshold response of N₂O efflux to the N input. This response is decided by the competition for the available N between a plant and soil microbe, and the N₂O production usually will be suppressed until plant N demands have been fully satisfied (Peng et al., 2011; McSwiney and Robertson, 2005; Kim et al., 2012). Previous studies showed similar results on other ecosystems (Kim et al., 2012; Malhi et al., 2006; McSwiney and Robertson, 2005; Velthof et al., 1996). For example, McSwiney and Robertson (2005) showed that in the agroecosystem, the N₂O flux remains low until the N addition reaches 134 kg N ha⁻¹, at which point the fluxes increase sharply.

Previous studies reported that the annual emissions are greater when rainfall coincides with N fertilizer applications (Barton et al., 2008; Smith et al., 2012; Cantarel et al., 2011). While contrary results were observed in our study. The current study showed that the cumulative N₂O effluxes in the N addition treatments were significantly lower in rainy 2011 than in droughty 2010. The interannual variations in N₂O efflux could be attributed to differences in the seasonal distribution of precipitation (Fig. 2). Some studies have shown that the seasonal distribution of rainfall affects ecosystem productivity, biological diversity, and soil nutrients cycling (Fay et al., 2000; Weltzin et al., 2003; Peng et al., 2012), as well as the biological activity of microbes and plants. Thus, nitrification and denitrification processes driven by microbial activity and N availability must be affected. Collins et al. (2008) proposed a threshold-delay-nutrient dynamics (TDND)

model to describe nutrient supply and demand in arid ecosystems, and the model predicted that plants were more sensitive to drought stress than soil microbe because their metabolism was more easily restrained during drought conditions. Kim et al. (2010) observed in a 6-years study of fertilized grassland that the N₂O emission in a drought year was two times higher than those in normal precipitation years because the N uptake of plants reduced during a drought period, and N input was beyond the plant N uptake demand. The experiment performed in the Baiinxile Pasture by Bai (1999) indicated that the positive effects of 10 day precipitation from late June to late August were observed on the primary productivity, which was critical to plant growth in terms of water requirement. The 15-year research also showed that the reverse pattern was observed in the effect of 10 day precipitation from mid-April to mid-June. In the current study, the soil moisture was low in July 2010, and drought conditions restrained plant metabolism, including plant growth and N uptake. By contrast, the rainfall was concentrated in late June and early July in 2011, which significantly ameliorated the soil moisture conditions. Furthermore, the WFPS fluctuated at high levels for almost two weeks. Plant growth benefited from such favorable soil water condition; thus, the plant N demand increased accordingly; plants became better competitors for soil N than microbes, and the N₂O flux exhibited higher threshold responses to N input.

The cumulative N₂O fluxes of the W treatments were all higher than those of the D treatments for the same N addition levels, both in 2010 and in 2011. The simulated rainfall induced larger N₂O effluxes. The water addition did not markedly increase plant N uptake, and its effect seemed inconsistent with the effect of seasonal distribution of precipitation on N₂O effluxes mentioned above. A possible reason for this result was that different soil moisture thresholds controlled plant and microbial activities (Austin et al., 2004; Collins et al., 2008; Austin, 2011; Dijkstra et al., 2012). Huxman et al. (2004) found that microbial populations were sensitive to small precipitation events. However, the plants were more responsive to heavy precipitation events or to multiple small rainfall events. For the W treatments, the amount of artificial water added did not exceed 12 mm of rainfall, and the water was added in days without natural precipitation. The amount of added water did not exceed the water threshold for plants, but it still could increase the microbial metabolism. The microbes could then occupy the more favorable condition for N competition with plants, resulting in larger N₂O effluxes. However, this hypothesis lacks support by microbiological data and should be validated by further research.

5. Conclusion

From the perspective of temporal dynamics, water and N addition both increased the soil N₂O emissions in 2–3 days. Nitrogen addition caused significant increases in N₂O efflux within the first 2–3 weeks, and the influence of water addition were concentrated within 3–4 days. The N₂O cumulative effluxes increased when the N input levels increased whether in the D treatment or in the W treatment. Furthermore, the simulated precipitation also increased the cumulative N₂O effluxes in all N input levels, especially significantly increased in the high N treatment level (N20). Beside these, the variation in soil N₂O effluxes reflected the coupling effects between water and N, and the response of N₂O effluxes to the increasing N deposition and precipitation could vary because of the different precipitation distributions and intensities, which resulted in a significant interannual variation in the influence of water and N addition on soil N₂O effluxes. These contradictory results indicated that the relationship of water and N inputs with N₂O efflux is complex and closely related with the complex interactions of soil water, temperature, available N contents, as well as the biological

activity of microbes and plants. These factors significantly influenced the soil N₂O effluxes and should be considered when predicting the future changes of N₂O effluxes from temperate grassland soils.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 41330528, 41373084, 41203054), the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-EW-302), and the Special Fund for Agro-scientific Research in the Public Interest (No. 201203012). We are grateful to the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) for providing field assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.still.2015.01.008>.

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