JOURNAL OF ENVIRONMENTAL SCIENCES 56 (2017) 192–201

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Effects and mechanism of freeze-thawing cycles on the soil N_2O fluxes in the temperate semi-arid steppe

Liqin Wang[1,2], *Yuchun Qi*[1,*], *Yunshe Dong*[1], *Qin Peng*[1], *Shufang Guo*[1,2], *Yunlong He*[1,2], *Zhaolin Li*[1,2]

1. *Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China. E-mail: wangliqin@sina.cn*

2. *University of the Chinese Academy of Sciences, Beijing 100049, China*

ARTICLE INFO

Article history:
Received 9 March 2016
Revised 29 July 2016
Accepted 19 September 2016
Available online 28 October 2016

Keywords:
Temperate semi-arid steppe
Nitrous oxide
Freeze-thawing period
Water and nitrogen addition

ABSTRACT

High nitrous oxide (N_2O) emissions during freeze-thawing period (FTP) have been observed in many different ecosystems. However, the knowledge about the dynamic of soil N_2O emissions and its main driving mechanism during the freeze-thawing processes in grassland ecosystem is still limited. An *in-situ* experiment was conducted during the FTP on the sites with 0 and 15% surplus of the average rainfall and two levels of N addition (0,10 g N/(m^2·year)) during growing season (marked as W0N0, W15N0, W0N10, W15N10, respectively) to explore the effects of water and N background on soil N_2O emissions during FTPs and the relationship between soil N_2O emissions and environmental factors. The results indicated that water and N treatments conducted during growing season did not show significant effect on the N_2O effluxes of FTP, but the soil mineral N contents of W0N10 treatment were significantly higher than those of W0N0, W15N0, W15N10 treatments ($p < 0.05$). The soil PLFA concentrations of microbial groups monitored during 2015 spring freeze-thawing period (2015S-FTP) were lower than those during winter freeze-thawing period of 2014 (2014W-FTP), while cumulative soil N_2O emissions of 2015S-FTP were higher than those of 2014W-FTP. The correlations between soil N_2O effluxes and most of the measured environmental factors were insignificant, multiple stepwise regression analysis indicated that the soil temperature, soil NH_4^+-N content and air temperature were the major environmental factors which significantly influenced the N_2O effluxes during 2014W-FTP, and air temperature and soil water content were the significant influencing factors during 2015S-FTP.

Introduction

Freeze-thawing cycles have a great influence on the physico-chemical and biological properties of the soil, which thereby change the soil N_2O emissions through affecting the nitrogen (N) migration and transformation in terrestrial ecosystem.

Previous studies have shown that soil N_2O emissions during freeze-thawing period account for large part of annual emissions, N_2O emitted from Guelph agricultural soil (Wagner-Riddle et al., 1997), Norway spruce forest soil (Goldberg et al., 2010), Colorado short-grass steppe soil (Mosier et al., 1996; Martin et al., 1998) during freeze-thawing period contributed 65%, 84%, 20%–40% respectively to annual N_2O emissions. N_2O emission during freeze-thawing period is an important contributor to climate change, which, in turn, affects the intensity and frequency of freeze-thawing action

* *Corresponding author*. E-mail: qiyc@igsnrr.ac.cn (Yuchun Qi).

http://dx.doi.org/10.1016/j.jes.2016.09.013
1001-0742/

(Sharratt, 1993; Kattenberg et al., 1996; Isard and Schaetzl, 1998; Decker et al., 2003; Hugh, 2007). In China, 78% of grassland is distributed in the regions above 30 degrees north latitude, where the regions belong to seasonal frozen soil area. Therefore, it's of great significance for accurate estimation of annual soil N_2O emissions to study on the soil N_2O emissions during freeze-thawing period in the temperate grassland of China.

The Intergovernmental Panel on Climate Change (IPCC) fourth assessment report showed that temperature in most parts of China and the mean annual precipitation of North China would increase significantly in the 21st century (IPCC, 2007). In addition, monitoring located in temperate steppe of Inner Mongolia has shown that N deposition was high and may have harmful effects on grassland ecosystem (Zhang et al., 2013). Moreover, fertilization could also be promoted with the requirements of more fine management. The change of water and N input could change the migration and transformation of soil N, and therefore inevitably affect soil N_2O emissions. Under this background, studies on the N_2O effluxes and their main driving mechanisms during freeze-thawing period under different water and N nutrient condition could provide a very important scientific support for grassland management, the rationality of land use evaluation, and the greenhouse gas reduction. However, little information so far has been provided on the individual and interactive effects of water and N on soil N_2O emission from the soil of freeze-thawing period in Chinese temperate grasslands. Based on this, an *in situ* experiment was conducted during the freeze-thawing period in a semi-arid temperate typical steppe of Inner Mongolia, China. The objectives of this study were as follows: (1) to determine the effects of water and N addition during growing season on N_2O emissions of freeze-thawing period; (2) to analyze the relationship between soil N_2O emission and environmental factors, and to explore the driving mechanisms of N_2O emission during freeze-thawing period.

1. Materials and methods

1.1. Site description

The *in situ* experiment was conducted at a *Leymus chinensis* steppe (43°33′N, 116°40′E, 1225 m above sea level) in the Xilin River Basin of Inner Mongolia, China. The site was characterized by a continental, semi-arid temperate climate. The mean annual temperature ranged from −0.3 to 1°C, and the mean annual precipitation ranged from 350 to 450 mm. The soil was classified as dark chestnut soil according to Chinese classification or Calcic-OrthicAridisol according to US soil taxonomy classification, with 60% sand, 21% clay and 19% silt. The depths of soil and humus layer were approximately 100 to 150 cm, 20 to 30 cm respectively.

Twelve 8 m × 8 m experimental plots, separated by 1 m buffers, were established in May 2014 and randomly designed with four treatments and three replicates to simulate different levels of nitrogen deposition or precipitation, and the N and water addition were conducted during growing season. The treatments were as follows: (1) control, without N or water addition (W0N0); (2) with water addition and without N (W15N0); (3) with N addition atarate of 10 g N/(m^2·year) and without water (W0N10); (4) with N addition atarate of 10 g N/(m^2·year) and with water addition (W15N10). Approximately 51.7 mm/year of water (equivalent to 15% of mean annual precipitation) was manually added to the designated experimental plots using backpack sprayers in June, July, August, and September. The amount of water addition (51.7 mm/year) is based on report which predicts that semi-arid grassland in north China may increase 12%–18% of the annual precipitation in the future 100 years (Jiang et al., 2008; Wang, 2007). The N additions were conducted using NH_4NO_3 and the amount of N addition (10 g N/(m^2·year) was based on the current and future 30 years N deposition level with the underlying assumption that large doses of N addition over a short period would effectively mimic small doses of N deposition over a long period and the assumption that N is not lost by different ways such as seepage, immobilization in deep soil layers, or volatilization in an N addition experiment of 100 kg N/(ha·year) over 6 years (Qi et al., 2014; Liu et al., 2013; Dise and Stevens, 2005; Galloway and Cowling, 2002). The added N was split into halves and applied in late June and early August of rainy season each year, considering that the main pulsing N inputs in this area are often along with rainfall.

1.2. Sampling and analyzing

Gas samples were collected by static closed opaque chambers with inner dimensions of 50 cm (length) × 40 cm (width) ×30 cm (height). In order to avoid over-fast temperature increases in the chamber, the chambers were made of 8 mm black acrylic material, and the chamber's surface was covered with reflecting tin foil (Dong et al., 2000; Zou et al., 2004; Qi et al., 2007). The whole freeze-thawing period was divided into 2014 winter freeze-thawing period (2014W-FTP) and 2015 spring freeze-thawing period (2015S-FTP). Gas sampling was conducted every other day during 2014W-FTP (from October 17, 2014 to November 29, 2014) and once a day during 2015S-FTP (from March 14, 2015 to April 15, 2015).

The air temperature, soil temperatures at depth of 0, 5, 10 cm, and internal chamber temperature were measured by DHM2 mechanical ventilated thermometer, SN2202 digital thermo detectors (Sinan Instruments Plant of Beijing Normal University), and temperature sensors, respectively, simultaneously with the gas sampling. In addition, soil temperatures at depth of 5, 10, 20 cm were recorded hourly by DS1922L iButton in order to monitor the daily changes of soil temperature at different depths. Soils analyzed for soil NH_4^+-N, NO_3^--N and soil water content were collected synchronously with gas sampling, and soil NH_4^+-N and NO_3^--N were extracted with 2 mol/L KCl and measured by an automated flow injection analyzer (Braun and Lübbe, Norderstedt, Germany) and soil water content was determined using oven-drying method.

Soils analyzed for soil composition of microbe and enzymes were collected at the beginning and the end of each freeze-thawing period. The community composition of microbe was evaluated by Phospholipid fatty acids (PLFA) (Budge et al., 2011), and the PLFA extraction method was based on the study of Frostegård et al. (1991) and Sun et al. (2015). The activity of protease was measured by ninhydrin colorimetric method, urease by sodium phenate colorimetric method, nitrate reductase by phenol disulfonic acid colorimetric method, and nitrite reductase by alpha-naphthylamine colorimetric method. The above enzyme activity determinations were the methods of Guan (1986).

1.3. Data analysis

The N_2O efflux was calculated as follows:

$$F = \rho \times h \times \frac{\Delta c}{\Delta t} \times \frac{273}{273 + T}$$

where, F (μg/(m^2·hr)) refers to the N_2O efflux, ρ (μg/m^3) is the N_2O gas density, and h (m) is the chamber height. $\Delta c/\Delta t$ (m^3/(m^3·hr)) denotes the change in gas concentrations inside the chamber during the sampling period, and T (°C) is the average chamber temperature during the sampling period.

The statistical analyses, *i.e.*, one-factor analysis of variance (ANOVA), Pearson's correlation and multiple stepwise regression were performed using SPSS 18.0. The graphs were prepared using Origin 8.5. The cumulative N_2O emissions were calculated by using Matlab 23 to interpolate the N_2O fluxes measured during sampling periods (Peng et al., 2011; Liu, 2013).

2. Results

2.1. Seasonal variation of freeze-thawing characteristics

During 2014W-FTP, the frozen soil depth of the initial stage (from October 17 to November 5) varied from 0 to 8 cm with a slow freezing rate and thereafter (from November 6 to November 29) the frozen soil depth ranged from 19 to 69.5 cm with a fast freezing rate. 2015S-FTP started from March 14, the depth of thawing layer varied between 0 and 7 cm from March 14 to March 26, and thereafter (from March 28 to April 9) the depth of thawing layer ranged from 11.5 to 100 cm with a fast thawing rate. The melted soil refreezed when there was a drop in air temperature on April 7, and the refrozen layer started to melt from the top down as the air temperature rises, but did not melt completely until April 12. So there appeared a freezing layer at depth of 6 to 16 cm in the soil profile from April 9, 2015 to April 11, 2015 (Fig. 1).

2.2. Variations in soil temperature, soil water content and mineral N content during the two freeze-thawing periods

2.2.1. Soil temperature

Soil temperatures at depth of 5, 10, 20 cm were recorded hourly during the freeze-thawing period. The diurnal variation of soil temperature showed that the soil temperature at depth of 5, 10, 20 cm during 2014W-FTP started to rise at mainly about 9:00–10:00 a.m., 10:00–12:00 a.m., 12:00–16:00 a.m., respectively, and the daily peak temperature was reached at about 1:00–5:00 p.m., 2:00–7:00 p.m., 4:00 p.m.–1:00 a.m. (the next day), respectively; soil temperatures at depth of 5, 10 cm declined to below 0 all day from November 7, and soil temperature at depth of 20 cm declined to below 0 all day from November 8. During 2015S-FTP, the soil temperature at depth of 5, 10 cm started to rise at 8:00–10:00 a.m., 9:00–12:00 a.m., respectively, and the daily peak temperature was reached at about 2:00–5:00 p.m., 4:00–10:00 p.m., respectively, but the dynamic of soil temperature at depth of 20 cm was not that regular; soil temperature at depth of 20, 10, 5 cm rose to above 0 all day from March 27, April 9, and April 12, respectively. From the point of the whole freeze-thawing period, the diurnal freeze-thawing cycles mainly occurred at depth of 0 to 5 cm and 5 to 10 cm, and the curve of soil temperature at depth of 20 cm changing with time was comparatively smooth. The number of freeze-thawing cycles at depth of 5 to 10 cm was less than that at depth of 0 to 5 cm (Fig. 2).

2.2.2. Soil water content

Soil water contents at depth of 0 to 10 cm were improved to a certain extent by the intermittent rainfall during October 29, 2014 to November 1, 2014, but the influence of the rainfall persisted for a shorter period of time because of the big soil evaporation caused by the high wind velocity and dry air of the study area. The influence mainly focused on the depth of 0to 10 cm, and the soil water contents at depth of 10 to 20 cm were relatively stable. A fluctuant reduction in soil water contents was found at depth of 0to 10 cm from March 14, 2015 to April 2, 2015. The soil water contents (0–10 cm and 10–20 cm) increased rapidly as the snow, which fallen on April 2, melted on April 11. And the maximum soil water content of 0–10 cm depth reached 22.37%. Soil water contents of different treatments showed similar dynamic. For the soil water contents at the soil layer of 0 to 10 cm, only difference between W15N10 and the rest treatments in

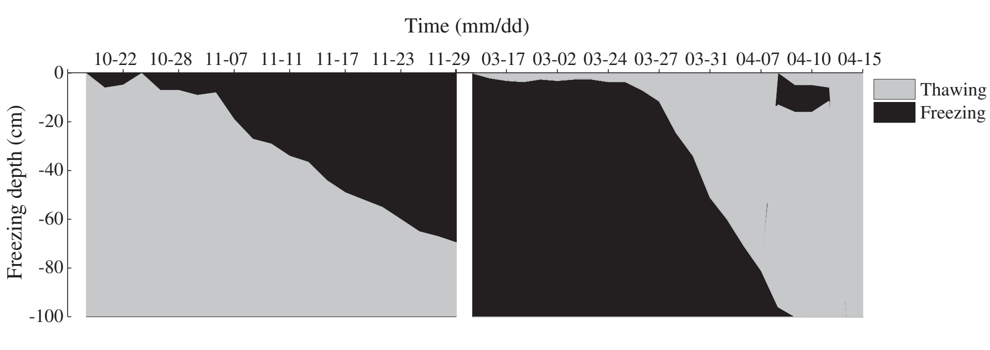

Fig. 1 – Seasonal freeze-thawing dynamics of the studied soil.

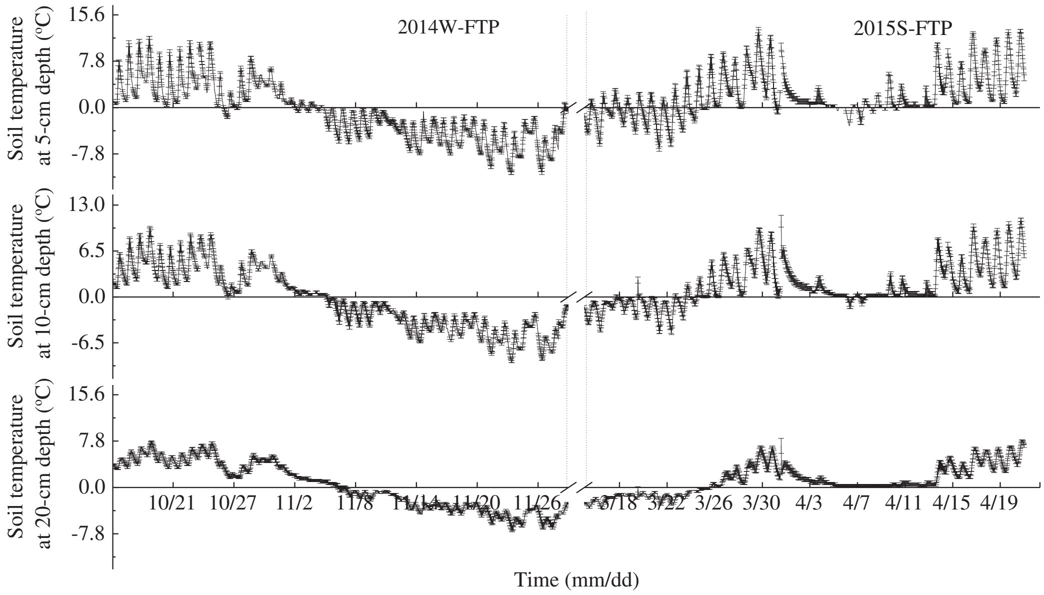

Fig. 2 – Soil temperatures at three soil depths (5, 10, and 20 cm) which were recorded hourly for the two observed periods.

2014W-FTP and difference between W15N0 and W15N10 in 2015S-FTP reached a significant level ($p < 0.05$), and for soil water contents at depth of 10 to 20 cm in both freeze-thawing periods, there were no significant differences between any two treatments ($p > 0.05$). In addition, for difference in mean soil water contents (0–10 cm and 10–20 cm) between 2014W-FTP and 2015S-FTP of the same treatment, only differences between the two freeze-thawing periods of W15N0 and W0N10 at depth of 10 to 20 cm reached a significant level of 0.05.

2.2.3. Mineral N content

During 2014W-FTP and 2015S-FTP, the temporal dynamics of mineral N contents for different treatments were basically consistent, and the soil NO_3^--N contents of W0N10 were significantly higher than those of W0N0, W15N0, W15N10 ($p < 0.05$). According to the result of ANOVA, 2014W-FTP can be divided into two stages (the first stage is from October 17 to November 7 and the second stage is from November 8 to November 29), the mean NO_3^--N content of the second stage was slightly higher than that of the first stage. As for 2015S–FTP, the NO_3^--N contents after March 26 were significantly higher and fluctuated more remarkably than those before March 26.

Similar to the dynamics of soil NO_3^--N content, the mean soil NH_4^+-N content of W0N10 treatment was also significantly higher than those of W0N0, W15N0, W15N10 ($p < 0.05$). But the change of soil NH_4^+-N content didn't show obvious stage characteristics (Fig. 4).

2.3. Changes of soil microbial community composition and enzyme activity caused by freeze-thawing periods

Fig. 5 showed that there was no consistent regularity among treatments, but stage characteristics of data about microbes and enzyme were obvious. The PLFA concentrations of the total PLFA were all higher in 2014W-FTP than those in 2015S-FTP. And the differences in total PLFA between the two freeze-thawing periods were statistically significant ($p < 0.05$). Except for W15N0 treatment in 2014W-FTP, the protease activities of the rest treatments were not improved after each of the two freeze-thawing periods, and instead, decreased to some degree; protease activities were relatively stable in 2015S-FTP, and there was no sharp decrease after freeze-thawing period just like in 2014W-FTP. Compared with the urease activities at the beginning of 2014W-FTP, the urease activities under W0N0, W15N0, W0N10, W15N10 treatments at the end of 2014W-FTP increased by 47.05%, 52.57%, 33.66%, 29.90% respectively, and the urease activities under W0N0, W15N0, W0N10, W15N10 treatments at the end of 2015S-FTP increased by 1.31%, 31.21%, 25.69%, 112.28% respectively compared with those at the beginning of 2015S–FTP. Except for W15N10 treatment, urease activities at the beginning and end of 2015S-FTP were relatively high than those of 2014W-FTP, respectively. Except for individual treatment, the activities of nitrate reductase and nitrite reductase were slightly increased after each of the two freeze-thawing periods, and the activities of nitrate reductase were significantly lower in 2015S-FTP than in 2014W-FTP ($p < 0.05$), data about nitrite reductase did not show regularity between the two freeze-thawing periods.

2.4. Response of N_2O emission to freeze-thawing cycles

The fluctuation characteristics of N_2O emitted from different treatments were basically consistent during 201 W-FTP and 2015 S-FTP. Except for significant difference between W0N0 and W15N0 of 2015S-FTP, the rest levels of water and N addition conducted during growing season of 2014 did not show significant effect on the N_2O effluxes during the both freeze-thawing periods ($p > 0.05$) (Fig. 6). The N_2O effluxes varied between (−4.591 ± 0.546) μg/(m²·hr) (W0N10) and (32.256 ±7.490) μg/(m²·hr) (W0N0) during 2014W-FTP, and between (12.230 ± 2.154) μg/(m²·hr) (W15N10) and (125.846 ± 3.121) μg/(m²·hr) (W0N10) during 2015S-FTP. The cumulative N_2O effluxes during 2015S-FTP were significantly higher than those during 2014 W-FTP ($p < 0.05$) (Fig. 7).

2.5. Correlations between the N_2O effluxes and the environmental factors during freeze-thawing period

The Pearson correlations (Table 1) and multiple stepwise regression (Table 2) analysis were used to explore the relationship between the N_2O effluxes and environmental factors during the two freeze-thawing periods. During both freeze-thawing periods, most of the correlations between the N_2O effluxes and environmental factors were weak and did not reach significant level of 0.05.

During 2014W-FTP, the N_2O effluxes were significantly and positively correlated with the soil NH_4^+-N contents in W15N0 ($p < 0.05$), W0N0 ($p < 0.01$) and W15N10 ($p < 0.01$). Multiple stepwise regression results indicated that soil NH_4^+-N content and temperature were the main factors which could explain large part of the variation in the N_2O efflux in W0N0, W15N0, W15N10 treatments. But correlation between N_2O effluxes and soil NH_4^+-N contents in W0N10 did not reach significant level ($p > 0.05$), and there were no environmental factors included in the regression equation.

During the 2015S-FTP, the N_2O effluxes were significantly and positively correlated with air temperature in W0N0 ($p < 0.01$), W0N10 ($p < 0.05$) and W15N10 ($p < 0.05$); multiple stepwise regression analysis showed that the air temperature and the soil water content at depth of 0 to 10 cm were the major environmental factors that influenced the N_2O effluxes. Correlations between N_2O effluxes and environmental factors in W15N0 were relatively weak compared with those in other treatments, and there were also no environmental factors included in the regression equation.

3. Discussion

3.1. Characteristics of soil N_2O emissions during freeze-thawing period under different levels of water and N condition

As for the effects of water and N input conducted during growing seasons on soil N_2O emissions during freeze-thawing period, Li et al. (2012) studied alpine grassland (sampling frequency were twice a month during winter and every 2–3 days during thawing period in spring) and found that there was no significant difference between the N input treatments and the blank control group. In this study, we increased the sampling frequency (sampled once every two days during 2014W-FTP and once a day during 2015S-FTP except for snowmelt period) to capture the effects of the freeze-thawing cycles, we found the similar results. In addition, the water addition (W15N0) conducted during growing season had no significant difference compared with the blank control group (W0N0) either. As for the stage characteristic, many studies have showed that N_2O effluxes during spring period were obviously higher than winter period (Wagner-riddle et al., 2007; Norman et al., 2008; Dusenbury et al., 2008; Song et al., 2006). In this study, we also found that the cumulative N_2O effluxes during 2015S–FTP were significantly higher than those during 2014 W–FTP ($p < 0.05$).

In this study, the average N_2O efflux during 2015S-FTP was 29.579 (W15N0)-38.052 μg/(m²·hr) (W0N0), and the maximum efflux was up to (125.846 ± 3.121) μg/(m²·hr) (W0N10). Holst et al. (2008) monitored the Xilin River Basin during March 12, 2006 to May 11, 2006, the results showed the average N_2O efflux was (8.2 ± 0.5) μg/(m²·hr), and the maximum flux was 75 μg/(m²·hr). The intensity and frequency of soil freeze-thawing cycles have a close relationship with the temperature and precipitation in winter (Sharratt, 1993; Isard and Schaetzl, 1998; Decker et al., 2003). So, the freeze-thawing cycles would have an obvious inter-annual variation which would result in inter-annual changes of soil N_2O efflux. By comparing the climate factors, we found that precipitation in this study was

Table 1 – Correlations between the N_2O effluxes and the environmental factors.

Treatment	Soil water content (%)		T_a (°C)	T_s (°C)			NO_3^--N [a] (mg/kg)	NH_4^+-N [a] (mg/kg)	TN [a] (mg/kg)
	0-10 cm	10–20 cm		5 cm	10 cm	20 cm			
2014 W–FTP									
W0 N0	0.048	−0.191	0.306	0.214	0.233	0.252	0.245	0.785**	0.688**
W15 N0	0.392	0.115	0.298	0.218	0.198	0.181	−0.310	0.579*	0.395
W0 N10	0.225	−0.395	0.312	0.187	0.173	0.151	−0.451	0.420	0.103
W15 N10	0.335	−0.035	0.268	0.171	0.192	0.199	−0.345	0.744**	0.390
2015S–FTP									
W0 N0	0.155	0.124	0.599**	0.535*	0.463*	0.484*	0.316	−0.052	0.189
W15 N0	0.371	0.094	0.337	0.183	0.105	0.206	0.130	−0.186	0.058
W0 N10	0.467*	0.403	0.472*	0.333	0.307	0.359	0.290	−0.307	−0.111
W15 N10	0.174	0.131	0.483*	0.336	0.261	0.324	0.296	−0.101	0.149

T_a: air temperature; T_s: soil temperature.
* and ** indicate the correlation was significant at $p < 0.05$ and 0.01 level, respectively.
[a] Quality contents in dry soil.

Table 2 – Stepwise regression equations between the N_2O effluxes and the environmental factors.

Statistical period	Treatment	Regression equation	F	α	R^2
2014W-FTP	W0N0	$Y = 0.690\ T_{10} + 4.874N_a + 1.983$	20.008	0.000	0.755
	W15N0	$Y = 0.697\ T_5 + 4.166N_a + 1.134$	7.962	0.006	0.551
	W0N10	–			
	W15N10	$Y = 0.573T_a + 5.455N_a - 8.595$	17.204	0.000	0.726
2015S-FTP	W0N0	$Y = 2.408T_a + 1.965\ W_1 - 15.577$	9.630	0.001	0.503
	W15N0	–			
	W0N10	$Y = 2.706T_a + 4.002\ W_1 - 47.652$	15.211	0.000	0.575
	W15N10	$Y = 2.065T_a + 10.066$	6.087	0.023	0.233

T_5: soil temperature at 5 cm depth; T_{10}: soil temperature at 10 cm depth; N_a: NH_4^+-N contents in the 0 to 10 cm soil layer; W_1: soil water content in the 0 to 10 cm soil layer.
–: the regression equation was not available.

obviously more than that in the study of Holst et al. (2008). The differences in N_2O efflux between our study and Holst et al. (2008) may be caused by the inter-annual difference of climate, in addition, the difference in microclimate and geographical environment such as topography, terrain, and altitude may also be the important influencing factors. So, in order to evaluate and predict N_2O emission contribution of freeze-thawing period to annual emission accurately, a long-term monitoring during freeze-thawing period with a higher measured frequency should be conducted.

3.2. The changes of soil microbial biomass and enzyme activity during freeze-thawing period and their relationship with N_2O effluxes

The soil microbes and enzymes play a key role during the transformation process of soil nitrogen. In this study, the total PLFA concentrations, the PLFA concentrations of the bacteria and fungi were dramatically reduced after the deep frozen period between 2014W-FTP and 2015S-FTP, but there was no obvious regularity between treatments. The protease and urease are the key enzymes during the protein or peptide hydrolysis, and urea hydrolysis, respectively. In this study, the changes of the protease activities and urease activities indicated that the protein or peptide hydrolysis was weakened while urea hydrolysis was improved after each of the freeze-thawing periods. The nitrate reductase and nitrite reductase were the main enzymes in denitrification process (Abdelmagid and Tabatabai, 1987), the changes of the nitrate reductase indicated that the process of reducing NO_3^- to NO_2^- was weakened in 2015S-FTP compared with that in 2014W-FTP, and the changes of nitrite reductase showed that the process of reducing NO_2^- to NO was improved after each of the freeze-thawing periods while did not show obvious stage characteristics between 2014W-FTP and 2015S-FTP. The soil PLFA concentration of microbial groups monitored during 2015S-FTP were lower than those during 2014W-FTP, while soil N_2O emissions during 2015S-FTP were higher than those during 2014W-FTP. The number of freeze-thawing cycles in 2015S-FTP was more than that in 2014W-FTP (Fig. 2) and the precipitation in 2015S–FTP was more intense than that in 2014 W–FTP (Fig. 3). The more intense precipitation and more freeze-thawing cycles in 2015S-FTP may have a greater effect on soil physicochemical and biological properties than in 2014W-FTP. Although the soil microbe biomass decreased dramatically after a deep frozen period, the soil surviving microbes may be highly activated by the nutrients released from the destroyed microbial cells and physical disruption of soil aggregates caused by freeze-thawing cycles (Christensen and Tiedje, 1990; Christensen and Christensen, 1991; Edwards and Cresser, 1992). On the other hand, previous studies have shown that the soil N_2O emission during the process of thawing include physical release of the trapped N_2O (Goodroad and Keeney, 1984; Burton and Beauchamp, 1994; Kaiser et al., 1998), which produced and was stored temporarily in the soil frozen layer as well as the closed thin sheet of ice on the surface of the soil particle (Teepe et al., 2001; Koponen et al., 2004). The release of trapped N_2O may be another reason which resulted in higher soil N_2O emissions during 2015S-FTP than during 2014W-FTP. Finally, the data about the microbe and enzyme at both ends of the freeze-thawing periods may not sufficiently reflect the activity changes of soil microbe and enzyme in the whole freeze-thawing period, and there may have fluctuation between the two ends. So sampling frequency of soil microbe and enzyme should be increased in the future research, and the mechanism of the contribution of the trapped N_2O to soil N_2O emissions during freeze-thawing period is a key issue needs to be further explored.

3.3. The relationship between N_2O effluxes and environmental factors during freeze-thawing period

The correlations between soil N_2O effluxes and most of the selected environmental factors were weak. The low temperature or both low temperature and low effective soil water content may be the restriction factor(s), which limited the full play of the functions of the other environmental factors, and then weakened the correlations between soil N_2O effluxes and other environmental factors. In addition, N_2O emissions during freeze-thawing period contained physical release of the trapped N_2O during the frozen period, which may result in inconsistency between generation and emission of N_2O, and then weakened the correlations between soil N_2O effluxes and environmental factors. Meanwhile, there perhaps existed other influencing factors that we did not monitor in this study (Fig. 4).

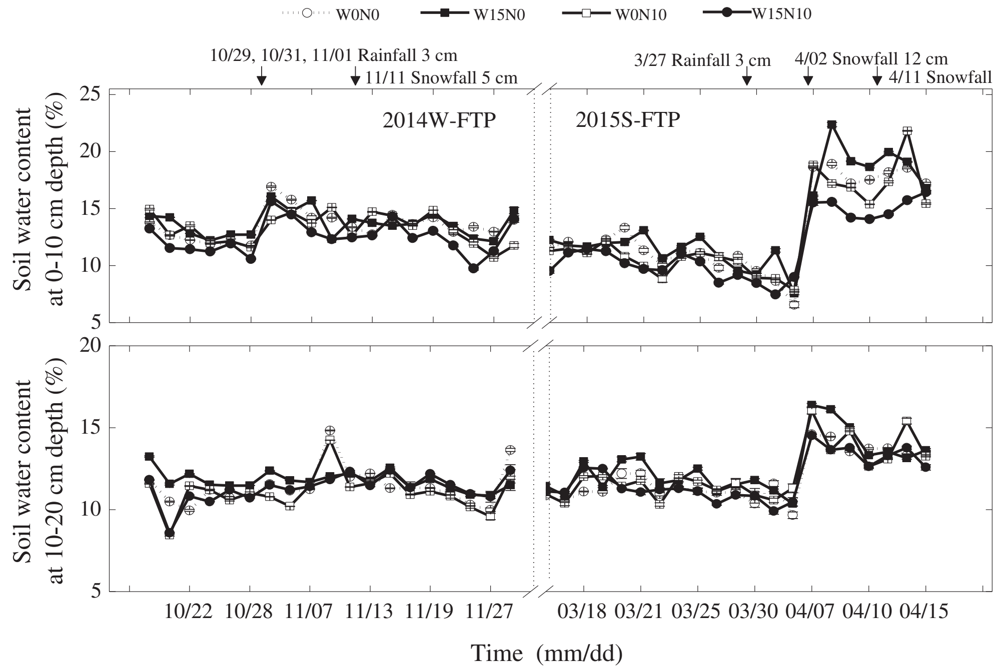

Fig. 3 – Soil water contents (*n* = 3) at two soil depths (0–10 and 10–20 cm) during the two observed periods.

The fluctuation characteristics of the soil mineral N content under different treatments were basically consistent in both freeze-thawing periods. The mineral N content of W0 N10 treatment was significantly higher than that of W0N0 treatment ($p < 0.05$), and also significantly higher than the mineral N content of W15N0, W15N10 treatments ($p < 0.05$).

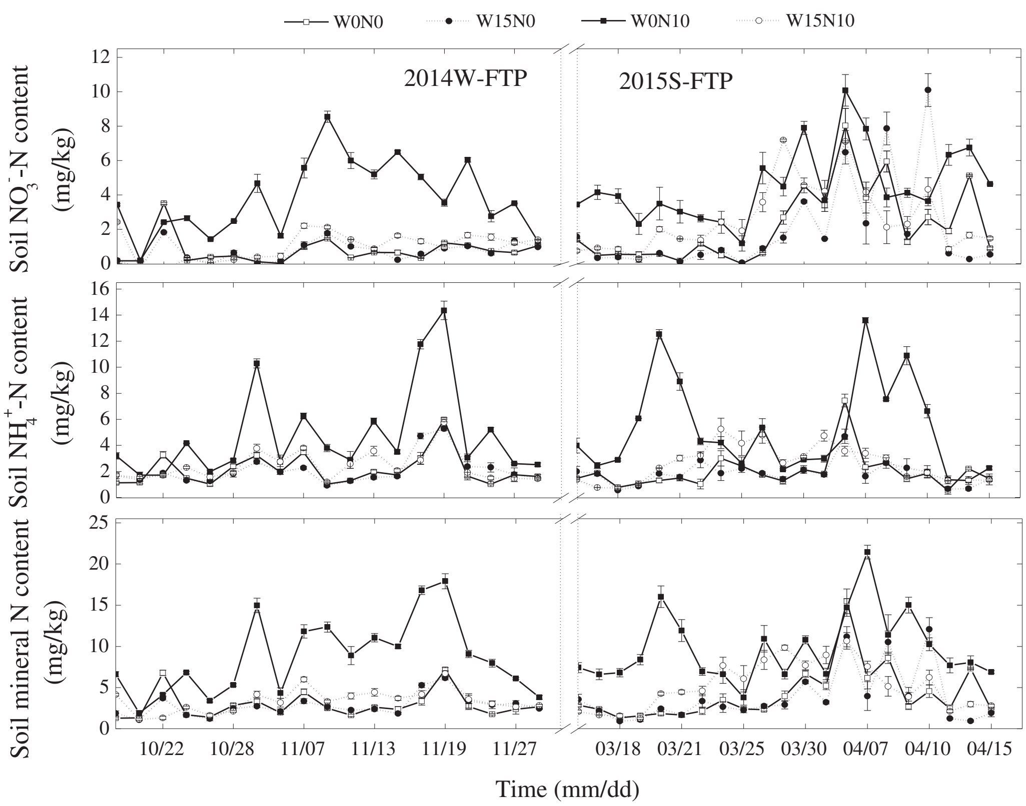

Fig. 4 – Variations in soil mineral N content (*n* = 3) for the different treatments at a depth of 0 to 10 cm during freeze-thawing periods.

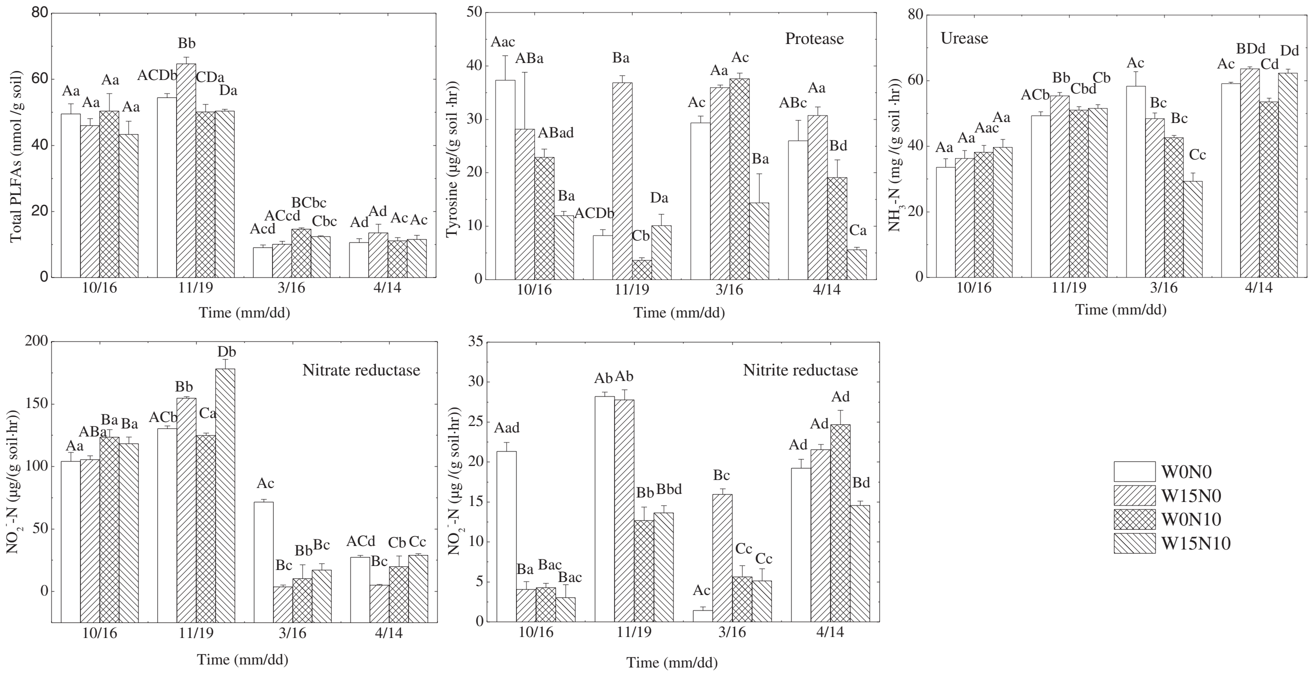

Fig. 5 – Changes of soil Phospholipid fatty acids (PLFAs) and enzyme activity caused by freeze-thawing cycles. Bars with different small letters denote sampling times of the same treatment differ significantly at $p < 0.05$, bars with different capital letters denote treatments at the same sampling time differ significantly at $p < 0.05$. The incubation temperature was 37°C during enzyme activity determination.

Although the mineral content of W0N10 treatment was significantly higher than that of other treatments, there was still no significant difference in N_2O effluxes between W0N10 and other treatments ($p > 0.05$). The effect of soil mineral N content of W0N10 was limited, and this may be caused by the possible restriction factors (low temperature or both the low temperature and low soil water content). In addition, soil mineral N content itself may be an another restriction factor, and the difference in soil mineral N content between W0N10 and other treatments was not large enough to make a difference in soil N_2O emissions (Fig. 6).

Multiple stepwise regression analysis showed that the soil temperature, soil NH_4^+-N content and air temperature were the significant environmental factors that influenced the N_2O

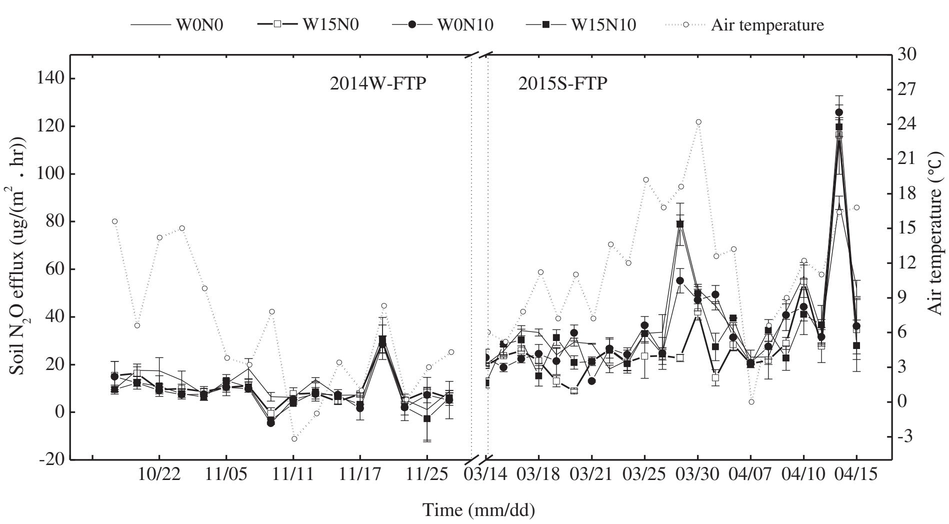

Fig. 6 – Dynamics of soil N_2O effluxes during the 2014W-FTP and 2015S-FTP under different water and N treatments. FTP: freeze-thawing period.

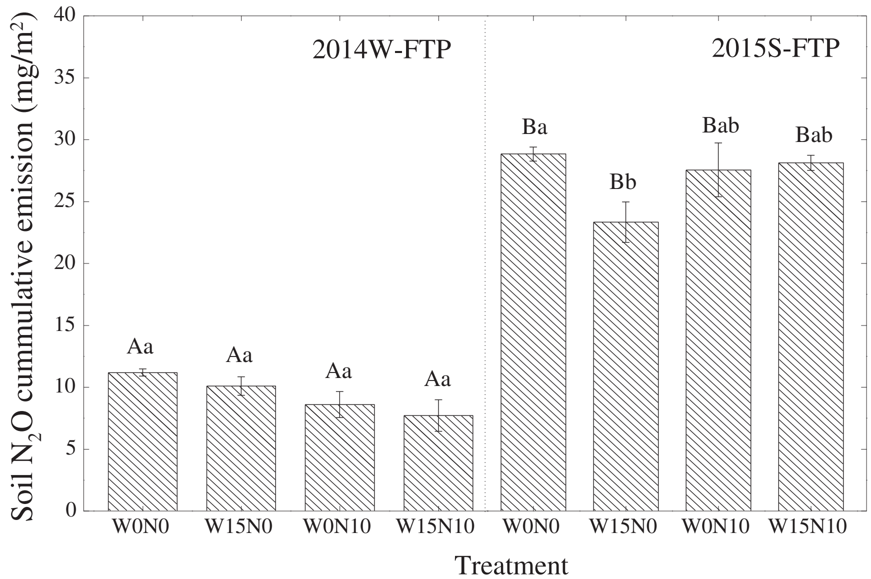

Fig. 7 – Cumulative N_2O emissions of the different treatments. Error bars indicate the standard errors of the mean (n = 3). Bars with different small letters denote treatments of the same freeze-thawing period differ significantly at p < 0.05, bars with different capital letters denote 2014W-FTP and 2015S-FTP of the same treatment differ significantly at p < 0.05.

effluxes during the 2014W-FTP, and air temperature and the soil water content were the significant environmental factors during 2015S-FTP. The temperature factor was included in the multiple stepwise regression equations of both periods, which means temperature factor was the main factor during the freeze-thawing periods. With the increasement of precipitation during 2015S-FTP, soil water content was included in the stepwise regression equations in 2015S-FTP. But the R^2 were not high enough, which also indicated that there may exist other factors affected the soil N_2O emissions jointly. Laboratory simulation is easy to control the environment variables, and it is better to conduct field experiment together with laboratory simulation in the future studies (Fig. 7).

4. Conclusions

The short-term changes of precipitation and N deposition did not show remarkable differential influence on soil N_2O emissions of freeze-thawing periods. The soil PLFA concentrations of microbial groups monitored during 2015S-FTP were lower than those monitored during 2014W-FTP, while soil N_2O emissions of 2015S-FTP were higher than those of 2014W-FTP, as may be caused by the enhanced activity of surviving microbe in 2015S-FTP and the physical release of the N_2O trapped by soil during the whole freeze-thawing process and frozen period. Multiple stepwise regression analysis showed that the soil temperature, soil NH_4^+-N content and air temperature were the major environmental factors that influenced the N_2O effluxes during 2014W-FTP, and air temperature and the soil water content during 2015S-FTP. The soil microbe driving mechanism and the contribution of the trapped soil N_2O to soil N_2O emissions need to be further studied. To make the possible reasons more certain and further explore mechanisms of the freeze-thawing cycles on soil N_2O emission, the combination of laboratory simulation experiments and long-term field investigation with high monitoring frequency should be carried out in the future.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 41373084, 41330528, and 41203054). We are very grateful to the Inner Mongolia Grassland Ecosystem Research Station for their assistance. The authors thank the editors and the anonymous reviewers for their valuable comments on the manuscript.

REFERENCES

Abdelmagid, H.M., Tabatabai, M.A., 1987. Nitrate reductase activity of soils. Soil Biol. Biochem. 19 (4), 421–427.

Budge, K., Leifeld, J., Egli, M., Fuhrer, J., 2011. Soil microbial communities in (sub) alpine grasslands indicate a moderate shift towards new environmental conditions 11 years after soil translocation. Soil Biol. Biochem. 43 (6), 1148–1154.

Burton, D.L., Beauchamp, E.G., 1994. Profile of nitrous oxide and carbon dioxide concentrations in a soil subject to freezing. Soil Sci. Soc. Am. J. 58 (1), 115–122.

Christensen, S., Christensen, T.B., 1991. Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. Eur. J. Soil Sci. 42 (4), 637–647.
Christensen, S., Tiedje, J.M., 1990. Brief and vigorous N_2O production by soil at spring thaw. Eur. J. Soil Sci. 41 (1), 1–4.
Decker, K.L.M., Wang, D., Waite, C., Scherbatskoy, T., 2003. Snow removal and ambient air temperature effects on forest soil temperatures in northern Vermont. Soil Sci. Soc. Am. J. 67 (4), 1234–1243.
Dise, N.B., Stevens, C.J., 2005. Nitrogen deposition and reduction of terrestrial biodiversity: evidence from temperate grasslands. Sci. China C Life Sci. 48 (z2), 720–728.
Dong, Y.S., Zhang, S., Qi, Y.C., Chen, Z.Z., Geng, Y.B., 2000. Fluxes of CO_2, N_2O and CH_4 from a typical temperate grassland in Inner Mongolia and its daily variation. Chin. Sci. Bull. 45 (17), 1590–1594.
Dusenbury, M.P., Engel, R.E., Miller, P.R., Lemke, R.L., Wallander, R., 2008. Nitrous oxide emissions from a Northern Great Plains Soil as influenced by nitrogen management and cropping systems. J. Environ. Qual. 37, 542–550.
Edwards, A.C., Cresser, M.S., 1992. Freezing and its effects on chemical and biological properties of soil advances in soil. Science 18, 59–79.
Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14 (3), 151–163.
Galloway, J.N., Cowling, E.B., 2002. Reactive nitrogen and the world: 200 years of change. AMBIO J. Hum. Environ. 31 (2), 64–71.
Goldberg, S.D., Borken, W., Gebauer, G., 2010. N_2O fluxes in a Norway spruce forest due to soil frost: concentration and isotope profiles shed a new light on an old story. Biogeochemistry 97 (1), 21–30.
Goodroad, L.L., Keeney, D.R., 1984. Nitrous oxide emissions from soils during thawing. Can. J. Soil Sci. 64 (2), 187–194.
Guan, Y.S., 1986. Soil Enzyme and the Research Methods. China Agriculture Press, Beijing.
Holst, J., Liu, C., Yao, Z., Brüggemann, N., Zheng, X., Giese, M., et al., 2008. Fluxes of nitrous oxide, methane and carbon dioxide during freezing–thawing cycles in an Inner Mongolian steppe. Plant Soil 308 (1), 105–117.
Hugh, A.L.H., 2007. Soil freeze–thaw cycle experiments: trends, methodological weaknesses and suggested improvements. Soil Biol. Biochem. 39 (5), 977–986.
IPCC, 2007. Climate change 2007: the physical science basis. In: Solomon, S., Qin, D., Manning, M., et al. (Eds.), Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
Isard, S.A., Schaetzl, R.J., 1998. Effects of winter weather conditions on soil freezing in southern Michigan. Phys. Geogr. 19 (1), 71–94.
Jiang, Z.H., Zhang, X., Wang, J., 2008. Projection of climate change in China in the 21 century by IPCC-AR4 models. Geogr. Res. 27 (4), 787–799.
Kaiser, E.A., Kohrs, K., Kücke, M., Schnug, E., Munch, J.C., Heinemeyer, O., 1998. Nitrous oxide release from arable soil: importance of N-fertilisation, crops and temporal variation. Soil Biol. Biochem. 30 (12), 1553–1563.
Kattenberg, A., Giorgi, F., Grassi, A., Meehl, G.A., Mitchell, J.F.B., Stouffer, R.J., 1996. Climate models—projections of future climate. In: Houghton, J.T., MeiraFilho, L.G., Callander, B.A., et al. (Eds.), Climate Change 1995: the Science of Climate Change. Cambrige University Press, New York, pp. 285–357.
Koponen, H.T., Flojt, L., Martikainen, P.J., 2004. Nitrous oxide emissions from agricultural soils at low temperatures: a laboratory microcosm study. Soil Biol. Biochem. 36 (5), 757–766.
Li, K.H., Gong, Y.M., Song, W., Lv, J.L., Chang, Y.H., Hu, Y.K., et al., 2012. No significant nitrous oxide emissions during spring thaw under grazing and nitrogen addition in an alpine grassland. Glob. Chang. Biol. 18 (8), 2546–2554.
Liu, X.C., 2013. The Effects of Water and Nitrogen Coupling on the Soil N_2O Fluxes in the Temperate Semi-arid Grassland. (PhD Thesis). University of Chinese Academy of Sciences, Beijing, China.
Liu, X.J., Zhang, Y., Han, W.X., Tang, A.H., Shen, J.L., Cui, Z.L., et al., 2013. Enhanced nitrogen deposition over China. Nature 494 (7438), 459–463.
Martin, R.E., Scholes, M.C., Mosier, A.R., Ojima, D.S., Holland, E.A., Parton, W.J., 1998. Controls on annual emissions of nitric oxide from soils of the Colorado short-grass steppe. Glob. Biogeochem. Cycles 12 (1), 81–91.
Mosier, A.R., Parton, W.J., Valentine, D.W., Ojima, D.S., Schimel, D., Delgado, J.A., 1996. CH_4 and N_2O fluxes in the Colorado short-grass steppe: impact of landscape and nitrogen addition. Glob. Biogeochem. Cycles 10 (3), 387–399.
Norman, J., Jansson, P.E., Farahbakhshazad, N., Butterbach-Bahl, K., Li, C.S., Klemedtsson, L., 2008. Simulation of NO and N_2O emissions from a spruce forest during a freeze/thaw event using an N-flux submodel from the PnET-N-DNDC model integrated to CoupModel. Ecol. Model. 216 (1), 18–30.
Peng, Q., Qi, Y.C., Dong, Y.S., Xiao, S., He, Y., 2011. Soil nitrous oxide emissions from a typical semiarid temperate steppe in inner Mongolia: effects of mineral nitrogen fertilizer levels and forms. Plant Soil 342 (1), 345–357.
Qi, Y.C., Dong, Y.S., Liu, J.Y., Domroes, M., Geng, Y.B., Liu, L.X., et al., 2007. Effect of the conversion of grassland to spring wheat field on the CO_2 emission characteristics in Inner Mongolia, China. Soil Tillage Res. 94 (2), 310–320.
Qi, Y.C., Liu, X.C., Dong, Y.S., Peng, Q., He, Y.T., Sun, L.J., 2014. Differential responses of short-term soil respiration dynamics to the experimental addition of nitrogen and water in the temperate semi-arid; steppe of Inner Mongolia, China. J. Environ. Sci. 26 (4), 834–845.
Sharratt, B.S., 1993. Freeze–thaw and winter temperature of agricultural soils in interior Alaska. Cold Reg. Sci. Technol. 22 (1), 105–111.
Song, C.C., Wang, Y.S., Wang, Y.Y., Zhao, Z.C., 2006. Emission of CO_2, CH_4 and N_2O from freshwater marsh during freeze–thaw period in northeast of China. Atmos. Environ. 40, 6879–6885.
Sun, L.J., Qi, Y.C., Dong, Y.S., He, Y.T., Peng, Q., Liu, X.C., et al., 2015. Interactions of water and nitrogen addition on soil microbial community composition and functional diversity depending on the inter-annual precipitation in a Chinese steppe. J. Integr. Agric. 14 (4), 788–799.
Teepe, R., Brumme, R., Beese, F., 2001. Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biol. Biochem. 33 (9), 1269–1275.
Wagner-Riddle, C., Thurtell, G.W., Kidd, G.K., 1997. Estimates of nitrous oxide emissions from agricultural fields over 28 months. Can. J. Soil Sci. 77 (2), 135–144.
Wagner-Riddle, C., Furon, A., Mclaughlin, N., Ivan, L., Barbeau, J., Jayasundara, S., et al., 2007. Intensive measurement of nitrous oxide emissions from a corn-soybean-wheat rotation under two contrasting management systems over 5 years. Glob. Chang. Biol. 13, 1722–1736.
Wang, Y.M., 2007. The Influence of Grazing Intensity on the Hydrological Effects of *Stipa grandis* Steppe. (PhD Thesis). Inner Mongolia Agricultural University, Inner Mongolia, China.
Zhang, J., Kang, R.H., Zhao, B., Huang, Y.M., Ye, Z.X., Duan, L., 2013. Monitoring nitrogen deposition on temperate grassland in Inner Mongolia. J. Environ. Sci. 34 (9), 3552–3556.
Zou, J.W., Huang, Y., Zheng, X.H., Wang, Y.S., Chen, Y.Q., 2004. Static opaque chamber-based technique for determination of net exchange of CO_2 between terrestrial ecosystem and atmosphere. Chin. Sci. Bull. 49 (4), 381–388.