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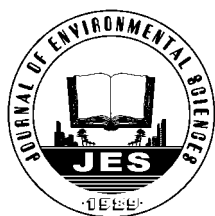
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## Differential responses of short-term soil respiration dynamics to the experimental addition of nitrogen and water in the temperate semi-arid steppe of Inner Mongolia, China

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### ABSTRACT

We examined the effects of simulated rainfall and increasing N supply of different levels on CO<sub>2</sub> pulse emission from typical Inner Mongolian steppe soil using the static opaque chamber technique, respectively in a dry June and a rainy August. The treatments included NH<sub>4</sub>NO<sub>3</sub> additions at rates of 0, 5, 10, and 20 g N/(m<sup>2</sup>·year) with or without water. Immediately after the experimental simulated rainfall events, the CO<sub>2</sub> effluxes in the watering plots without N addition (WCK) increased greatly and reached the maximum value at 2 hr. However, the efflux level reverted to the background level within 48 hr. The cumulative CO<sub>2</sub> effluxes in the soil ranged from 5.60 to 6.49 g C/m<sup>2</sup> over 48 hr after a single water application, thus showing an increase of approximately 148.64% and 48.36% in the effluxes during both observation periods. By contrast, the addition of different N levels without water addition did not result in a significant change in soil respiration in the short term. Two-way ANOVA showed that the effects of the interaction between water and N addition were insignificant in short-term soil CO<sub>2</sub> effluxes in the soil. The cumulative soil CO<sub>2</sub> fluxes of different treatments over 48 hr accounted for approximately 5.34% to 6.91% and 2.36% to 2.93% of annual C emission in both experimental periods. These results stress the need for improving the sampling frequency after rainfall in future studies to ensure more accurate evaluation of the grassland C emission contribution.

## Introduction

Soil respiration is one of the major processes that control the carbon (C) budget of terrestrial ecosystems and the largest fluxes in the global C cycle (Schlesinger and Andrews, 2000). Worldwide concerns about the C cycle and its effects on the environment have emphasized the need for an improved understanding and quantification of soil respiration processes (Mariko et al., 2007).

The production and emission of carbon dioxide (CO<sub>2</sub>) in

soil is a complicated biological process and is influenced jointly by various biotic and abiotic factors (Bowden et al., 2004; Harper et al., 2005; Li et al., 2011). In semi-arid and arid ecosystems, water and soil nutrient availability are considered the most important limiting factors in the primary production of ecosystems (Belnap, 2001; Austin et al., 2004; Guo et al., 2006; Zhao et al., 2009) and the mineralization of soil organic matter, because soil moisture and nutrients affect the supply of C substrates to plant roots and soil microorganisms (Illeris et al., 2003; Xiang et al., 2008; Ouyang et al., 2008).

N fertilization in the different biomes of northern tem-

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perate zones has been estimated to enhance C storage by 0.3 to 0.5 Pg C/year (Bowden et al., 2004). Several published field and laboratory studies have examined the response of soil CO<sub>2</sub> effluxes to changes in N availability. However, the results obtained have often been controversial. For example, some studies (Craine et al., 2001; Xu and Wan, 2008) found that an increased N input stimulated soil respiration, whereas other studies either did not find any effects (Raich and Tufekcioglu, 2000; Micks et al., 2004) or discovered negative effects (Burton et al., 2004; Mo et al., 2008). Bowden et al. (2004) and Peng et al. (2011) found an immediate change in soil respiration following the addition of N in the initial stage of their studies; however, the respiration rates of fertilized plots did not differ from those of the control with continuous N addition. The effects of N addition on soil respiration changed over time.

In addition, alterations in the amount and timing of rainfall events in arid and semi-arid ecosystems because of global changes also are likely to have important effects on the C balance of the entire ecosystem (Sponseller, 2007). Fluctuations in water availability, particularly the rapid increase in water potential after dry soil rewetting in grasslands or other water-limited soil, often result in an intense C emission pulse, and the CO<sub>2</sub> often increases by as much as 500% (Fierer and Schimel, 2003). By contrast, Bouma and Bryla (2000) indicated that heavy rainfall events might temporarily suppress soil respiration because of the reduced diffusion in fine-textured soil with high numbers of water-filled pore spaces. The responses of soil CO<sub>2</sub> emission to changes in either N availability or precipitation are complex and remain debatable. Furthermore, information on grassland ecosystems is limited, particularly on the temperate steppes of China.

Increasing anthropogenic N deposition and shifting precipitation regimes have become a global change phenomenon (Galloway and Cowling, 2002; IPCC, 2007). Anthropogenic N deposition, mainly originating from fertilizer application, fossil fuel combustion, and legume cultivation, has increased from less than 1 Tg N/year in the 1860s to 25 Tg N/year around the year 2000 (Matson et al., 2002; Lu et al., 2012). Given the increasing N concentration in the atmosphere, the global N deposition level is expected to double in the next 25 years (Moore et al., 2009). Liu et al. (2013) reported that the bulk N deposition in China increased significantly over time ( $P < 0.001$ ) with an average annual increase of 0.41 kg N/ha between 1980 and 2010. China has become the region with the third highest N deposition rate after America and Europe (Zhang et al., 2008). In addition, the anticipated global increase in precipitation per decade in this century is approximately 0.5% to 1% (IPCC, 2001). The precipitation in North China is expected to increase by 14 to 155 mm at the end of the 21st century according to the simulations of 13 IPCC global climate models (Jiang et

al., 2008). The significant changes in precipitation and N input may affect greenhouse gas effluxes and result in feedback on global climate change (Liu et al., 2008). Therefore, further research on the effect of altered N and water availability on soil C emission in the arid or semi-arid regions of Northern China is important to the accurate prediction of the C budget of the ecosystem in future climate change scenarios. Several studies on arid and semi-arid regions have shown a close relationship between N and water availability in the regulation of the ecosystem primary production (Zhang and Zak, 1998; Rao and Allen, 2010; Ronnenberg and Wesche, 2011). However, little information has been provided on the interactive effects of water and N on soil CO<sub>2</sub> emission. The short-term effects induced by water or N pulse inputs have often been ignored in previous research. Thus, a comprehensive study on the differential responses of grassland soil respiration to water and N availability, as well as the synthetic effects of water and N at different time scales, is important.

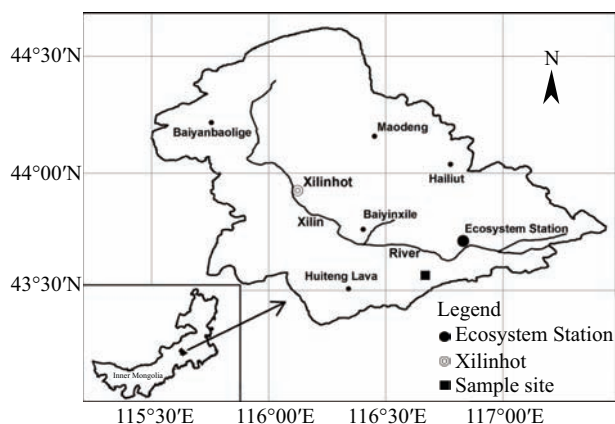
Grassland is the largest terrestrial ecosystem in China, covering an area of approximately  $4 \times 10^6$  km<sup>2</sup> or 40% of the land area of China (Chen and Wang, 2000). Approximately 78% of the grasslands in China are located in the temperate arid and semi-arid regions. A fragile eco-environment increases the sensitivity of grasslands to environmental change compared with other ecosystems (Warren et al., 1996; Wen, 1996). To the best of our knowledge, few studies have explored the short-term response of soil CO<sub>2</sub> fluxes to the coupled changes in water and N availability in the semi-arid region of China. Given the limited studies in this field, a temperate typical steppe in Inner Mongolia, China was investigated. The long-term and short-term responses of soil respiration to changes in the water and N availability were systematically studied. This current study, focused on the short-term effect of elevated N and water availability on soil respiration, was part of a long-term research study. The objectives of this study were as follows: (1) identify the short-term differential responses of soil respiration to the increased N and water availability and their coupled variations, as well as explore the intrinsic mechanisms; (2) characterize the contribution of short-term CO<sub>2</sub> pulses to annual C emission after the episodic addition of water and N.

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## 1 Materials and methods

### 1.1 Site description

The experiment site was located in the Xilin River Basin of Inner Mongolia, China (43°26'N to 44°39'N, 115°32'E to 117°12'E) and in the vicinity of the Inner Mongolian Grassland Ecosystem Research Station, Chinese Academy of Sciences (Fig. 1). The Xilin River Basin is situated in the core area of the Northeast China Transect (NECT) for



**Fig. 1** Map of the sampling site and the Xilin River Basin of Inner Mongolia, China.

the International Geosphere-Biosphere Program (IGBP) on global change study and the original features of the temperate typical steppe in this transect are both typical and representative of grasslands in China and even entire grasslands in Eurasia (Zhang et al., 1997). A *Leymus chinense* steppe (43°32'N, 116°40'E, 1257 m above sea level), which is one of the most widely distributed grassland types in the Xilin River Basin, was selected for investigation. The experimental site was characterized by a continental semi-arid temperate steppe climate with a mean annual temperature of  $-0.3^{\circ}\text{C}$  to  $1^{\circ}\text{C}$ . The temperature ranged from  $-21.41^{\circ}\text{C}$  in January to  $18.53^{\circ}\text{C}$  in July. The mean annual precipitation was approximately 350 to 450 mm, 70% of which fell between June and September. The soil was classified as dark chestnut soil according to Chinese classification or calcic-orthic aridisol according to US soil taxonomy classification. The texture of the soil is composed of 60% sand, 21% clay and 19% silt. The depths of the soil profiles were 100 to 150 cm with a 20–30 cm humus layer. The community was dominated by *L. chinensis*, *Stipa grandis*, *Agropyron michnoi*, and *Cleistogenes squarrosa*. The experiment site had been used for grazing prior to enclosure, with a grazing intensity of approximately 2.25 sheep per hectare. The characteristics of the soils in the experimental plots are listed in **Table 1**.

## 1.2 Experimental design

A randomized block design was used to prepare 5 replicates for each of 8 treatments to examine the short-term

and long-term effects of water and N additions. In May 2010, 40 plots measuring  $8\text{ m} \times 8\text{ m}$  and separated by 1 m buffers were established in the selected *L. chinense* steppe. The simulated experiments on short-term effect were conducted in the relatively dry June of 2010 and in the rainy August of 2011 as part of a long-term study. The eight treatments were: (1) control, i.e., without N and without water addition (CK); (2) with N addition at a low rate of  $5\text{ g N}/(\text{m}^2\cdot\text{year})$  without water (LN); (3) with N addition at a medium rate of  $10\text{ g N}/(\text{m}^2\cdot\text{year})$  without water (MN); (4) with N addition at a high rate of  $20\text{ g N}/(\text{m}^2\cdot\text{year})$  without water addition (HN); (5) with water addition without N (WCK); (6) with N addition at a low rate of  $5\text{ g N}/(\text{m}^2\cdot\text{year})$  and water (WLN); (7) with N addition at a medium rate of  $10\text{ g N}/(\text{m}^2\cdot\text{year})$  and water (WMN); (8) with N addition at a high rate of  $20\text{ g N}/(\text{m}^2\cdot\text{year})$  and water (WHN).

In the manipulative experiment, 640 L water (equivalent to approximately 10.0 mm rainfall) was manually added to the experimental plots using backpack sprayers on June 24, 2010 and August 3, 2011. Simultaneously,  $\text{NH}_4\text{NO}_3$  was ground into fine powder, weighed, and then spread evenly on the fertilized plots. Rainfall of approximately 4 to 10 mm, which occurred in higher frequency in the study area during growing season, was considered an ecologically effective precipitation and was sufficient to cause the soil surface to alternate in a dry-wet-dry cycle (Du et al., 2006; Hao et al., 2010). The amount of N addition was based on the current and future 50 years N deposition level. The simulated studies applied large doses of N over a short period, with the underlying 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 ways such as seepage, immobilization in deep soil layers, or volatilization in an N addition experiment of  $100\text{ kg N}/(\text{ha}\cdot\text{year})$  over 5 years, the cumulative N is  $500\text{ kg N}/\text{ha}$  or approximately 25 years of N deposition at a rate of  $21.1\text{ kg N}/(\text{ha}\cdot\text{year})$ , which is the average annual bulk deposition of N in China in 2010 (Dise and Stevens, 2005; Liu et al., 2013).

## 1.3 Soil respiration rate measurements

The  $\text{CO}_2$  gas samples for the short-term pulse experiment were mainly collected by using a static opaque chamber during a dry period (from June 24, 2010 to June 26, 2010) and a relatively rainy period (from August 3, 2011

**Table 1** Characteristics of the *L. chinense* steppe soil \*

Soil sampling depth (cm)	Total organic C (g/kg)	Total N (g/kg)	C/N ratio	$\text{NH}_4^+-\text{N}$ (mg/kg)	$\text{NO}_3^--\text{N}$ (mg/kg)	Bulk density ( $\text{g}/\text{cm}^3$ )
0–10	16.52 ( $\pm 1.05$ )	1.43 ( $\pm 0.27$ )	11.55 ( $\pm 1.51$ )	3.98 ( $\pm 0.03$ )	6.54 ( $\pm 0.50$ )	1.28 ( $\pm 0.01$ )
10–20	14.05 ( $\pm 1.41$ )	1.35 ( $\pm 0.28$ )	10.40 ( $\pm 1.17$ )	3.91 ( $\pm 0.08$ )	3.38 ( $\pm 0.14$ )	1.34 ( $\pm 0.03$ )
20–30	13.83 ( $\pm 1.65$ )	1.21 ( $\pm 0.29$ )	11.43 ( $\pm 1.47$ )	3.84 ( $\pm 0.52$ )	3.85 ( $\pm 0.21$ )	1.35 ( $\pm 0.03$ )

Data are mean ( $\pm$  SE,  $n = 3$ ).

to August 5, 2011). The chamber had an area of 50 cm (length) × 40 cm (width) × 30 cm (height) and was made of 8 mm black acrylic material with a tinfoil reflecting film attached to the external surface to avoid over-fast temperature increases in the chamber. The effectiveness of the opaque chamber for measuring soil respiration has been reported previously (Dong et al., 2000; Zheng et al., 2002; Zou et al., 2004).

In this study, soil respiration rates were measured before the experimental addition of water and N, then at 2, 5, 24, and 48 hr. The sampling chambers were placed on a relatively flat plot (aboveground vegetation was cut to ground level 1 day before sampling), and three duplicates of each sampling were set. During measurement, the sampling chamber was placed into a groove (2 cm × 2 cm × 2 cm) at the four outer edges of the stainless steel frame and was carefully sealed with distilled water. The stainless steel frame was inserted into the soil at a depth of 5 cm. The lid of the chamber was installed with the following: an air disturbing fan driven by a 12 V lead-acid battery for air circulation, a highly precise temperature sensor connected with a digital thermometer, a gas channel that consists of a PVC tube, a silica gel pipe connected to a 100 mL syringe, and a three-way stopcock for gathering gas. Gas samples were extracted from the chamber at 0, 7, 14, and 21 min after capping. For each sample, approximately 200 mL of gas was extracted from the chamber and placed into the polyethylene-coated aluminum bags for CO<sub>2</sub> gas concentration analysis. CO<sub>2</sub> concentrations were measured in the laboratory using a LI-6252 infrared CO<sub>2</sub> analyzer (LICOR Inc., USA) shortly after the sampling.

Air temperature, soil temperature, and the internal chamber temperature were measured simultaneously during each gas sampling. The temperature in the chamber was measured using a temperature sensor. The air temperature was measured by a DHM2 mechanical ventilated thermometer, and the soil temperatures at 0, 5, and 10 cm were measured by a SN2202 digital thermo detector (Sinan Instruments Co., Beijing Normal University, China).

#### 1.4 Soil sampling and analysis

Soils were collected synchronously with gas sampling at depths of 0 to 10 cm, 10 to 20 cm, and 20 to 30 cm by using a handheld auger. Each sample comprised a mixture of nine samples from the same layer of the same treatment. After all of the visible extraneous materials were removed by hand, the soil samples were sieved (< 2 mm) and divided into two sub-samples. One sub-sample was air-dried at ambient temperature, ground, and sieved through 0.15 mm mesh for total organic C (TOC) and total N (TN) analyses. The other sub-sample was maintained fresh in the dark at 4°C to assess microbial biomass C (MBC). Gravimetric water moisture was determined using the oven-drying method at 105°C for 24 hr. The samples were analyzed for TOC and TN using the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> oxidation method and

the micro-Kjeldahl method, respectively (Bremner, 1996). Soil MBC was extracted using the chloroform fumigation method and was quantified by a TOC analyzer (Vario TOC Cube, Elementar, Germany).

#### 1.5 Data analysis

The CO<sub>2</sub> efflux was calculated as follows:

$$F = \frac{\Delta m}{\Delta t} D \frac{V}{A} = h_c D \frac{\Delta m}{\Delta t} \quad (1)$$

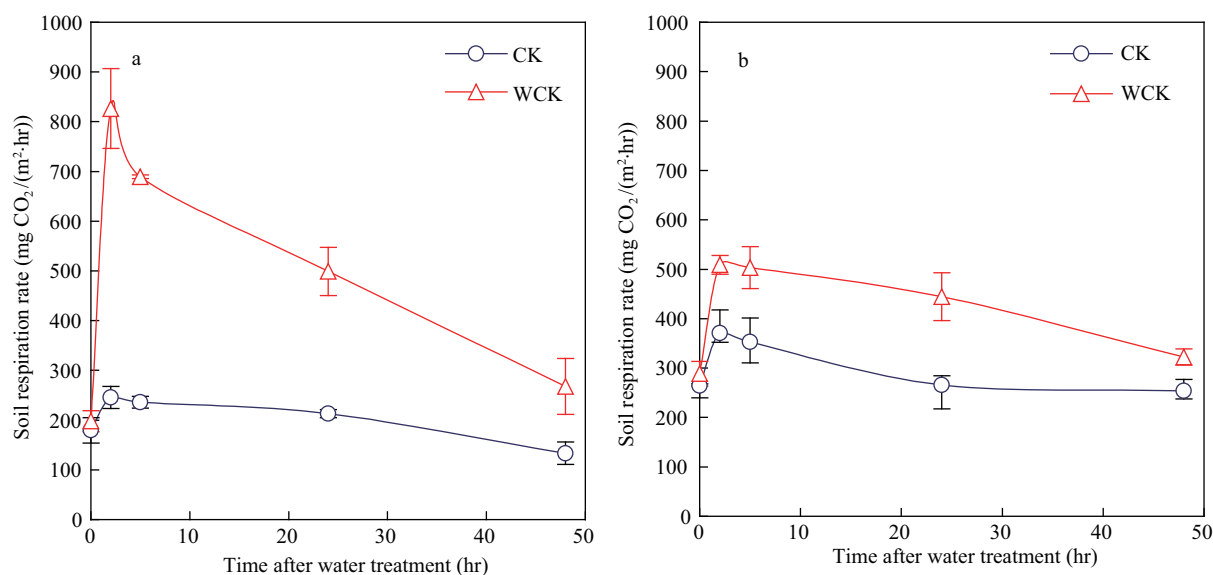
where,  $F$  (mg/(m<sup>2</sup>·hr)) refers to CO<sub>2</sub> efflux,  $V$  (m<sup>3</sup>) is the volume of the sampling chamber,  $A$  (m<sup>2</sup>) is the land area covered by the chamber,  $D$  (mol/m<sup>3</sup>) is the gas density of the chamber ( $D = n/v = P/RT$ ,  $n$  (g/mol) is the molar mass of CO<sub>2</sub>,  $v$  (m<sup>3</sup>) is the gas volume,  $P$  (Pa) is the air pressure,  $T$  (K) is the temperature inside the chamber, and  $R$  (J/(mol·K)) is the gas constant.  $\Delta m/\Delta t$  denotes the linear slope of the concentration change over the measurement period.  $h_c$  (m) represents the height of the sampling chamber.

To describe the response dynamics of soil respiration to water addition, two indexes were selected: (1) AVR-2h, which is the time-weighted average respiration rate measured during the first 2 hr; (2) CFLUX-48h, which is the total C emission over the whole duration of the experiment (Sponseller, 2007; Jin et al., 2009). Statistical analyses of the experimental data were performed using the SPSS 13.0 software package (SPSS Inc., 2001). The differences in soil respiration rate and MBC content between plots with and without water addition were determined by the paired-samples  $t$ -test. Statistically significant differences among different N addition treatments were identified using one-factor analysis of variance (ANOVA). The two-way ANOVA was used to detect the significance of the effects of the main factors, i.e., N addition (N) and water addition (W), and the effect of the interaction factors (N × W) on each sampling period. Graphs were prepared using Excel® 2007 and Coreldraw1 12.0.

## 2 Results

### 2.1 Responses of soil CO<sub>2</sub> effluxes to a precipitation pulse under different ambient soil moisture conditions

The temporal dynamics of soil respiration rates in the water addition experiments in June 2010 and August 2011 were similar (Fig. 2). The soil respiration rates had similar values in the plots with and without water addition before the manipulative experiment. During the relatively dry observation period of 2010, natural precipitation did not occur for approximately 2 weeks. Only a light shower of approximately 1.1 mm occurred one week before the experiment. Following the simulated precipitation events,



**Fig. 2** Short-term dynamics of soil respiration rates in response to a 10 mm precipitation pulse during the observation periods of late June 2010 (a) and early August 2011 (b). CK refers to the treatment without water and N addition; WCK refers to the treatment with water addition without N. Error bars indicate the standard errors of the mean ( $n = 3$ ).

CO<sub>2</sub> emission from the treatment with single water addition (WCK) increased immediately and reached the maximum of 826.22 mg CO<sub>2</sub>/(m<sup>2</sup>·hr) at 2 hr after soil rewetting, thus showing an increase of approximately 316.92% compared with the initial emission value. The CO<sub>2</sub> effluxes then decreased gradually but remained at a relatively high level within 5 hr, and after that, CO<sub>2</sub> emissions decreased sharply and reverted to the background level within 48 hr. Relative to the pulse emissions generated in the WCK treatment, the soil respiration rates of CK remained at a low level throughout the experimental period and displayed a low peak to valley ratio. During the relatively rainy experiment period of August 2011, a certain pulse effect of water addition was present but the peak value of soil respiration in the plots with water addition was only 76.35% higher than the initial emission value. The differences in CO<sub>2</sub> effluxes between WCK and CK in 2010 and 2011 were all significant ( $p < 0.05$ , paired-sample *t*-test); however, the CO<sub>2</sub> pulse caused by the rapid increase in water availability was weaker in the rainy August 2011 than in dry June 2010.

By calculating the AVR-2h and CFLUX-48h, we found that the AVR-2h and CFLUX-48h values of WCK were significantly higher than the values of CK (**Table 2**). However, the increases in the magnitudes of soil respiration differed between the two experiments in 2010 and 2011. The AVR-2h value of WCK during the experiment period of 2010 was 0.140 g C/(m<sup>2</sup>·hr), which was 2.41 times as much as that of the AVR-2h value of CK (0.058 g C/(m<sup>2</sup>·hr)). The CFLUX-48h values of the CK and WCK treatments were 2.609 and 6.487 g C/m<sup>2</sup>, respectively. CO<sub>2</sub> effluxes after water application alone showed an increase of approximately 148.64% over 48 hr. During the

**Table 2** Comparison of AVR-2h and CFLUX-48h values between treatments with and without water addition

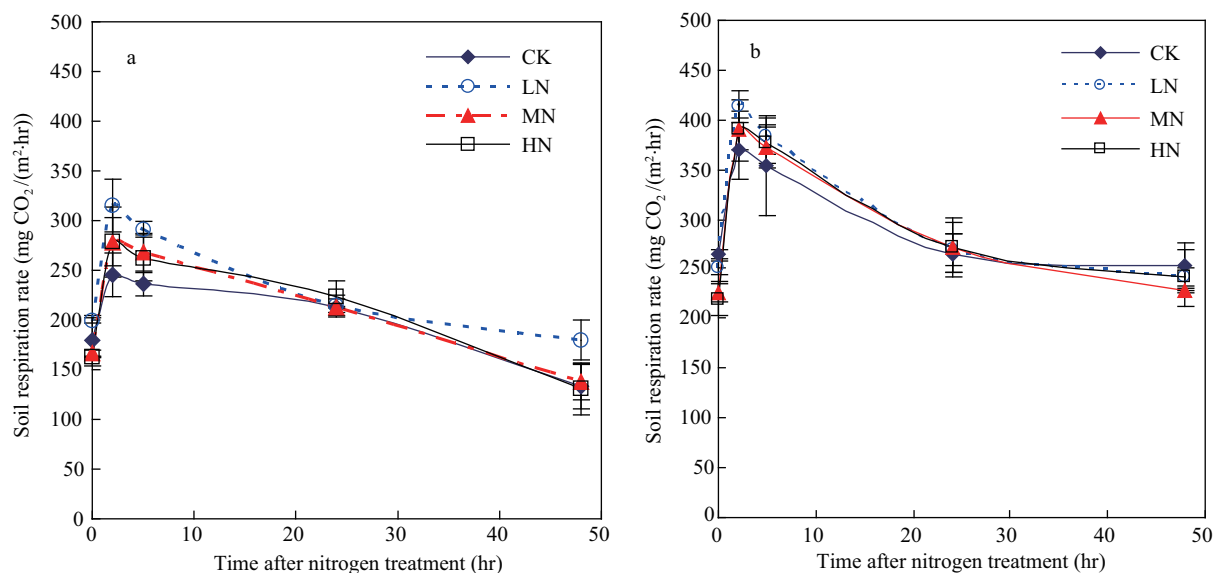
Sampling period	Index	CK	WCK
June 24–26, 2010	AVR-2h (g C/(m <sup>2</sup> ·hr))	0.058	0.140
	CFLUX-48h (g C/m <sup>2</sup> )	2.609	6.487
August 3–5, 2011	AVR-2h (g C/(m <sup>2</sup> ·hr))	0.092	0.109
	CFLUX-48h (g C/m <sup>2</sup> )	3.772	5.596

second experiment period in August 2011, the AVR-2h and CFLUX-48h values in the CK treatments were both higher than those in 2010; the opposite result occurred in the WCK plots. The AVR-2h and CFLUX-48h values in WCK were only 17.72% and 48.36% higher than those in CK. Similar to the effect on the emission peak, the pulse effect of water addition on the mean flux of 2 hr and the cumulative C emission over 48 hr were weaker in August 2011 than in June 2010.

## 2.2 Short-term effects of N addition on soil CO<sub>2</sub> effluxes

The dynamics of soil respiration rates in both the fertilized and unfertilized plots were similar during the study period; the highest fluxes were observed at 2 hr after N input in both years (**Fig. 3**). The peaks of the different N treatments were 278.67 ± 24.30 (HN), 278.40 ± 35.38 (MN), 315.03 ± 26.27 (LN), and 245.47 ± 22.07 (CK) mg CO<sub>2</sub>/(m<sup>2</sup>·hr) in June 2010 (**Fig. 3a**), and 389.70 ± 18.85 (HN), 389.71 ± 30.33 (MN), 413.98 ± 15.60 (LN) as well as 370.96 ± 31.32 (CK) mg CO<sub>2</sub>/(m<sup>2</sup>·hr) in August 2011 (**Fig. 3b**). The maximum CO<sub>2</sub> emission rates under the different N treatments were approximately 1.13 to 1.28 times and 1.05 to 1.12 times as much as those of CK during both observation periods. The high respiration rates remained

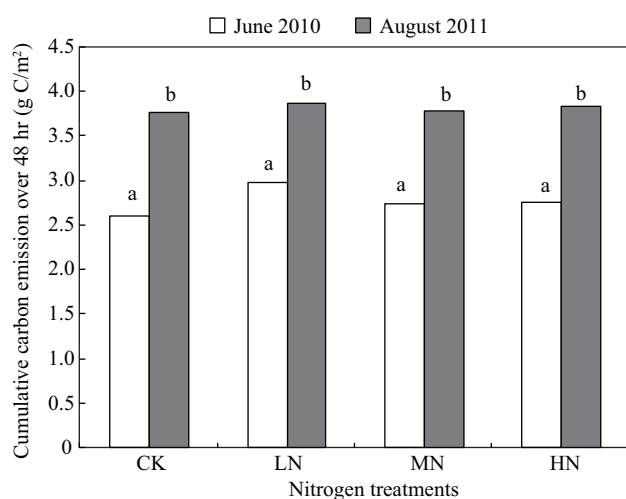




**Fig. 3** Short-term dynamics of soil respiration rates after the N addition during the observation periods of late June 2010 (a) and early August 2011 (b). Error bars indicate the standard errors of the mean ( $n = 3$ ). CK refers the treatment without water and N addition, LN, MN and HN refer the treatments with the N addition at rates of 5, 10 and 20 g N/(m<sup>2</sup>·year) respectively and without water.

until approximately 5 hr after N input in all treatments. Then, decreasing CO<sub>2</sub> effluxes with small fluctuations were observed until 48 hr.

Cumulative CO<sub>2</sub> fluxes over the 48 hr period were calculated for each of the fertilized and unfertilized treatments (**Fig. 4**). In the dry experiment period of 2010, the C losses throughout the 48 hr period were 2.76 g C/m<sup>2</sup> (in HN), 2.74 g C/m<sup>2</sup> (in MN), and 2.99 g C/m<sup>2</sup> (in LN), which were 5.8%, 4.9%, and 14.5% higher than the losses in the CK plot (2.61 g C/m<sup>2</sup>), respectively. The addition of different N levels increased the soil respiration rates to a certain degree. However, one-way ANOVA showed that the differences between the three N fertilization treatments and CK did not reach the 0.05 significance level. In the



**Fig. 4** Cumulative CO<sub>2</sub> effluxes over 48 hr in the soil treated with different N levels during different observation periods. Bars with different letters denote treatments that differ significantly at  $p < 0.05$ .

relatively wet experiment period of 2011, the cumulative CO<sub>2</sub> effluxes had the following order: LN (3.87 g C/m<sup>2</sup>) > HN (3.83 g C/m<sup>2</sup>) > MN (3.78 g C/m<sup>2</sup>) > CK (3.77 g C/m<sup>2</sup>). The highest C loss occurred in the LN treatment rather than in MN or in HN treatments, and the pulse effect caused by N input in August 2011 was weaker than that in June 2010. Similar to that of June 2010, the C loss during the entire 48 hr period in the experiment of August 2011 was not elevated significantly by N addition, and no significant differences were observed among the three levels of N addition treatment in both years either (one-way ANOVA and Fisher's LSD,  $p > 0.05$ ). This result implied that the addition of different N levels did not cause any significant changes in the short-term CO<sub>2</sub> effluxes of the temperate steppe soils of the study area. The differences in the soils that underwent the same N treatment level between the two experimental periods were all significant ( $p < 0.05$ ).

### 2.3 Interactive effects of N and water addition on soil CO<sub>2</sub> pulse emission

Global change involves simultaneous changes in multiple factors, which could have complex interactive influences on ecosystem biogeochemical processes (Zhou et al., 2006). When averaged over the entire study period, one can see that on one hand, the CO<sub>2</sub> effluxes in treatments with the same N levels were all significantly greater in treatments with water than without water addition (**Table 3**). The smallest detectable difference in soil CO<sub>2</sub> effluxes between treatments with and without water addition was 274.06 and 115.35 mg CO<sub>2</sub>/(m<sup>2</sup>·hr) in June 2010 and August 2011, respectively. The mean effluxes of different N treatments with water were 147.22% and 46.55% higher

**Table 3** Comparison of mean soil CO<sub>2</sub> effluxes (mg CO<sub>2</sub>/(m<sup>2</sup>·hr)) over 48 hr among treatments with different water and N addition

Sampling period	Treatment	Mean of CO <sub>2</sub> efflux		With water/without water
		Without water addition	With water addition	
June 24–26, 2010	CK	199.32 ± 45.74 a	495.56 ± 267.91 b	2.49
	LN	228.21 ± 59.49 a	532.33 ± 266.54 b	2.33
	MN	209.00 ± 61.62 a	483.06 ± 167.86 b	2.31
	HN	210.81 ± 63.51 a	583.92 ± 248.46 b	2.77
	Average	211.84	523.72	2.47
	CV (%)	5.68	8.64	
August 3–5, 2011	CK	288.15 ± 55.65 a	427.50 ± 102.48 b	1.48
	LN	295.67 ± 80.73 a	458.54 ± 126.40 b	1.55
	MN	288.98 ± 78.51 a	414.06 ± 74.05 b	1.43
	HN	292.86 ± 78.47 a	408.21 ± 85.47 b	1.39
	Average	291.42	427.08	1.47
	CV (%)	1.20	5.26	

Values followed by the same letter in the same column are not significantly different at  $p < 0.05$ .

than those of treatments without water addition in both observation periods. Water addition also enlarged the variation coefficient (CV) of the soil respiration rates among different N treatments. However, water addition did not lead to a significant change in the difference of the four N treatments in both observation periods.

On the other hand, the CO<sub>2</sub> effluxes were significantly increased by water addition. However, the stimulating effects of water addition on soil respiration were different among treatments with different N levels. The ratios between the mean CO<sub>2</sub> effluxes in treatments with the same N addition level with and without water application were compared. The comparison showed that only the ratio for high N addition in June 2010 and that for low N addition in August 2011 were higher than the WCK/CK ratio in the same sampling period. The high N addition in June 2010 and the low N addition in August 2011 enhanced the effect of the water application on soil respiration, whereas the other N treatments offset the positive influence of water on soil respiration to a certain extent.

The above-mentioned analyses showed that a certain interaction exists between water and N availability. However, the results of the two-way ANOVA showed that the CO<sub>2</sub> effluxes were significantly ( $p < 0.01$ ) affected by water but were insignificantly affected by N addition rates and the interactive effect between N and water (Table 4).

### 3 Discussion

#### 3.1 Response and mechanisms of soil CO<sub>2</sub> emission to N input

Most studies on N-limited ecosystems showed a pronounced effect caused by fertilization or the combination of fertilization and irrigation on ecosystem C emission

**Table 4** Results of two-way ANOVA on the effects of N addition (N), water addition (W), and the interactive effect of N and water on soil respiration in both observation periods (with five replications)

	F value		P value	
	June 2010	August 2011	June 2010	August 2011
N	0.18	0.38	0.91	0.77
W	24.86	16.51	0.00	0.00
N × W	0.09	0.16	0.96	0.92

(Lai et al., 2002; Xu and Wan, 2008; Peng et al., 2011). However, our data implied that higher soil CO<sub>2</sub> effluxes occurred in the LN application rather than in the MN or HN treatments, and the responses of short-term soil CO<sub>2</sub> effluxes to N addition level were not statistically significant in treatments both with and without water application in the two sampling periods. Similar results have also been reported in other ecosystems (Micks et al., 2004; Wilson and Al-Kaisi, 2008; Jiang et al., 2010). The lack of N effect could be attributed to the counteracting response of autotrophic and heterotrophic respiration. Plant respiration rate increases with increasing tissue N and root biomass under N fertilization (Vose et al., 1997; Tu et al., 2011). However, N addition has also been shown to decrease heterotrophic respiration by decreasing microbial activity (Burton et al., 2004; Philips and Fahey, 2007; Zhang et al., 2012). Generally, NH<sub>4</sub>NO<sub>3</sub> application would result in short-term increases in soil CO<sub>2</sub> effluxes, depending upon the hypothesis that microbial activity in soils is N limited rather than C limited (Micks et al., 2004; Peng et al., 2011). The microbial growth is limited when the soil micro biota are under the status of C deficiency because of insufficient energy and substrate. A decrease in soil microbial respiration partly offsets the increase in plant respiration, thus resulting in no measurable difference among N treatments. The results indicated a pre-existing

C limitation in the study site. In the present study, the soil of the study area was significantly threatened by desertification and pasture degradation because of long-term over-grazing; the background levels of the C/N ratio in the surface soil were in the range of 10 to 15 (Table 1), which was considerably lower than the most appropriate microbial growth and respiration C/N ratio level of 25. N addition further aggravated the lack of C resources. Our findings implied that the quantitative relationship between C and N should be considered when evaluating the response of C emission to the change in soil N availability and predicting the ecosystem C budget.

N and water had an insignificant synergistic effect on soil C emissions. This result could be attributed to the inverse effects of water and N on community composition and diversity as well as on ecosystem evapotranspiration, etc. N addition often weakened the positive effect of water application on grassland ecosystems by changing the species composition, suppressing plant diversity, or stimulating ecosystem evapotranspiration (Zavaleta et al., 2003; Harpole et al., 2007; Niu et al., 2009). Similar results showing a lack of additive effects of water and N addition were also obtained by Harpole et al. (2007) and Niu et al. (2009) in their long-term experiments on net ecosystem C exchange (NEE) in semi-arid temperate grassland ecosystems.

### 3.2 Response and mechanisms of soil CO<sub>2</sub> emission to rainfall pulse

In contrast to the effect of N addition, the effect of simulated precipitation on soil respiration rates was significant. The cumulative soil CO<sub>2</sub> emissions over the 48 hr study period were increased by a factor of 1.5 to 2.5 in both observation periods. Similar results were reported in some other studies. Sponseller (2007) found that CO<sub>2</sub> production that was integrated over 48 hr increased 4.3-fold and 2.1-fold in fields with different habitats in the Sonoran Desert ecosystem in the United States immediately after experimental rewetting. The measurements conducted by Jin et al. (2009) in the desert shrubland of *Artemisia ordosica* on Ordos Plateau, China, showed that soil respiration rates increased 2.8 to 4.1 times immediately after water addition. The total CO<sub>2</sub> production over 48 hr in the treatments with water addition was 1.2 to 1.6 times as much as the total CO<sub>2</sub> production in the control group. In the present study, no significant difference was found in the soil temperature between treatments with and without water. The soil temperatures at depths of 0, 5, and 10 cm during both sampling periods were all in the range of 20 to 35°C, which was relatively suitable for microbial respiration. One of the possible explanations for the CO<sub>2</sub> pulse could be that surface soil rewetting stimulated C and net N mineralization, which increased the availability of the substrate for microbial activities (Austin et al., 2004; Muhr et al., 2010). Another explanation was that dry

soil rewetting caused macroaggregate disruption, which resulted in enhanced macroaggregate turnover and the loss of macroaggregate-associated organic matter (Denef et al., 2001; Mikha et al., 2005). Meanwhile, the replacement of soil air by precipitation might also cause an enriched CO<sub>2</sub> pulse immediately after water application (Chen et al., 2005). In addition, some studies argued that soil rewetting initiated a marked increase in soil microbial biomass, which would also be responsible for the rapid CO<sub>2</sub> emission pulse (Austin et al., 2004; Sponseller, 2007; Unger et al., 2010). However, in the present study, an insignificant decrease in the soil MBC contents ( $p = 0.078 > 0.05$ , paired-samples *t*-test) was found (data not shown) in the WCK treatment. Therefore, the “substrate supply” mechanism might have dominated the process of CO<sub>2</sub> pulse emission in the research area. The processes associated with soil rewetting (macroaggregate disruption, organic matter redistribution, desorption, stimulated C and net N mineralization, etc.) supply substrate pulses to microbes, thus resulting in the substantial release of CO<sub>2</sub> to the atmosphere (Xiang et al., 2008). These observations are consistent with the idea that short-term losses in soil CO<sub>2</sub> are driven by a small pool of labile organic matter characterized by rapid turnover (Franzuebbers et al., 2000) and that the intensity and duration of the pulse effect depends largely on the amount and quality of available substrates. Extending the duration of the wetted condition would have little or no effect on short-term C losses in resource-poor soil (Sponseller, 2007). In the present study, the CO<sub>2</sub> effluxes quickly returned to near-background levels at 48 hr because of the rapid depletion of the labile substrate, although the soil moisture did not fully decrease to the background level at that time.

The respiratory pulse caused by water addition was stronger in the dry observation period of June 2010, wherein soil respiration increased by 2.5-fold of the value of CK, than in the wet observation period of August 2011, wherein only a 1.5-fold increase was observed. The difference in CO<sub>2</sub>-pulse magnitude between the June 2010 and August 2011 observation periods might be caused by the long time interval between the simulated precipitation and last natural rain event in June 2010. In the 2010 observation period, natural precipitation did not occur for approximately two weeks prior to the simulated experiment, with the exception of a light shower of approximately 1.1 mm one week before. Thus, the soil remained extremely dry, and the soil moisture at the depths of 0 to 10 cm was only 4.20% of the gravimetric water content prior to the experiment. However, the experiment of 2011 was conducted in rainy August, and the dry period prior to the simulated precipitation was only 5 days. The soil moisture at the depths of 0 to 10 cm was approximately 7.24%, and a high background level of soil moisture existed prior to the water application. The increased amplitude of soil moisture after water addition was also higher in the observation period of

June 2010 than in the observation period of August 2011 (Fig. 5). The results confirmed that the response of the CO<sub>2</sub> pulse to the simulated precipitation depended on both the length of the drought prior to precipitation and the extent of changes in soil water potential. This result had been proven in previous studies on other ecosystems (Sponseller, 2007; Unger et al., 2010). The disrupted soil macroaggregate and the dead microbial biomass accumulated during the dry period were important respiratory substrates during soil rewetting.

### 3.3 Contribution of short-term CO<sub>2</sub> pulse to annual total C emission

On the basis of long-term experimental data of CO<sub>2</sub> effluxes in the two years (unpublished data), the estimation results of the annual soil respiration sum were obtained by using integral quadrature between the dynamic curves of the respiration rates in the current time interval and time axis (Table 5). Table 5 shows that the annual soil respiration sum in treatments with water and N varied between 110.69–125.55 g C/(m<sup>2</sup>·year) and 205.10–229.88

g C/(m<sup>2</sup>·year) in June 2010 and August 2011, respectively. The cumulative CO<sub>2</sub> effluxes of different water and N treatments over the 48 hr study period accounted for approximately 5.34% to 6.91% and 2.36% to 2.93% of annual estimates, which were calculated based on the measurements without intensive samplings during all the rainy days or the N addition days, in 2010 and 2011 respectively. This result was in good agreement with the result of 1.4% to 2.8% obtained by Sponseller (2007) in a desert ecosystem.

A single respiration pulse induced by water and N addition had little effect on cumulative annual CO<sub>2</sub> emissions. However, the experimental area, which was situated in the temperate semi-arid continental climate zone, was characterized by low rainfall, remarkable precipitation variability, and frequent light rainfall events during dry periods (Chen and Gong, 2005). On the basis of the analysis of precipitation records of the experimental site for 5 consecutive years, effective rainfall of over 5 mm occurred at an average of 13 times a year during the growing season. Rainfall of over 10 mm, which we evaluated in

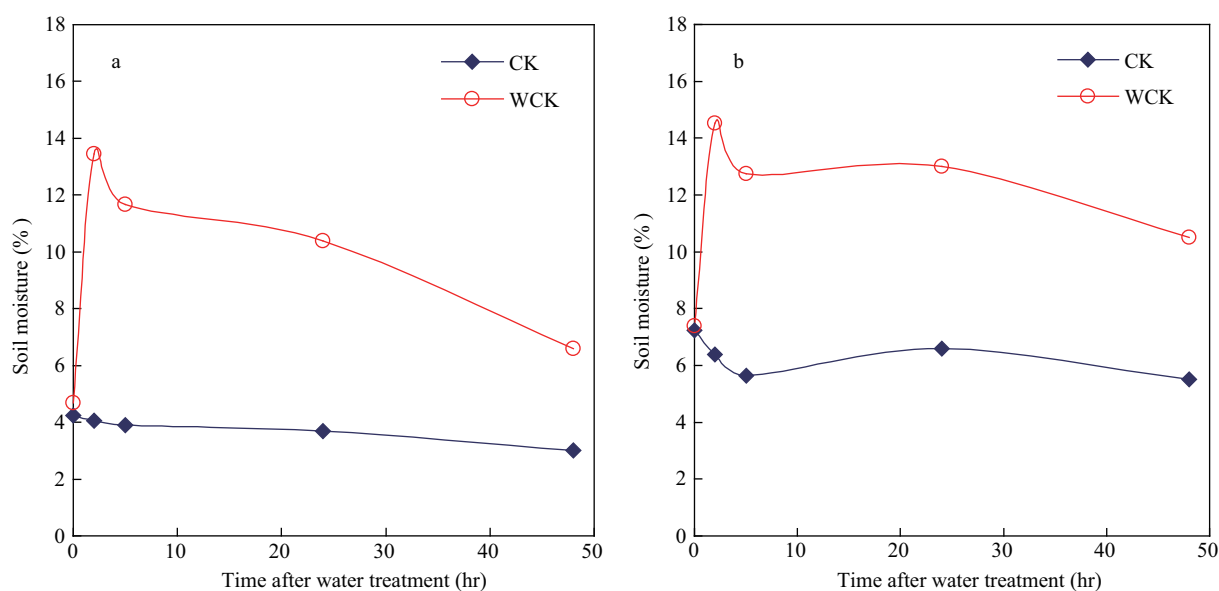


Fig. 5 Changes in soil gravimetric water content at soil depths of 0 to 10 cm following simulated rainfall during the observation periods of late June 2010 (a) and early August 2011 (b).

Table 5 Contribution of a CO<sub>2</sub> pulse to annual total C emission\*

Sampling period	Item	WCK	WLN	WMN	WHN
June 24–26, 2010	A (g C/m <sup>2</sup> )	6.49	6.97	6.32	7.64
	B (g C/(m <sup>2</sup> ·year))	121.59	125.55	117.74	110.69
	A/B (%)	5.34	5.55	5.37	6.91
August 3–5, 2011	A (g C/m <sup>2</sup> )	5.60	6.00	5.42	5.34
	B (g C/(m <sup>2</sup> ·year))	209.62	205.10	229.88	213.71
	A/B (%)	2.67	2.93	2.36	2.50

\* A refers to the cumulative CO<sub>2</sub> effluxes over the 48 hr study period; B refers to the annual CO<sub>2</sub> emission; WCK refers to the treatment with water addition without N; WLN, WMN and WHN refer to the treatments with water and the N addition at rates of 5, 10 and 20 g N/(m<sup>2</sup>·year) respectively.

this study, occurred 7 times a year on average. According to the rainfall frequency and the associated CO<sub>2</sub>-C losses, the short-term C loss after rainfall still accounted for a considerable part of annual total C emission, although the C emission losses perhaps progressively decreased because of the reduction in substrate concentration and the proportion could also slightly decrease if the annual total CO<sub>2</sub> emission were calculated with intensive data, including data obtained from samplings during all the rainy days and during N addition.

## 4 Conclusions

The cumulative soil CO<sub>2</sub> effluxes of different water and N treatments over the 48 hr study period accounted for approximately 5.34% to 6.91% and 2.36% to 2.93% of annual total C emission, respectively, in the two sampling periods. There was a distinct influence of rain pulses on short-term soil C emission, but the pulse of CO<sub>2</sub> caused by the rapid increase in water availability depended largely on the length of the drought period prior to rainfall. No statistically significant main effect of N or interactive effects between water and N addition were found on soil CO<sub>2</sub> effluxes during the short-term observations, and the availability of soil C resources should be considered when evaluating the response of C emission to the change in soil N input. The pulse effects on soil respiration induced by precipitation and N deposition usually had a short duration but made an important contribution to the annual total CO<sub>2</sub> efflux. The improvement of the sampling frequency after rainfall or anthropogenic N addition is necessary to ensure the accurate evaluation on soil C loss in future studies in arid and semi-arid grassland regions.

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