



## Effects of changing C and N availability on soil respiration dynamics in a temperate grassland in northern China

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### ABSTRACT

Soil carbon (C) availability may play a crucial role in regulating how soil respiration responds to the increase of nitrogen (N) inputs. However, the related research is sporadic so far and has not adequately verified this speculation or addressed the topic in a widespread temperate grassland ecosystem. This lack of knowledge hinders our further accurate prediction of C emissions from terrestrial ecosystems. To improve our knowledge of the role of soil C resources in regulating Rs responses to N inputs, we established a situ field experiment with three levels of N addition (0, 50, and 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and two levels of glucose (G) addition (0 and 480 kg C ha<sup>-1</sup> yr<sup>-1</sup>) to determine the Rs dynamics in a temperate semiarid grassland in China. The results showed that only N addition had no significant effects on Rs as well as microbial biomass C (MBC) during our 168-hour observation period, whereas G addition and G addition together with N addition had positive effects on Rs and MBC. The regression analysis further showed that the cumulative CO<sub>2</sub> efflux was significantly positively related to soil MBC concentrations during the 168-hour observation period. Altogether, our findings verify that soil C availability is an important driver affecting the Rs response to N addition. No response of Rs to increased N availability resulting from N input was attributed to microbial C limitation in a semiarid grassland during a 168-hour observation.

### 1. Introduction

Soil respiration (Rs) represents the efflux of carbon dioxide (CO<sub>2</sub>) from soil, and constitutes the second-largest carbon (C) flux between atmosphere and terrestrial ecosystems (Raich and Schlesinger, 1992). Globally, this process emits 75–80 Pg CO<sub>2</sub>-C yr<sup>-1</sup> to atmosphere, which is > 10 times the CO<sub>2</sub> emission from fossil fuel combustion (Raich and Schlesinger, 1992). Thus, even minor alterations in Rs induced by environmental changes can have strong influences on the CO<sub>2</sub> concentration in the atmosphere (Raich et al., 2002).

Soil nitrogen (N) availability has been regarded as one of the essential environmental factors that can strongly affect Rs (Fernández-Martínez et al., 2014; Liang et al., 2015; Peng et al., 2017). Due to human activities, such as the consumption of fossil fuels and agricultural N fertilizer, anthropogenic reactive N inputs have increased from 34 Tg N yr<sup>-1</sup> in 1860 to 100 Tg N yr<sup>-1</sup> in 1995 on the global scale; this value will reach 200 Tg N yr<sup>-1</sup> by 2050 (Galloway et al., 2008). These reactive N emissions to the atmosphere undergo a series of

physical-chemical transformations during the transport process and then return to the Earth's surface via dry and wet deposition (Dentener et al., 2006). The markedly elevated N deposition into the terrestrial ecosystem inevitably changes the N availability in the soil (Luo et al., 2016) and may further alter Rs via changing plant growth (Xu and Wan, 2008), the quality and quantity of litter return to soil (Janssens et al., 2010; Wang et al., 2017), the formation of soil organic matter (Riggs and Hobbie, 2016; Tian et al., 2016) and some other environmental factors (Xu and Wan, 2008; Zhang et al., 2014).

An increasing number of studies conducted in recent years have focused on how N deposition impacts Rs in the terrestrial ecosystem; however, large uncertainties still exist in this field. Numerous studies have found that Rs shows divergent responses to gradient increases in N addition levels (Lee and Jose, 2003; Li, Liu, et al., 2015; Luo et al., 2016; Micks et al., 2004). For example, Zhu et al. (2016) found that low-level N addition exerted positive effects on Rs, whereas high-level N addition had negative effects on Rs in a semiarid grassland. Hasselquist et al. (2012) also showed that Rs was significantly elevated

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by low N addition treatments compared with that in a control plot, while no significant effects were induced by a high N addition treatment in a boreal forest. The inconsistent results of Rs responses to N additions are possibly attributed to different amounts of available C resources in the soil, according to the speculation of some previous research (Illeris et al., 2003; Mo et al., 2008; Phillips and Fahey, 2007; Qi et al., 2014). These researchers supposed that when the available C resource was abundant in soil, Rs responded positively to the N addition. However, when the available C resources were poor, Rs had a negative or neutral response to the N addition even if the added N was in the same level (Chen et al., 2014; Eberwein et al., 2015; Liang et al., 2015; Vourlitis and Fernandez, 2015). Moreover, in some field experiments, researchers have found that relatively high N addition levels can result in microbial C limitation due to an increase in N availability and a decrease in C availability (Peng et al., 2017; Treseder, 2008; Zhang et al., 2014). Several studies in agricultural ecosystems have also observed that Rs is obviously stimulated by straw-C amendment in the N fertilization plots compared with that of only N addition plots (Bhattacharyya et al., 2012; Dossou-Yovo et al., 2016). However, whether C availability regulates Rs responses to different N addition levels has yet to be adequately verified and lacks enough field experiment research. So far, only some studies have focused on agricultural ecosystems. In these ecosystems, improved soil C availability induced by artificial C resource inputs, such as straw-C return, has always been observed for the increase in crop yield in long-term N fertilization (Bhattacharyya et al., 2012; Dossou-Yovo et al., 2016; Liu et al., 2014). In comparison with agricultural ecosystems, relatively fewer studies have focused on grassland ecosystems in regard to how C availability regulates Rs responses to N deposition.

In fact, grasslands are another important terrestrial ecosystem, accounting for 30% of the earth's soil C stock and playing a critical role in the global C cycle (Cahill et al., 2009; Parton et al., 1995). In China, one of the global N deposition hotspot areas (Liu et al., 2013; Vet et al., 2014; Zhao et al., 2017), grasslands are the largest, most widespread vegetation and cover 40% of the national land area. Among them, approximately 78% of grasslands are distributed in the temperate semi-arid and arid regions of northern China (Kang et al., 2007). Due to the severe climate conditions and decades of overgrazing and clipping management, these regions have suffered large scale degradation and desertification, and the soil exhibits in large variations in C availability and remains N-scarce conditions, which easily makes the grassland ecosystem sensitive to changing amounts of external N inputs (Peng et al., 2011; Qi et al., 2014; Luo et al., 2016). When considering how Rs in grassland ecosystems responds to external N inputs, we cannot neglect the possible crucial role of the variations in soil C availability.

Here, a field experiment was conducted in a typical temperate semi-arid grassland in Inner Mongolia, China. The objective of this study was to investigate the effects of labile C added using glucose (G) on Rs responses to different N input levels. Our study tested two hypotheses: (1) C availability is an important driver affecting Rs responses to N addition; (2) microbial C limitation contributes to the no responses of Rs to N addition.

## 2. Materials and methods

### 2.1. Study site

The experimental site is situated in the *Leymus chinensis* steppe zone (43°33'51.3"N, 116°40'44.1"E; 1225 m a.s.l.), which is a type of grassland that is primarily distributed in the Xilin River Basin of Inner Mongolia, northern China. This region is characterized by a continental semiarid climate with a mean yearly temperature of  $-0.4^{\circ}\text{C}$  and the average annual rainfall is approximately 350 mm to 450 mm, with > 70% of the precipitation occurring during the growing season (June–September). The vigorous growth period of plants occurs from mid-June to late August every year. The soil type is dark chestnut (Chinese classification) or a Calcic-Orthic Aridisol (USDA Soil Taxonomy classification), which is characterized by a particle size distribution of 21% clay, 19% silt, and 60% sand. During the experimental period, the soil profile depth ranged from 100 to 150 cm, and the humus layer was 20–30 cm. The soil properties of the experimental field sites are shown in Table 1.

### 2.2. Experimental design

The long-term and short-term experiments to monitor Rs were set up in 2014 under a completely randomized block design. A total of 18 plots of  $8 \times 8$  m was randomly established with a 1 m wide buffer zone and there was a 1 m interval between plots. In one block, treatments involving three levels of N addition with and without G addition ( $480 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ ) were randomly arranged in each plot:  $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (the control, CN),  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (low N addition, LN) and  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (high N addition, HN);  $0 \text{ kg N ha}^{-1} \text{ yr}^{-1} + 480 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  (CN plus G addition, CNG),  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1} + 480 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  (LN plus G addition, LNG) and  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1} + 480 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  (HN plus G addition, HNG). The treatments were distributed in the experimental plots and replicated three times. We added N fertilizer ( $\text{NH}_4\text{NO}_3$ ) in late June and late July 2014, and the amount of each application was half of the total amount of N added. Based on the current rate of N deposition ( $22.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) in northern China (Liu et al., 2013), LN and HN represent twice the current and projected N deposition rates, respectively. After each application of N fertilizer, labile C in the form of G (40% C per gram) was applied twice per plot using 1/4 of the total amount of added G; these two applications were separated by a two-week interval. The amount of added G was approximately equal to the annual average MBC content in situ (approximately 2% of soil organic C), which was a critical threshold for the occurrence of priming effects (Blagodatskaya and Kuzyakov, 2008). The N fertilizer and G were added as a fine powder that facilitated even coverage of each plot.

### 2.3. Measurements of soil respiration rates

To capture the potential short-term impacts of G on  $\text{CO}_2$  effluxes after late N fertilization in this study, soil  $\text{CO}_2$  flux was measured at 0, 3, 6, 24, 72, 120 and 168 h after each addition of G. To determine Rs,  $\text{CO}_2$  gas samples were taken using a static opaque chamber (Peng et al., 2011; Zou et al., 2004), which is mainly comprised of a sampling chamber and a stainless steel frame with four grooved edges. A sampling chamber ( $50 \text{ cm} \times 42 \text{ cm} \times 30 \text{ cm}$ ) was made of 0.8 mm-thick stainless steel that was packaged in white heat insulation board to

**Table 1**

The properties of the soil in the experimental site (mean  $\pm$  S.E.,  $n = 3$ ).

Soil sampling depth (cm)	Bulk density ( $\text{g cm}^{-3}$ )	Total organic C ( $\text{g kg}^{-1}$ )	Total N ( $\text{g kg}^{-1}$ )	C:N ratio (%)	$\text{NH}_4^+\text{-N}$ ( $\text{mg kg}^{-1}$ )	$\text{NO}_3\text{-N}$ ( $\text{mg kg}^{-1}$ )
0–10	$1.30 \pm 0.05$	$18.97 \pm 0.41$	$1.80 \pm 0.09$	$10.54 \pm 0.58$	$4.15 \pm 0.28$	$2.54 \pm 0.50$
10–20	$1.28 \pm 0.05$	$18.19 \pm 0.52$	$1.47 \pm 0.07$	$10.28 \pm 0.68$	$3.81 \pm 0.69$	$1.38 \pm 0.31$
20–30	$1.29 \pm 0.05$	$16.26 \pm 0.47$	$1.14 \pm 0.05$	$13.17 \pm 1.20$	$3.60 \pm 0.24$	$0.84 \pm 0.30$

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