



# Responses of soil organic carbon turnover to nitrogen deposition are associated with nitrogen input rates: Derived from soil $^{14}\text{C}$ evidences<sup>☆</sup>

Qiqi Tan<sup>a</sup>, Guoan Wang<sup>a,\*</sup>, Xuejun Liu<sup>a</sup>, Tianxiang Hao<sup>a</sup>, Wenbing Tan<sup>b</sup>

<sup>a</sup> Beijing Key Laboratory of Farmland Soil Pollution Prevention-control and Remediation, Department of Environmental Sciences and Engineering, College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, China

<sup>b</sup> State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing, 100012, China



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## ABSTRACT

Elevated atmospheric nitrogen (N) deposition has exerted profound influences on ecosystems. Understanding the effects of N deposition on the dynamics of soil organic carbon (SOC) is important in the studies of global carbon cycle. Although many studies have examined the effects of N deposition on SOC turnover using N addition experiments, the effects were reported to be different across studies. Thus, we lack a predictive understanding of how SOC turnover respond to atmospheric N deposition. The inconsistent results could be associated with ecosystem types and N addition rates. This study mainly wants to confirm the argument that the response of SOC turnover to N deposition is related with N input rates. We conducted a field experiment with multiple N addition levels (0, 3, 6, 12, and 24 g N m<sup>-2</sup>·yr<sup>-1</sup>) in Inner Mongolia Grassland, China. To better reveal the responses of SOC turnover to N enrichment, this study measured the soil  $^{14}\text{C}$  contents, because it can indicate SOC turnover directly. Compared with the control treatment (0 g N m<sup>-2</sup>·yr<sup>-1</sup>), N addition inhibits SOC turnover at the addition rate of 3 g N m<sup>-2</sup>·yr<sup>-1</sup>, whereas SOC turnover is not affected when N addition rate was 6, 12, and 24 g N m<sup>-2</sup>·yr<sup>-1</sup>. Our results suggest that N input rates affect the responses of SOC turnover to N enrichment. Thus, this study can confirm the argument mentioned above. Based on this study, it should be considered in the climate prediction model that varied atmospheric N deposition levels across regions may have different impacts on local SOC turnover. In addition, we also carried out a soil incubation to compare between the results obtained in incubation and that in  $^{14}\text{C}$  measurements. Two results are found to be inconsistent with each other. This indicates that soil respiration from incubation experiments could not comprehensively assess the effects of N deposition on SOC turnover.

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## 1. Introduction

During the past two centuries, anthropogenic activities have resulted in a dramatic increase in atmospheric nitrogen (N) deposition in the world (Kaiser, 2001; Galloway et al., 2004, 2008; Liu et al., 2011, 2013; Gu et al., 2015; Song et al., 2017). N deposition has brought profound influences on ecosystems (Deng et al., 2018; Schulte-Uebbing and de Vries, 2018; Shi et al., 2018). The impact of increased N inputs on soil organic carbon (SOC) dynamics have been received considerable attentions (Mack et al., 2004; Janssens et al., 2010; Wieder et al., 2015; Chen et al., 2018). The SOC pool

comprises approximately 2/3 of the terrestrial organic carbon (Post et al., 1990; Amundson, 2001). Its alteration in response to N enrichment may significantly influence the carbon dioxide (CO<sub>2</sub>) concentration in atmosphere and thus has implications for global C cycle and climate change.

A significant body of work has concentrated on the effects of N enrichment on SOC turnover. However, the effects were observed to vary significantly from one study to another. Most previous studies showed that N enrichment inhibited SOC turnover (Zak et al., 2008, 2017; Frey et al., 2014; Fisk et al., 2015; Riggs et al., 2015; Riggs and Hobbie, 2016; Tan et al., 2017). For examples, Frey et al. (2014) observed that N addition with a rate of 5 g N m<sup>-2</sup>·yr<sup>-1</sup> induced soil C accumulation in the Harvard Forest. Riggs et al. (2015) found that N addition slowed grassland SOC turnover at a rate of 10 g N m<sup>-2</sup>·yr<sup>-1</sup>. Zak et al. (2017) reported that N addition increased SOC accumulation in hardwood forest at 3 g N m<sup>-2</sup>·yr<sup>-1</sup>. However,

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\* Corresponding author.

E-mail address: [gawang@cau.edu.cn](mailto:gawang@cau.edu.cn) (G. Wang).

few positive and no significant effects had also been observed (Prescott, 1995; Cleveland and Townsend, 2006; Huang et al., 2011; Finn et al., 2015; Meyer et al., 2018). For example, Huang et al. (2011) showed that N addition enhanced SOC decomposition in Kinleith forest at a rate of  $25 \text{ g N m}^{-2} \cdot \text{yr}^{-1}$ . Prescott (1995) reported no effect of N addition ( $11.2 \text{ g N m}^{-2} \cdot \text{yr}^{-1}$ ) on SOC turnover. Chen et al. (2015) claimed that the effects of N addition on ecosystem carbon dynamics depended on ecosystem type and N addition rate. Thus, the varied responses of SOC turnover to N enrichment observed in previous studies could be associated with different ecosystems and different N addition rates. This study wanted to confirm the argument that the responses of SOC turnover to N deposition relate to N input rates. We conducted a field experiment with multiple N addition rates over nine years in Inner Mongolia Grassland, China.

Many researchers have examined the effect of N enrichment on SOC turnover by soil laboratory incubation (Fisk et al., 2015; Riggs et al., 2015; Riggs and Hobbie, 2016). However, the  $\text{CO}_2$  efflux from soil incubation only reflects the decomposition of easily degradable organic compounds. Thus, we argue that the effect of N enrichment could not be assessed comprehensively by soil respiration from incubation experiments. Soil  $^{14}\text{C}$  contents reflect the holistic dynamics of SOC containing the fractions with residence time of decades to millennia. Soil  $^{14}\text{C}$  contents can indicate SOC turnover rates directly (Cherkinsky and Brovkin, 1993; Gaudinsky et al., 2000; Torn et al., 2009; Trumbore, 2009; Trumbore et al., 2016). Larger  $^{14}\text{C}$  content means faster turnover rate of SOC (Bird et al., 2002). Thus, the measurement of soil  $^{14}\text{C}$  has been treated as a useful tool to study SOC dynamics. This study measured the soil  $^{14}\text{C}$  to better explore the influence of N deposition on SOC turnover. Meanwhile, we also conducted a soil laboratory incubation to compare the difference in the responses of SOC decomposition to N enrichment derived from the  $^{14}\text{C}$  measurements and the incubation experiment respectively.

## 2. Materials and methods

### 2.1. Site description

This study was conducted in Duolun County ( $42^\circ 02' \text{ N}$ ,  $116^\circ 17' \text{ E}$ ,  $1324 \text{ m a.s.l.}$ ), Inner Mongolia, China. Mean annual temperature (MAT) is  $2.1^\circ \text{ C}$ . Mean monthly temperature ranges from  $18.9^\circ \text{ C}$  in July to  $-17.5^\circ \text{ C}$  in January. The area is semi-arid; mean annual precipitation (MAP) is  $386 \text{ mm}$ , with 91% distributed from May to October. The vegetation type is temperate steppe with the dominant species of *Stipa krylovii*, *Cleistogenes squarrosa*, *Agropyron cristatum*, *Artemisia frigida* and *Potentilla acaulis* (Song et al., 2011). The soil is classified as a Calcic Luvisol according to the Food and Agriculture Organization (FAO) classification. Its mean bulk density is  $1.31 \text{ g cm}^{-3}$  and soil pH is 7.12 (Hao et al., 2018). Our manipulation experiment was carried within an area with  $30 \times 35 \text{ m}$ , which has been fenced off since 2001 to prevent disturbance of grazing. The topography of this area is quite flat, thus, the spatial heterogeneity could be negligible. The ambient N deposition rate at the study site is about  $1.5 \text{ g N m}^{-2} \cdot \text{yr}^{-1}$  (Xu et al., 2015).

### 2.2. Experimental design

The N addition experiment had been applied since 2005. A randomized block design was used with 6 treatments. Each treatment had 5 replicates (a total of 30 plots). Each plot is  $5 \times 5 \text{ m}$  in size and the distance between any two adjacent plots is  $1 \text{ m}$ . The N addition rates were 0, 3, 6, 12, 24, and  $48 \text{ g N m}^{-2} \cdot \text{yr}^{-1}$ . The  $3 \text{ g N m}^{-2} \cdot \text{yr}^{-1}$  treatment started in 2006. Experiments still continue today except the  $48 \text{ g N m}^{-2} \cdot \text{yr}^{-1}$  treatment, which ended

in 2009 in order to study the recovery capacity of grassland ecosystem after high N fertilization. Urea was first used in 2005, and then  $\text{NH}_4\text{NO}_3$  was applied since 2006. Each year, the amount of fertilizer to be applied to each plot was subdivided into 3 equal parts and sprayed in early June, July and August. The sampling of soil and plants were carried out in the 0, 3, 6, 12 and  $24 \text{ g N m}^{-2} \cdot \text{yr}^{-1}$  treatments. These treatments will be referred to as N0, N3, N6, N12 and N24 treatments below. Due to the high cost of  $^{14}\text{C}$  measurements, we took plant and soil samples in only three of the five (randomly selected) plots within each treatment.

### 2.3. Plant sampling

Plant sampling was conducted in late August of 2014. A quadrat of  $1 \text{ m}^2 (1 \times 1 \text{ m})$  was randomly established in each plot and it was at least  $50 \text{ cm}$  away from the edge of the plot in order to avoid the edge effect. All living plant species at the soil surface within the quadrat were clipped and treated as the aboveground plant sample. Subsequently, three  $5\text{-cm}$  diameter cores were collected within the quadrat to depth  $50 \text{ cm}$  with  $10 \text{ cm}$  intervals. Roots in these cores were regarded as the belowground plant sample.

### 2.4. Soil sampling

In each plot, five soil cores ( $3.5 \text{ cm}$  diameter,  $0\text{--}5 \text{ cm}$  depth) were randomly collected by using soil auger and then mixed as one representative sample for the plot. In addition, we also collected three soil samples using a cutting ring ( $5 \text{ cm}$  diameter  $\times$   $5 \text{ cm}$  height) to measure soil bulk density. The fresh soil samples were stored in ice boxes and then brought back in the laboratory. In the laboratory, all soil samples (i.e. soil cores collected by using soil auger) were sieved with a  $2 \text{ mm}$  sieve, and divided into three parts. The first part was maintained fresh and prepared for microbial respiration experiment and the analysis of soil microbial composition. The second part was air-dried for the determination of soil physical and chemical properties and soil biochemical composition. The third part was stored in the refrigerator at  $4^\circ \text{ C}$ .

### 2.5. Plant chemical analysis

All the samples of aboveground and belowground parts were cleaned with deionized water, oven dried at  $65^\circ \text{ C}$  and weighed. Subsequently, the oven-dried plant samples were ground into a fine powder. The C and N concentrations of plant samples were determined using an elemental analyzer (FlashEA 1112; CE Instruments, Wigan, UK). The standard deviations for the measurements of plant C% and N% both were less than 0.1%. Finally, the measurements of lignin contents were conducted according to the method of Wang et al. (2015).

### 2.6. Soil physical and chemical analysis

Soil pH was measured in a soil water suspension (1:2.5 soil to water ratio) using the pH electrode. Samples for the measurement of soil bulk density were oven-dried at  $105^\circ \text{ C}$  until a constant weight was reached, and then the soil bulk density was calculated to be the weight of dry soil divided by the volume of the cutting ring. Soil particle size distribution was determined using a laser particle size analyzer (Malvern Masterizer, 2000, UK) after removing the calcium carbonates with  $0.2 \text{ M HCl}$  and organic matter with  $30\% \text{ H}_2\text{O}_2$ .

### 2.7. Soil DRIFTS analyses

Diffuse reflectance Fourier transform mid-infrared spectroscopy

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