



# Soil extractable carbon and nitrogen, microbial biomass and microbial metabolic activity in response to warming and increased precipitation in a semiarid Inner Mongolian grassland

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## ABSTRACT

Few studies have examined the long-term responses of soil labile organic carbon (C) and nitrogen (N) and microbial activities to climate change in semiarid and arid regions. Here we investigated soil extractable organic carbon (EOC) and nitrogen (EON), microbial biomass and microbial metabolic activities at two depths of 0–10 and 10–20 cm in response to single and combined effects of warming and increased precipitation in a semiarid grassland of northern China since April 2005. Soil EOC and EON pools were measured using KCl and hot water extractions, and microbial metabolic activities were measured using MicroResp. Results showed that warming had no effects on EOC, EON and microbial biomass C (MBC) and N (MBN) in the two extracts as well as the ratio of MBC to MBN at the two depths, but increased precipitation significantly increased MBC, MBN, EON and microbial quotient at the 0–10 cm depth. Warming significantly decreased microbial metabolic activities at both soil depths, but significantly increased microbial metabolic diversity (H) and evenness (E) at the 10–20 cm depth. Increased precipitation significantly decreased microbial metabolic activities, but significantly increased H and E at the two depths. Warming and increased precipitation significantly interacted to affect microbial metabolic activities at the two depths as well as H and E at the 10–20 cm depth. Redundancy analysis determined that microbial quotient, i.e., the ratio of MBC to total C, pH and  $\text{NH}_4^+ - \text{N}$  greatly accounted for the variances in the soil microbial metabolic profiles, but the ratio of EOC to EON, moisture and microbial quotient largely accounted for the variances in the soil microbial metabolic profiles specifically at the 10–20 cm depth, implying that microbial physiology such as microbial quotient rather than the amounts of labile organic C and N pools exerted more influence on driving the patterns of microbial metabolic profiles. Our results indicated that soil EOC and EON, microbial biomass and microbial metabolic activities at the two depths differentially responded to warming and increased precipitation in this semiarid region.

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## 1. Introduction

Global mean temperature is predicted to increase by 1.8–4.0 °C at the end of this century (IPCC, 2007). In concurrent with the rising temperature, global and regional precipitation regimes are predicted to change as well (IPCC, 2007). Changes in temperature and precipitation can greatly affect carbon (C) and nitrogen (N) cycling in terrestrial ecosystems (Allison and Treseder, 2008; Liu et al., 2009). Soils store large amounts of C and any minor reductions in soil C stock in

response to climate change can release more C to the atmosphere (IPCC, 2007), resulting in positive feedback to global warming. Therefore, results from long-term manipulative climate change experiments will be helpful for predicting the responses of soil C and ecosystem functions to climate change.

Soil extractable organic C and N serve as indicators for labile organic C and N pools, which are the most active fractions of soil organic matter since they are sensitive to changes in management practices (Zhou et al., 2011). As labile organic C and N pools are accessible to soil microorganisms, they can function as an important short-term reservoir of nutrients for microbes and plants (Schimel et al., 2007). Warming was reported to increase soil labile organic C and microbial biomass in a tall prairie (Belay-Tedla et al., 2009). However, in semiarid and arid regions, warming can lead to water loss from soils via evapotranspiration, thus exacerbating soil water stress (Niu et al.,

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2008). In contrast, increased precipitation can remarkably influence soil respiration (Liu et al., 2009) and ecosystem respiration in semiarid regions (Niu et al., 2008) via increases in soil water availability.

As soil microorganisms mediate C and N cycling, microbial activities are responsible for decomposition of soil organic C (Conant et al., 2008; Davidson and Janssens, 2006). Soil microbial activities can be greatly affected by many factors such as soil moisture (Wan et al., 2007), initial soil nutrient conditions (Xu et al., 2010) and plant communities (Bai et al., 2010). Most experiments have shown that warming can increase soil microbial activities (e.g. Bardgett et al., 2008; Luo, 2007), but a few studies report that warming can decrease or have no effect on microbial activities (Allison and Treseder, 2008; Liu et al., 2009; Zhang et al., 2005). By comparison, many studies have reported that increased precipitation can stimulate soil microbial activities (Hungate et al., 2007; Shen et al., 2009). In addition, most of these studies have focused on a single factor. Interactions among multiple climate factors, such as changes in temperature and precipitation regimes, can affect soil ecosystem in ways that are not easily predictable from measuring a single factor (e.g., Henry et al., 2005). Mechanistic understanding of the relative contribution of the key regulatory factors of soil microbial activity is essential for accurate predictions of soil C and N cycling in response to climate change.

Here, we took advantage of a long-term, ongoing multifactor experiment with experimental warming and increased precipitation in an Inner Mongolian steppe established in April 2005 (Liu et al., 2009; Yang et al., 2011). Given the importance of soil water availability in this semiarid region (Liu et al., 2009), we hypothesized that warming would decrease extractable organic C and N, microbial biomass and microbial metabolic activities, while increased precipitation would increase them. We also investigated if warming and increased precipitation exerted additive or non-additive effects on soil extractable organic C and N, microbial biomass and metabolic activities. The objectives of this study were to (1) examine the individual and combined effects of experimental warming and increased precipitation on soil extractable organic C and N, microbial biomass and microbial metabolic activities, and (2) quantify relative contributions of key regulatory factors influencing soil microbial metabolic activities under warming and increased precipitation at depths of 0–10 and 10–20 cm.

## 2. Materials and methods

### 2.1. Study site

This study site was established in late April 2005 in a semiarid temperate steppe in Duolun County (42°02'N, 116°17'E, 1324 m a.s.l.), Inner Mongolia, China. The region is characterized as a moderate temperature zone with a monsoon climate. Long-term mean annual precipitation and mean annual temperature are approximately 383 mm and 2.1 °C, respectively. About 90% of the total precipitation falls during the period from May to October and monthly mean temperature ranges from −17.5 °C in January to 18.9 °C in July. The soil in this area is classified as chestnut according to Chinese classification or Haplic Calcisols according to the FAO classification with 62.8 ± 0.1% sand, 20.3 ± 0.1% silt, and 16.9 ± 0.1% clay, respectively. Soil bulk density is 1.31 ± 0.02 g cm<sup>−3</sup> at the 0–10 cm depth. The plant community at our experimental site is dominated by *Stipa krylovii*, *Artemisia frigida*, *Potentilla acaulis*, *Cleistogenes squarrosa*, *Allium bidentatum*, and *Agropyron cristatum* (Yang et al., 2011).

### 2.2. Experimental design

This experiment used a paired and nested design with four treatments (Yang et al., 2011; Zhou et al., 2013). There were three blocks with an area of 44 × 28 m for each block. There was a pair of 10 × 15 m sub-blocks in each block, in which one plot was assigned

as the increased precipitation treatment and the other one as the ambient precipitation treatment. Each 10 × 15 m sub-block was divided into four 3 × 4 m plots with two warmed plots and two unwarmed plots arranged randomly. The distance between any two plots was 1 m. Thus, there were totally 24 plots with six replicates for each treatment [control (C), warming (W), increased precipitation (P), and warming plus increased precipitation (WP)]. There were six sprinklers arranged in two rows in each of the precipitation treatment plot, with each sprinkler covering a circular area with a diameter of 3 m. A total of 120 mm precipitation (approximately 30% of mean annual precipitation at this study site) was applied under the increased precipitation treatment in July and August at a rate of approximately 15 mm week<sup>−1</sup>. Each warmed subplot was heated continuously by a 165 × 15 cm SR-2420 infrared radiators (Kalglo Electronics, Bethlehem, PA, USA) suspended 2.5 m aboveground since April 28, 2005 [the heaters were turned off over the winter from November 16, 2007 to March 15, 2008]. One 'dummy' heater with the same shape and size as the infrared radiator was used to simulate the shading effect of the infrared radiator in the unwarmed control subplot. Records showed that warming significantly elevated soil temperature by 0.98 °C ( $P < 0.01$ ) and increased precipitation significantly improved soil moisture by 1.23% v/v ( $P < 0.01$ ) at the depth of 10 cm over the whole experimental period during 2005–2009 (Yang et al., 2011). Detailed information describing soil moisture and temperature in each plot has been provided previously for this experimental site (Liu et al., 2009; Yang et al., 2011).

### 2.3. Soil sampling and measurements of soil properties

Soil samples were collected from all 24 plots in early August 2010. In each plot, one soil core for each depth of 0–10 and 10–20 cm was collected using a soil auger (8 cm diameter). After passing through a 2-mm sieve, the soil samples were stored at 4 °C prior to analysis. The sub-samples were air-dried then stored at room temperature for hot water extraction. Soil moisture was determined after sub-samples were oven-dried at 105 °C overnight. Soil total C (TC) and N (TN) were determined using an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyzer (Isoprime-EuroEA 3000). Soil pH was measured at a 1:2.5 dry soil/water ratio. These data are presented in Fig. S1.

### 2.4. Measurements of soil extractable organic C and N

Soil extractable organic C (EOC) and N (EON) pools were measured using KCl and hot water extractions (Zhou et al., 2012). Briefly, field moist soil samples (5 g) were extracted with 30 ml of 2 M KCl in an end-to-end shaker for 1 h and filtered through a Whatman no. 42 paper. Concentrations of inorganic N were measured on a Lachat Quickchem automated analyzer (Quick Chem method 10-107-064-D for  $\text{NH}_4^+$  and 10107-04-1-H for  $\text{NO}_3^-$ ). For hot water extraction, air-dried soil samples (5 g) were extracted with 50 ml of hot water and following the same procedure as for the KCl extraction. Extractable inorganic N (EIN) was calculated as the sum of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N. The EOC and extractable total N (ETN) in the soil extracts were determined using a SHIMADZU TOC-VCPH/CPN analyzer (fitted with a TN unit). The EON was calculated by subtracting EIN from ETN for each soil sample.

### 2.5. Measurements of microbial biomass C and N

Soil microbial biomass C (MBC) and N (MBN) were measured by the chloroform fumigation–extraction method as described in Zhou et al. (2011). Briefly, two portions of 10 g field moist soil samples were weighed, with one portion fumigated with chloroform for 24 h and extracted with 0.5 M  $\text{K}_2\text{SO}_4$  in an end-to-end shaker for 1 h, then filtered through a Whatman no. 42 paper. The other portion

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