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Soil Microbial Responses to Experimental Warming and Nitrogen Addition in a Temperate Steppe of Northern China*1

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ABSTRACT

The responses of soil microbes to global warming and nitrogen enrichment can profoundly affect terrestrial ecosystem functions and the ecosystem feedbacks to climate change. However, the interactive effect of warming and nitrogen enrichment on soil microbial community is unclear. In this study, individual and interactive effects of experimental warming and nitrogen addition on the soil microbial community were investigated in a long-term field experiment in a temperate steppe of northern China. The field experiment started in 2006 and soils were sampled in 2010 and analyzed for phospholipid fatty acids to characterize the soil microbial communities. Some soil chemical properties were also determined. Five-year experimental warming significantly increased soil total microbial biomass and the proportion of Gram-negative bacteria in the soils. Long-term nitrogen addition decreased soil microbial biomass at the 0–10 cm soil depth and the relative abundance of arbuscular mycorrhizal fungi in the soils. Little interactive effect on soil microbes was detected when experimental warming and nitrogen addition were combined. Soil microbial biomass positively correlated with soil total C and N, but basically did not relate to the soil C/N ratio and pH. Our results suggest that future global warming or nitrogen enrichment may significantly change the soil microbial communities in the temperate steppes in northern China.

Key Words: arbuscular mycorrhizal fungi, global warming, Gram-negative bacteria, nitrogen enrichment, microbial biomass, microbial community

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Global temperature and nitrogen deposition are projected to increase significantly in the coming decades (Solomon *et al*., 2007; Gruber and Galloway, 2008), which may produce profound impacts on soil microbial communities in various ecosystems (Frey *et al*., 2008; Zhang *et al*., 2008; Zak *et al*., 2011; Zhou *et al*., 2012). The shifts in soil microbial communities can enhance ecosystem feedbacks to climate change through microbial decomposition of soil organic matters, resulting in more greenhouse gas (GHG) emissions to the atmosphere (Conrad, 1996; Schimel and Gulledge, 1998; Bardgett *et al*., 2008). Therefore, examining the responses of soil microbial communities to global warming and nitrogen enrichment are critical to the projection of future changes in ecosystem functions and atmospheric GHG concentration because of the massive organic carbon pools in the soils in terrestrial ecosystems (Schimel and Gulledge, 1998; Jobbágy and Jackson, 2000; Singh *et al*., 2010).

Scientists have put much effort into studying the impacts of warming and nitrogen enrichment on soil microbial communities individually. Many experiments, such as laboratory soil incubation experiments (Zogg *et al*., 1997; Feng and Simpson, 2009), seasonal temperature gradient-based experiments (Waldrop and Firestone, 2006; Björk *et al*., 2008), soil translocation experiments (Budge *et al*., 2011; Vanhala *et al*., 2011), and *in-situ* ecosystem warming experiments (Zhang *et al*., 2005; Rinnan *et al*., 2007), have reported the warming effects on soil microbial community biomass

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and composition in various biomes across the world. It is shown that soil microbial communities of a forest in Petersham, USA (Frey *et al.*, 2008), a grassland in western Alberta, Canada (Feng and Simpson, 2009), a subarctic heath in the Swedish Lapland (Rinnan *et al.*, 2007), and a tundra in Antarctica (Yergeau *et al.*, 2012) all would be greatly modified by global warming. As regards to nitrogen deposition, it is anticipated that the effects of nitrogen addition on soil microbes are related with the experimental duration, the total amount of nitrogen added and the plant community (Treseder, 2008; Liu and Greaver, 2010; Liu *et al.*, 2010). Low-level and short-term nitrogen addition can relieve the nitrogen deficiency in soils and increase soil microbial biomass (Vitousek and Howarth, 1991; Allison *et al.*, 2009). However, intensive and long-term nitrogen deposition may have negative effects on soil microorganisms by acidifying soil, increasing osmotic pressures, depleting soil minerals, and increasing aluminum toxicity (Aber *et al.*, 1998; Treseder, 2008; Niu *et al.*, 2011; Li *et al.*, 2012). Furthermore, nitrogen addition can change plant community structure and carbon allocation by, *e.g.*, decreasing the proportion of the legumes and modifying the plant shoot to root ratio, which would also modified soil microbial community (Xia and Wan, 2008; Liu and Greaver, 2010).

So far, few studies have reported soil microbial responses to the combined effects of global warming and nitrogen enrichment and no consistent conclusions have been drawn from the limited results. For example, Bell *et al.* (2010) showed that warming and nitrogen addition had no interactive effect on soil microbial community in a temperate old field in Canada. Ma *et al.* (2011) found that combined warming and nitrogen addition significantly decreased the ratio of bacterial to fungal biomass in a grassland in northeastern China; however, Gutknecht *et al.* (2012) have recently suggested that global warming and nitrogen enrichment have positive interactive effects on this ratio at the Jasper Ridge Biological Preserve in California, USA. Hence, much more multi-factor research is needed to fully understand how global warming and nitrogen enrichment affect soil microbial communities.

Temperate steppe are one of the largest ecosystems by area in China and it is pivotal to maintain the ecosystem functions, such as carbon and water cycling and biodiversity, in northern China (Kang *et al.*, 2007; Han *et al.*, 2009; Fang *et al.*, 2010). Global change may have profound impacts on the steppe ecosystem because the temperature and nitrogen availability in this region may continue to increase in the 21st century according to the projection of global change models (Dentener *et al.*, 2006; Ding *et al.*, 2007; Solomon *et al.*, 2007). Understanding the impacts of global change on the steppe ecosystem is necessary for effectively managing the ecosystem in the near future. Taking the opportunity of a long-term field experiment in a temperate steppe of northern China (Wan *et al.*, 2009; Xia *et al.*, 2009), the current study aimed to investigate the individual and interactive effects of experimental warming and nitrogen addition on soil microbial biomass and community structure, and to determine the relationships between soil microbial biomass and soil properties in the steppe ecosystem.

MATERIALS AND METHODS

Study area

This study was conducted at the Duolun Ecological Station (42°02′ N, 116°17′ E, 1 324 m above sea level), in Inner Mongolia, China. The study area is a temperate steppe with a typical temperate monsoon climate. Mean annual temperature is 2.1 °C with the minimum and maximum temperatures being −17.5 °C in January and 18.9 °C in July, respectively. The mean annual precipitation is 383 mm, 90% falling between May and October. This area received 325.6 mm rainfall during the entire growing season of 2010 (May to October), which was very close to the long-term average precipitation of 345 mm. The soils of the study area are very sandy with 62.75% sand, 20.30% silt, and 16.95% clay. The mean bulk density of the soils is 1.31 g cm^{-3}. The dominant plant species are perennial grasses and herbs, including *Stipa krylovii*, *Artemisia frigida*, and *Leymus chinensis.* There are also some legumes existing, such as *Astragalus galactites, Astragalus scaberrimus, Gueldenstaedtia stenophylla*, and *Melilotoides ruthenica*.

Experimental design and soil sampling

The long-term field experiment began in 2006 (Wan *et al.*, 2009; Xia *et al.*, 2009), including four treatments: control (CK), diurnal experimental warming (EW), nitrogen addition (ND), and diurnal warming plus nitrogen addition (WN). Each treatment had six replicate plots and the size of each plot was 3 m × 4 m. The warming treatment was achieved using MSR-2420 infrared radiators (Kalglo Electronics Inc., Bethlehem, USA), which were suspended 2.25 m above the ground. In each control or nitrogen addition plot, one 'dummy' heater with the same shape and size was used to simulate the shading effects of the infrared radiator. The warming treatment started on April 23, 2006, with significantly increased mean soil temperature by 1.79 °C