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Short- and long-term warming alters soil microbial community and relates to soil traits

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ABSTRACT

Climatic warming abnormally alters the structure and function of terrestrial ecosystems. It is vital to understand the response of belowground biota such as soil microbial communities to warming regimes, especially in native arid areas. The present *in situ* experiment was established to determine the effects of climatic warming regimes on soil microbe communities and the relationships between soil microbial groups and soil physicochemical features in a desert grassland ecosystem. Two warming regimes—long-term moderate warming (T1) and short-term acute warming (T2)—were established to simulate different climatic change scenarios. Soil from each plot was collected in 2014 at the late stage of the experiment, and phospholipid fatty acid (PLFA) profiling analysis was performed to assess the composition of the soil microbial communities. It was found that warming induced a severe water deficit stress. The T2 warming regime significantly increased the ratio of bacteria to fungi (B/F), and the ratio of Gram-positive to Gram-negative bacteria (GP/GN) in August. Belowground biomass (BGB) and soil organic carbon (SOC) were significantly correlated with all of the soil microbial groups in August. The changes in B/F and GP/GN ratios might indirectly induce changes in microbial structure. It was concluded that alterations in the structure of soil microbial communities may strongly depend on growing seasons and that soil nutrient status might have a profound impact on soil microbial communities' responses to climatic warming.

1. Introduction

By the end of this century, the average global surface temperature is projected to increase from 1.0 °C to 3.7 °C due to emission of greenhouse gases. Drought events will be more severe and frequent, particularly in arid areas, due to the decreased precipitation and/or increased evaporation resulting from ongoing warming (Dai, 2013; IPCC, 2014; Trenberth et al., 2014). Similarly, an enhanced soil drought induced by climatic warming in the semiarid area of northern China is also observed (Zhang et al., 2017). Global warming will lead to dramatic, abnormal alterations in the structure and function of terrestrial ecosystems, such as limited vegetation productivity and ecosystem biodiversity (e.g. Tylianakis et al., 2008; Xu et al., 2016).

Soil microbes are critical for terrestrial ecosystems to maintain their structure and function (Allison and Martiny, 2008; Bardgett and Van der Putten, 2014; Wardle et al., 2013; Sayer et al., 2017). They play a vital role in the decomposition process and regulate soil nutrient cycling (Swift et al., 1979; Schmidt et al., 2011; Waring et al., 2013; Canarini et al., 2017; Waldrop et al., 2017). Climatic change can modify the structure of soil microbial communities by, for example, altering the ratio between bacteria and fungi species (Schimel et al., 2007; Allison and Martiny, 2008; Sayer et al., 2017; Melillo et al., 2017). Relative to soil bacteria, soil fungi are often recognised to have high resistance to water deficit stress (Schimel et al., 2007; Yuste et al., 2011; De Vries et al., 2012; Fuchslueger et al., 2014; Nielsen and Ball, 2015). A recent report based on a meta-analysis indicated that a reduction in rainfall could significantly reduce the total microbial biomass and bacterial abundance of soil but leave fungal abundance unchanged (Ren et al., 2018). Under drought conditions, a decrease in the B/F ratio was found in both laboratory and field experiments (Canarini et al., 2017). However, greater loss of fungal taxa was found in a nutrient-poor grassland ecosystem with chronic summer drought and warming (Sayer et al., 2017). In European shrubland, antecedent warming and summer drought did not markedly influence the size or composition of microbial communities (Rousk et al., 2013). However, the results concerning the responses of soil microbial communities to warming are still

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controversial (Yuste et al., 2011; Fuchslueger et al., 2014; Sayer et al., 2017).

In China, about 22.6% of grasslands are classified as desert steppes, which are vulnerable because they feature less vegetation cover and harsh environmental conditions (Maestre et al., 2012; Hou et al., 2013; Yu et al., 2014). Currently, desert steppe ecosystems are severely degraded due to inappropriate land use and negative climatic change (Kang et al., 2007; Xu et al., 2016). Many studies have indicated that warming and warming-induced drought are major constraints on grassland functioning in the most vulnerable arid areas (e.g. Maestre et al., 2012; Xu et al., 2016). However, the changes in belowground processes, such as those of soil microbial communities, that occur in response to various climatic warming regimes are still poorly understood in desert steppes (Wall and Virginia, 1999; Xu et al., 2016). In addition, fewer *in situ* experiments with different warming scenarios have been performed relative to laboratory incubation experiments (e.g. Joergensen et al., 1990; Steinweg et al., 2008). Therefore, we performed a warming experiment *in situ*, hypothesising that, (i) due to the different effects of long-term and short-term warming on soil resource status, soil microorganisms will respond to warming differently and (ii) warming may change the structure of soil microbial communities due to differences in the tolerance of different soil microorganisms.

2. Methods and materials

2.1. Experimental site

This study was conducted in a desert steppe located northwest of Bailingmiao City (110°19′53.3″E, 41°38′38.3″N; 1409 m above sea level), Damao County, Inner Mongolia, China. At the site, the mean annual temperature (based on climate records from 1955 to 2014) is 4.3 °C, with a range from −39.4 °C to 38.1 °C. In addition, the mean annual precipitation is 256 mm, approximately 70% of which occurs from June to August. According to the Chinese classification (Calcic Kastanozems in the FAO soil classification), the soil type is chestnut. The soil has an average bulk density of 1.23 g·cm^{-3} and a pH of 7.4 (0–20 cm of surface soil). The site has remained ungrazed for more than three decades and is dominated by *Stipa tianschanica* var. klemenzii, which is accompanied by *Cleistogenes squarrosa*, *Neopallasia pectinate* and *Erodium stephanianum* (Hou et al., 2013; Liu et al., 2016).

2.2. Experimental design

The experiment was established with three treatments—control (without warming) (T0), moderate long-term warming (T1) and acute short-term warming (T2)—using a randomised complete block design. The T1 and T2 treatments had three replicates, and T0 had six replicates, resulting in a total of 12 plots (2 m × 2 m) with a 3 × 4 matrix. The moderately warmed plots were warmed during the growing season (early June to late August) for four years (2011–2014), and the acutely warmed plots were warmed only in the 2014 growing season. The warming pots were warmed by 800 W infrared lamps with a length of 1.0 m (GHT220-800, Beijing Sanyuan Huahui Electric Light Source Co. Ltd., Chaoyang, Beijing, China). The lamps were suspended 1.5 m and 1.0 m above the moderately and acutely warmed plots, respectively. To minimise the risk of experimental error, we installed 'dummy' heaters in the control plots and separated the adjacent plots by 1 m buffer zones.

2.3. Soil temperature and moisture

We monitored soil temperature at a depth of 10 cm and soil moisture at a depth of 0–20 cm using a thermocouple (HOBO S-TMB-M006, Onset Computer Corporation, Bourne, MA, USA) and a humidity transducer (HOBO S-SMA-M005, Onset Computer Corporation, Bourne, MA, USA), respectively, at the centres of each plot. Data were continuously recorded by an automatic data logger (HOBO H21-002, Onset Computer Corporation, Bourne, MA, USA) every half hour (Liu et al., 2016).

2.4. Soil sampling

Soil from each plot was randomly sampled at a depth of 0–20 cm (10 cm inside diameter) on 13 July and 20 August 2014 and then passed through a 1 mm sieve to separate roots. Soil from each plot was divided into three aliquots. The first aliquot was stored at 4 °C for soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), soil ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) tests. The second aliquot was stored in ice boxes and then frozen at -70 °C for phospholipid fatty acid (PLFA) measurements. The third aliquot was air-dried to test the soil organic carbon (SOC) content.

2.5. Soil and microbial analyses

Roots were separated from soil samples and were washed, then oven-dried at 70 °C to achieve a constant weight for calculation of the belowground biomass (BGB). Soil organic carbon (SOC) was measured by heating a mixture of H_2SO_4 and $K_2Cr_2O_7$ and performing titration with $FeSO_4$ (Nelson and Sommers, 1982; Chen et al., 2008; Edwards and Jefferies, 2013). The concentrations of NH_4^+-N and NO_3^--N were extracted by potassium chloride (KCl) solution, and a flow injection analyser (SEAL Auto Analyzer 3, SEAL Analytical, Inc., Mequon, Wisconsin, USA) was used to obtain data (Liu et al., 2014). Soil (0–10 cm; 10–20 cm depth) from each plot was oven-dried at 105 °C for over 24 h to determine the soil water content (SWC). MBC and MBN were measured using the chloroform fumigation extraction method, and carbon and nitrogen content were calculated as the difference between fumigated and nonfumigated samples (Rinnan et al., 2009; Liu et al., 2014).

The PLFA method was used to investigate the soil microbial community. PLFAs were extracted from 8 g of soil (dry weight) with a chloroform:methanol:phosphate buffer mixture (1:2:0.8, v/v/v), as described by Bossio et al. (1998). The obtained lipids were eluted with chloroform, acetone and methanol on solid-phase extraction columns to obtain the phospholipids. Then, the phospholipids were subjected to mild alkaline methanolysis and the extractions were re-dissolved in hexane with an internal standard of 19:0. Samples were analysed using a gas chromatograph (Agilent 6850, USA), and the peaks were identified with MIDI peak identification software (Microbial ID Inc., Newark DE, USA). Bacterial biomass was represented by PLFA peaks of i15:0, a15:0, 15:0, i16:0, 16:1ω7, 16:1ω9, i17:0, a17:0, 17:0, cyl7:0, 18:1ω7 and cyl9:0 (Frostegård and Bååth, 1996). The biomass of Gram-positive bacteria (GP) was represented by branched PLFAs and the sum of i15:0, a15:0, i16:0, i17:0 and a17:0 (Moore-Kucera and Dick, 2008; Yang et al., 2017). The biomass of Gram-negative bacteria (GN) was represented by cyclopropyl, mono PLFAs and the sum of cy17:0, 16:1ω7, 16:1ω9, 18:1ω7 and cy19:0 (Zogg et al., 1997). Fungal biomass was quantified by the sum of the PLFAs 18:2ω6,9 and 18:1ω9 (Bååth, 2003). The PLFA 16:1ω5 was not included since it is also found in arbuscular mycorrhizal fungi (AMF) (Frostegård and Bååth, 1996). Actinomycete biomass was quantified by the sum of 10Me16:0, 10Me17:0 and 10Me18:0 (Moore-Kucera and Dick, 2008; Yang et al., 2017). Protozoan biomass was quantified by the sum of 20:3ω6 and 20:4ω6 (Ringelberg et al., 1997).

2.6. Statistical analysis

All statistical analyses were performed using SPSS 16.0 (SPSS Institute Incorporated, Chicago, IL, USA). The normality of data was tested using the Shapiro-Wilk test. A two-way ANOVA was used to test the effects of warming and month and their interactions with soil nutrient content, belowground roots and soil microbial biomass. One-way