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Effects of elevated CO_2 and drought on the microbial biomass and enzymatic activities in the rhizospheres of two grass species in Chinese loess soil

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ABSTRACT

Elevated CO_2 and drought are key consequences of climate change and affect soil processes and plant growth. This study investigated the effects of elevated CO_2 and drought on the microbial biomass and enzymatic activities in the rhizospheres of *Bothriochloa ischaemum* and *Medicago sativa* in loess soil. Drought exerted significant species-specific negative effects on root and shoot biomass and microbial properties except for the soil basal respiration in the rhizospheres of *B. ischaemum* and *M. sativa*. Increased CO_2 exerted weak effects on plant biomass and enzymatic activities but demonstrated significant effects on the amounts of carbon and nitrogen in soil microbial biomass, basal respiration, substrate-induced respiration, and the metabolic quotients in the rhizospheres of *M. sativa* and *B. ischaemum*. The rhizosphere soil microbial index was a good aggregative indicator of the general state of the microbial properties of the rhizospheres. The interactive effects of elevated CO_2 and drought on plant growth and microbial properties significantly differed, indicating that elevated CO_2 significantly alleviated the effects of drought stress on the microbial properties of the rhizosphere. In addition, the effects of elevated CO_2 and drought on microbial biomass and enzymatic activities considerably varied between the two selected species. *M. sativa* generally experienced a better ameliorative effect than *B. ischaemum*.

1. Introduction

Anthropogenic activities such as urbanization and energy use, particularly fossil fuel consumption, have been dramatically increasing the concentration of atmospheric CO_2 (IPCC, 2014). Climate change is responsible for the frequent alteration of precipitation patterns and duration and increased drought events since the 1970s (IPCC, 2007). Knowledge regarding the effects of the components of climate change has considerably increased in the last few decades (García-Palacios et al., 2015). However, the interactive effects of these components on ecosystems remain uncertain because of the inextricable links and feedback between soil microbial communities and aboveground communities of plants, pathogens, herbivores, and parasites.

Numerous studies have described the effects of elevated CO_2 on ecosystem structure and function, species diversity, plant growth, plant production and physiological characteristics, soil fertility, and ecological processes. However, studies analyzing the plant-specific mechanisms of the effects of elevated CO_2 are still comparatively scarce and inconsistent because of the use of different experimental technologies, plant species, plant ages, and treatment times (Davey et al., 2006; Reddy et al., 2010). Furthermore, the effects of elevated CO_2 on the soil biota are indirect and mainly caused by plants because CO_2 concentrations are 10–50 times higher in the soil than in the atmosphere (Bruce et al., 2000). Changes in plant processes under elevated CO_2 levels alter the belowground inputs by plants, rhizodeposition, and recycling of rhizospheric material, which subsequently affect the number, activity, community structure, and metabolism of microorganisms (Kandeler et al., 2006).

Drought stress exerts considerable effects on general plant physiology; however, plant responses to drought are complex and vary via a series of parallel physiological, cellular, and molecular events depending on the plant species and the intensity, duration, and progression rate of the imposed drought stress. A drought-induced reduction in the photosynthetic performance advantages of C4 plants relative to C3 plants is a general phenomenon (Taylor et al., 2011). Moreover, drought stress can decrease plant nutrient uptake by reducing the nutrient supply available through mineralization (Sanaullah et al., 2012) as well as nutrient diffusion and mass flow in the soil (Lambers et al., 2008). These

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changes alter biomass allocation patterns, but the changes are inconsistent. In addition, drought may directly (e.g., changes in abiotic soil conditions) or indirectly (e.g., changes in the composition of plant communities) influence soil processes and the organisms that mediate these processes (Turner et al., 2003). Decreasing water potentials also reduce microbial activity (Baldrian et al., 2010) by reducing the energy available for the synthesis of biomass and restricting the diffusion of substrates to microorganisms (Schimel et al., 2007). The inconsistency in these conclusions is likely caused by the natural variations in soil microbial communities, soil type, the plant species under study, and methodological biases (Sanaullah et al., 2011).

Many studies have investigated the effects of climate change on biological systems and soil microbes, but few studies have examined the interactions among these factors such as those elevated CO_2 levels, drought, or warming. The stimulation caused by elevated CO_2 might be suppressed under other negative climatic/environmental stresses, such as drought, high temperature, and their combination. For example, plant growth and productivity responses to elevated CO_2 are constrained by drought, and this effect depends on nitrogen availability, plant species, drought intensity, and duration (Xu et al., 2007). Plant species have different physiological responses to global change factors; specifically, the growth of C3 species is stimulated regardless of water availability, whereas that of C4 species is stimulated under water deficiency. Several studies have focused on the interactive effects of components of global change on plants, but few studies have elucidated the mechanisms by which elevated CO_2 and drought interactively impact soils because of the complexity of plant–soil interactions (Kassem et al., 2008).

Nitrogen is a major growth-limiting nutrient in most non-fertilized terrestrial ecosystems (LeBauer and Treseder, 2008). Nitrogen limitation can change the effects of elevated CO_2, drought, and their interactive effects on ecosystems. For example, N limitation restricts the CO_2 fertilization effect (Dijkstra et al., 2008), exacerbates the effect of drought (Markelz et al., 2011), and limits the positive effect of elevated CO_2 under drought (Zong and Shangguan, 2014). Legumes, as drivers of N dynamics, are the most diverse and widespread group of plants with N_2 fixation capacity; nevertheless, the ability of nodules to both fix N_2 and assimilate nitrate can be altered by many climate change factors. For example, elevated CO_2 may stimulate growth and N_2 fixation in most symbiotic N_2-fixing plants when grown under environmental constraints, such as nutrient deficiency, drought, and low temperature (Aranjuelo et al., 2009a). However, the response is inconsistent and remains unclear (Guo et al., 2013; Rogers et al., 2009). To date, many studies have investigated the plant physiology of leguminous plants under the interactive effects of drought stress and elevated CO_2, but few of these studies have analyzed soil microbial characteristics.

Grasslands are easily impacted by changes in climate, including elevated CO_2 (Fay et al., 2003) and droughts (Knapp and Smith, 2001). *Bothriochloa ischaemum* is a C4 perennial grass that is important in reducing soil erosion, increasing water retention, and maintaining distinctive natural landscapes. *Medicago sativa* is a C3 leguminous plant grown on 1 million ha in China, a 31% increase since 2001 (Jia et al., 2006). Both of these plants are important drought-resistant and forage species for increasing livestock production and improving water use efficiency and soil fertility in arid and semi-arid regions of China (Xu et al., 1996). Previous studies have explored the responses to climate change of aboveground plant processes (Sanz-Sáez et al., 2012) and belowground ecosystem function (Sanaullah et al., 2012; Anderson et al., 2010) in relation to these two species. However, the interactive effects of elevated CO_2 levels and drought on the properties of the soil microbiota remain unclear. The Loess Plateau, which is one of the most severely eroded areas in the world, suffers from depleted soils, particularly nitrogen deficiency and drought (Jiang, 1997). Thus, the effects of elevated CO_2 and drought on the microbial characteristics and plant growth in the rhizospheres of the two dominant grass species in this region should be studied.

In the present study, we hypothesized that (1) elevated CO_2, drought, and plant species affect soil microbial biomass, respiration, and enzymatic activities and that (2) the effects of these three factors are interactive. We tested these hypotheses in a climate-controlled experiment using *B. ischaemum* and *M. sativa* with two soil moisture levels (well-watered and drought) and two atmospheric CO_2 concentrations (ambient and elevated). We measured the microbial properties in the rhizospheres and analyzed the interactive effects of elevated CO_2 level, drought, and plant species on these properties. On the basis of the results and the comparison with the control treatments, a theoretical basis and technological parameters for understanding the potential effects of global climate change on the properties of soil microbes were discussed.

2. Materials and methods

2.1. Experimental design

2.1.1. Facilities

The experiment was conducted in two identical and closed climate-controlled chambers (AGC-D001P, Qiushi Corp., China) at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Education, Yangling, Shaanxi, China (E108°4′27.95″, N34°16′56.24″). The chambers were equipped with an environmental control system (Qiushi Company, China) to supply CO_2 from a cylinder of compressed CO_2 controlled by a solenoid valve. The CO_2 concentration in the chambers was monitored and maintained at the target concentration via automatic injection. A HOBO data logger (MicroDAQ.com, Ltd., NH, USA) was fixed to the inside of each chamber to record the CO_2 concentration, air temperature, and relative humidity every 30 min. The photosynthetic photon flux density was measured using a Dual Radiation Meter (Apogee Instruments Inc., CA, USA) every 2 h.

2.1.2. Treatments

A total of 40 pots were used to plant *B. ischaemum* and *M. sativa*. These plants were treated with two levels of CO_2 and soil moisture. Twenty pots of each species were randomly divided into four groups of five replicates. These four groups received the following treatments: (i) C: control, 375 μmol mol^{-1} $CO_2 \times$ well-watered (80%–90% field capacity (FC)); (ii) D: drought (40%–45% FC), 375 μmol mol^{-1} $CO_2 \times$ drought stress; (iii) E: elevated CO_2 level, 750 μmol mol^{-1} $CO_2 \times$ well-watered; and (iv) ED: elevated CO_2 level and drought, 750 μmol mol^{-1} $CO_2 \times$ drought stress.

2.2. Plant materials and growth conditions

Seeds of *B. ischaemum* and *M. sativa* were collected from experimental fields at the Ansai Research Station of the Chinese Academy of Sciences (E109°19′23″, N36°51′30″). Loess soil was collected from the upper 20 cm of a cultivated field at the station and was sieved through a 2 mm plastic mesh before the experiment to achieve homogenization. The soil water content at FC and the wilting point were 18.4% and 3.8%, respectively. The pH was 8.55 ± 0.14, the soil organic matter content was 3.24 ± 0.24 g kg^{-1}, the total N and P content was 0.29 ± 0.02 and 0.51 ± 0.02 g kg^{-1}, respectively, and the hydrolyzable N and available Olsen P content were 43.79 ± 3.61 and 1.17 ± 0.09 mg kg^{-1}, respectively.

All seeds were soaked in deionized water for 24 h and were evenly sown in a plastic pot (20 cm × 15 cm, height × inner diameter) that each contained 3.5 kg of (oven-dried equivalent) soil. The pots for each CO_2 treatment were randomly placed into the two chambers for germination. Seven plant seedlings per pot were selected, and the remaining seedlings were removed. When 80% of the first leaves were observed, the CO_2 concentrations of the two chambers were set at 375 and 750 μmol mol^{-1} CO_2. The illumination cycle comprised 10.5 and 13.5 h