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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 × well-watered (80%–90% field capacity (FC)); (ii) D: drought (40%–45% FC), 375 μmol mol^{-1} CO_2 × drought stress; (iii) E: elevated CO_2 level, 750 μmol mol^{-1} CO_2 × well-watered; and (iv) ED: elevated CO_2 level and drought, 750 μmol mol^{-1} CO_2 × 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

of light and dark, respectively. The photosynthetic photon flux density was 280 μmol m^{-2} s^{-1} from 07:30 to 11:30 am, 560 μmol m^{-2} s^{-1} from 11:30 am to 14:30 pm, and 280 μmol m^{-2} s^{-1} from 14:30 to 18:00 pm. The chambers were maintained at a temperature of 27 ± 1 °C and a relative humidity of 45% ± 3%. No fertilizer was added to the pots during the experiment.

The soil moisture was maintained at 80%–90% FC during the first 10 weeks of the experiment, and the soil moisture treatments were applied over the following 7 weeks. The two established soil moisture treatments were 80%–90% and 40%–45% FC, which represented well-watered and drought conditions, respectively. Daily evapotranspiration was assessed by weighing the pots at 16:00 pm. Distilled water was added in the required amount through plastic pipes adjacent to the inner walls of the pots, which allowed the water to reach the bottom of the pots.

2.3. Sampling and analysis

2.3.1. Rhizosphere soil sampling

We randomly selected three pots as replicates for each treatment after 7 weeks of the soil moisture treatments for the collection of soil samples. Each pot was emptied, and the roots were manually separated from the soil. Rhizospheric soil collects within the space between the roots (Garcia et al., 2005) and adheres strongly to the roots. This soil was manually separated from the roots and thoroughly homogenized through a 2 mm sieve. The fresh soil was stored at 4 °C until the analyses of microbial biomass, respiration, and enzymatic activity. A subsample of soil from each pot was oven-dried at 105 °C for 24 h to determine the moisture content. All soil data were based on air-dried weights.

2.3.2. Plant analysis

The plants were harvested when the rhizospheric soil was collected. The plant samples were separated into shoots and roots, dried immediately at 70 °C to a constant weight, and then weighed. The shoot and root biomasses and root/shoot ratio were expressed as averages for all of the plants from each pot.

2.3.3. Analysis of rhizospheric soil

The soil microbial biomass and enzymatic properties were analyzed as described by Zhang et al. (2011). The soil microbial biomass C (SMBC) and N (SMBN) were measured through fumigation extraction using *kc* factors of 0.38 and 0.54, respectively. The activities of saccharase (SAC), urease (URE), alkaline phosphatase (ALP), and catalase (CAT) were assayed and expressed as mg glucose released g^{-1} soil h^{-1}, mg NH_4^+–N g^{-1} soil h^{-1}, mg phenol g^{-1} soil h^{-1}, and ml 0.1 mol $KMnO_4$ g^{-1} soil h^{-1}, respectively. The soil basal respiration (BR) was measured using the method described by Menyailo et al. (2003). Ten grams of soil (moistened to 50% FC with distilled water) were pre-incubated at 28 °C for 3 days and then in a sealed jar at the same temperature for 24 h. The CO_2 produced was trapped in 0.05 M NaOH, and the residual NaOH was titrated with 0.01 M HCl. Substrate-induced respiration (SIR) was determined using the same method for BR but with the addition of glucose to the soil as a source of C at 100 mg C kg^{-1} soil (Menyailo et al., 2003). BR and SIR were expressed as mg CO_2–C kg^{-1} day^{-1}. The metabolic quotient qCO_2 was calculated as the BR per unit of SMBC (Anderson and Domsch, 1993) and is expressed as mg CO_2–C g^{-1} biomass–C h^{-1}.

2.3.4. Rhizospheric soil microbial index (RSMI)

The RSMI, an indicator of the state of the microbial properties in the rhizosphere, was calculated as described by Zhang et al. (2011). The method of calculation involved three main steps as follows: (i) the selection of appropriate properties, (ii) the transformation and weighting of properties, and (iii) the combination of scores into an index. The appropriate properties and their weights were determined via principal component analysis (PCA), and Eq. (1) defines a sigmoidal-type curve that was used to transform the microbial property values into scores (*S*). Subsequently, the RSMI was calculated using Eq. (2).

$$S = a/\left(1 + (x/x_0)^b\right) \tag{1}$$

$$\mathrm{RSMI} = \sum_{i=1}^{n} W_i S_i \tag{2}$$

where *S* is the score of the selected property after transformation, *a* is the maximum score (in this case, $a = 1$), *x* is the value of the microbial property, x_0 is the mean value of each microbial property, *b* represents the slope of the equation, and *W* is the weighting factor of the microbial properties derived from the PCA.

2.4. Statistical analysis

All results are reported as the mean ± standard deviation. Plant species, moisture level, CO_2 concentration, and their interactive effects on the measured variables were tested using a three-way ANOVA. Comparisons among mean values were performed using Duncan's multiple range test calculated at $p < 0.05$. The individual contributions of the independent factors of plant species, water regime, CO_2 concentration, and their interactive effects on various parameters were calculated by dividing the sum of squares of the factors or their interactions by the total sum of squares and multiplying by 100 to obtain the percent contribution of these factors. The RSMI was calculated based on correlation analysis, scoring, and principal component analysis using SPSS 17.0 software. The relationships of the treatments with plant biomass and the microbial properties in the rhizosphere were identified through canonical correspondence analyses using CANOCO 5 with Monte Carlo permutation tests.

3. Results

3.1. Plant biomass and its allocation

Drought significantly decreased the shoot and root biomasses of *B. ischaemum* by 50.0% and 24.8%, respectively, but significantly increased the root/shoot ratio by 50.6% relative to the control (Fig. 1). Elevated CO_2 exerted no significant effects on the shoot or root biomasses or the root/shoot ratio of *B. ischaemum* (Fig. 1). Elevated CO_2 and drought significantly lowered the shoot biomass and increased the root biomass but had no significant effect on the root/shoot ratio. Drought also significantly decreased the shoot biomass and increased the root/shoot ratio of *M. sativa* but had no significant effect on the root biomass (Fig. 1). Elevated CO_2 significantly increased the shoot biomass and decreased the root/shoot ratio of *M. sativa* but had no significant effect on root biomass. The plants under both elevated CO_2 and drought had a significantly lower shoot biomass and a higher root biomass and root/shoot ratio than those under elevated CO_2 alone. Furthermore, elevated CO_2 and drought significantly increased the shoot and root biomasses and decreased the root/shoot ratio of *B. ischaemum* but had no significant effect on those of *M. sativa*. The shoot and root biomasses and root/shoot ratio were significantly influenced by interactions among the factors tested ($p < 0.05$) except for the change in the root/shoot ratio under the species × drought interaction (Table 1). The plant species explained 39.2%–94.8% of the variability in the biomasses of the roots, shoots, and their ratios; plant species, drought stress, CO_2 concentration, and their interactions explained 98.5%–99.3% of the variability according to the residual contributions of these effects (Table 1).

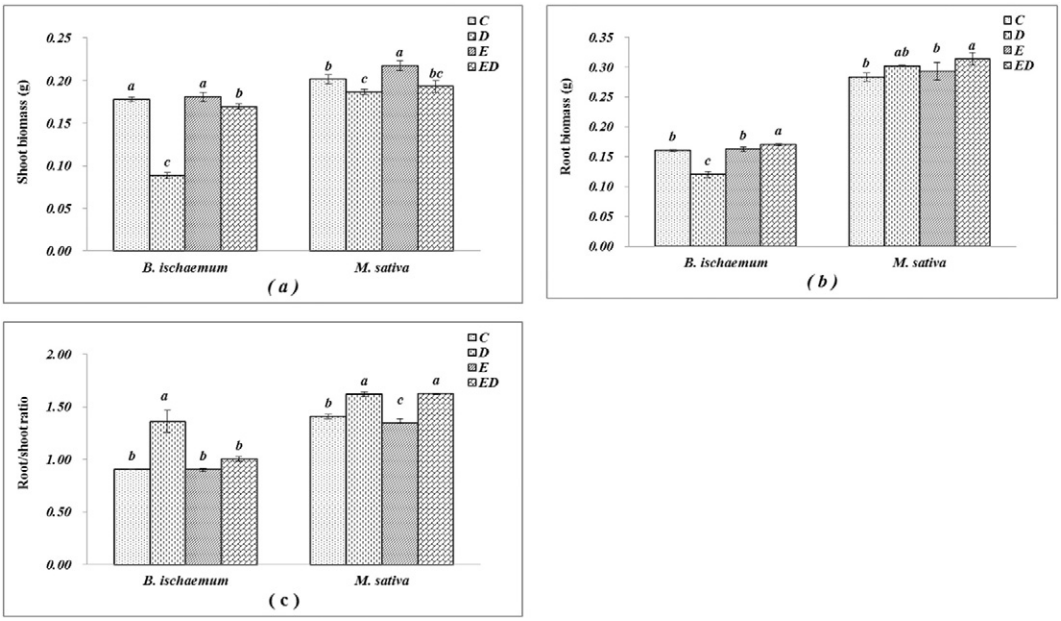

Fig. 1. Root, shoot biomass and root/shoot ratio of *B. ischaemum* and *M. sativa* under two CO_2 concentrations and two water regimes. Values with the same letter are not significantly different at $p < 0.05$. C: control; D: drought; E: elevated CO_2 and ED: elevated CO_2 and drought.

3.2. Soil biological properties

3.2.1. SMBC and SMBN

The SMBC and SMBN significantly decreased by 10.6% and 27.5%, respectively, in the rhizosphere of *B. ischaemum* under drought but showed no clear differences under elevated CO_2 (Fig. 2). The SMBC and SMBN did not significantly differ between elevated CO_2 alone and elevated CO_2 and drought combined but were higher under combined elevated CO_2 and drought conditions than under drought alone. Drought exerted no significant effect on the SMBC and SMBN for the rhizosphere of *M. sativa*, but elevated CO_2 alone significantly increased these parameters relative to the control. The SMBN was significantly higher under elevated CO_2 and drought than under elevated CO_2 alone, but the SMBC was similar under the two treatments. The SMBC and SMBN were higher under elevated CO_2 and drought than under drought alone, but this trend was not significant. Only the species × CO_2 × drought interaction significantly affected the SMBC and SMBN ($p < 0.05$), which were responsible for 12.6% and 11.9%, respectively, of the total variability (Table 1).

3.2.2. Soil microbial respiration and qCO_2

Drought significantly increased BR and qCO_2 but decreased SIR in the rhizospheres of both species (Fig. 3). BR, SIR, and qCO_2 in the rhizosphere of *B. ischaemum* under elevated CO_2 did not significantly differ from these values of these variables under the control conditions, but BR and qCO_2 were significantly lower and SIR was significantly higher for *M. sativa*. Compared with elevated CO_2 alone, elevated CO_2 and drought together exerted no significant effect on BR, SIR, or qCO_2 in the rhizosphere of *B. ischaemum* but significantly increased BR and qCO_2 and decreased SIR in the rhizosphere of *M. sativa*. Drought stress and CO_2 concentration were responsible for 21.0%–29.4% and 39.2%–41.2%, respectively, of the variability in BR, SIR, and qCO_2 (Table 1). BR,

Table 1
p-Values and contribution of independent factors (plant species, drought stress and CO_2) and their interactions to various parameters studied by three-way ANOVA.

	Species		CO_2		Drought		Species * CO_2		Species * drought		CO_2 * drought		Species * CO_2 * drought		Residual
	p	%	*p*	%	*p*	%	*p*	%	*p*	%	*p*	%	*p*	%	%
SB	<0.001	39.19	<0.001	13.33	<0.001	23.00	<0.001	4.48	<0.001	4.29	<0.001	5.50	<0.001	9.06	1.14
RB	<0.001	94.79	<0.001	1.53	NS	0.01	0.020	0.27	<0.001	1.45	0.001	0.72	0.002	0.57	0.66
R/S	<0.001	66.20	<0.001	3.33	<0.001	21.76	<0.001	1.86	NS	0.10	0.001	1.70	<0.001	3.51	1.54
SMBC	NS	3.32	0.001	33.57	NS	3.59	NS	1.51	NS	5.23	NS	3.70	0.032	12.61	36.45
SMBN	<0.001	32.90	<0.001	26.07	0.001	12.96	NS	1.61	NS	0.05	NS	0.94	0.002	11.91	13.56
BR	<0.001	2.68	<0.001	39.53	<0.001	29.38	NS	0.04	0.027	0.72	<0.001	24.22	0.003	1.49	1.94
SIR	NS	4.10	<0.001	39.21	<0.001	21.01	NS	1.63	NS	4.40	0.039	5.51	0.025	6.71	17.44
qCO_2	<0.001	3.70	<0.001	41.22	<0.001	26.84	NS	0.45	NS	0.01	<0.001	22.92	<0.001	3.12	1.73
SAC	<0.001	48.64	0.018	2.78	<0.001	21.99	NS	0.67	<0.001	17.45	NS	1.50	NS	0.62	6.35
URE	<0.001	37.13	0.001	13.06	0.042	4.08	NS	0.01	NS	1.72	<0.001	25.67	0.027	4.98	13.36
ALP	<0.001	48.83	<0.001	19.96	NS	0.12	0.008	3.47	0.031	2.16	<0.001	13.59	0.001	5.74	6.13
CAT	<0.001	63.41	0.001	11.59	NS	1.76	NS	2.69	NS	0.03	0.005	8.22	NS	0.03	12.28
RSMI	<0.001	36.92	<0.001	32.95	<0.001	11.77	NS	0.01	NS	0.70	<0.001	12.59	NS	0.53	4.54

SB: shoot biomass; RB: root biomass; R/S: shoot/root ratio; SBMC: soil microbial biomass carbon; SMBN: soil microbial biomass nitrogen; BR: basal respiration; SIR: substrate-induced respiration; qCO_2: metabolic quotient; SAC: soil saccharase; URE: urease; ALP: alkaline phosphatase; CAT: catalase; RSMI: the rhizosphere soil microbial index; NS: not significant at $p > 0.05$.

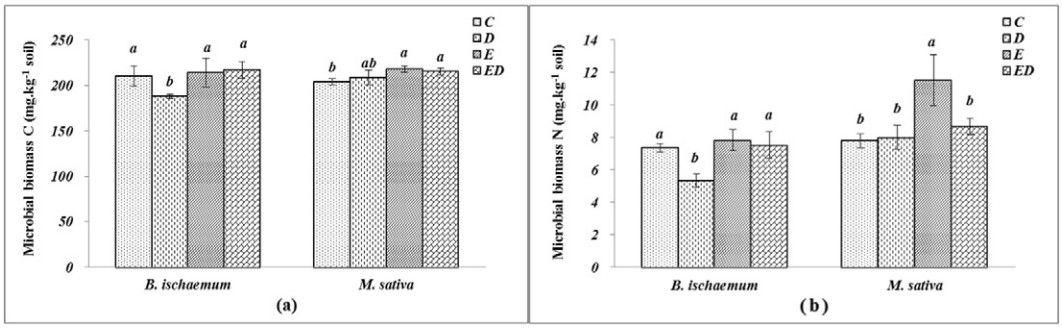

Fig. 2. Rhizosphere soil microbial biomass C and N of *B. ischaemum* and *M. sativa* under two CO_2 concentrations and two water regimes. Values with the same letter are not significantly different at $p < 0.05$. C: control; D: drought; E: elevated CO_2 and ED: elevated CO_2 and drought.

SIR, and qCO_2 were significantly affected by the CO_2 × drought and species × CO_2 × drought interactions (Table 1) but were not significantly affected by the species × CO_2 or species × drought interactions except for the effect of the species × drought interaction on BR ($p < 0.05$).

3.2.3. Soil enzymatic activities

Drought significantly decreased SAC, URE, ALP, and CAT activities in both plant species except for SAC activity in *B. ischaemum* (Fig. 4). Elevated CO_2 significantly affected SAC, URE, ALP, and CAT activities. The combination of elevated CO_2 and drought exerted no significant effect on SAC, URE, ALP, and CAT activities in the rhizosphere of *B. ischaemum*. However, the effects of elevated CO_2 and drought on soil enzymatic activities significantly differed for *M. sativa*. The combination of elevated CO_2 and drought significantly decreased SAC and increased ALP activities but demonstrated no influence on URE and CAT activities. In addition, the combination of elevated CO_2 and drought significantly increased SAC, URE, ALP, and CAT activities in the rhizospheres of both species, but the increase in SAC activity for *M. sativa* was not significant compared with the effect of drought alone. Plant species was the main factor influencing the variability in enzymatic activities and explained 37.1%–63.4% of the total variability. The significant effects of the species × drought interaction on SAC and ALP activities, the CO_2 × drought interaction on URE, ALP, and CAT activities, and the species × CO_2 × drought interaction are shown in Table 1.

3.2.4. Rhizosphere soil microbial index

Drought significantly decreased the RSMI by 52.2% and 36.7% in the rhizospheres of *B. ischaemum* and *M. sativa*, respectively (Fig. 5). Elevated CO_2 significantly increased the RSMI in the rhizosphere of *M. sativa* ($p < 0.05$). The RSMI did not significantly differ between elevated CO_2 alone and the combination of elevated CO_2 and drought but increased noticeably under elevated CO_2 and drought together relative to drought alone. Plant species, CO_2 level, and drought stress individually exerted significant effects ($p < 0.05$) on the RSMI, explaining 36.9%, 33.0%, and 11.8%, respectively, of the variability; nevertheless, only the CO_2 × drought interaction significantly affected the RSMI ($p < 0.05$) (Table 1).

3.3. Multivariate analysis

The relationship among the determination index of plant biomasses, soil microbial properties, and treatments is described in an ordination diagram in the horizontal and vertical directions (Fig. 6). The arrows represent the different treatment variables, and the direction of the

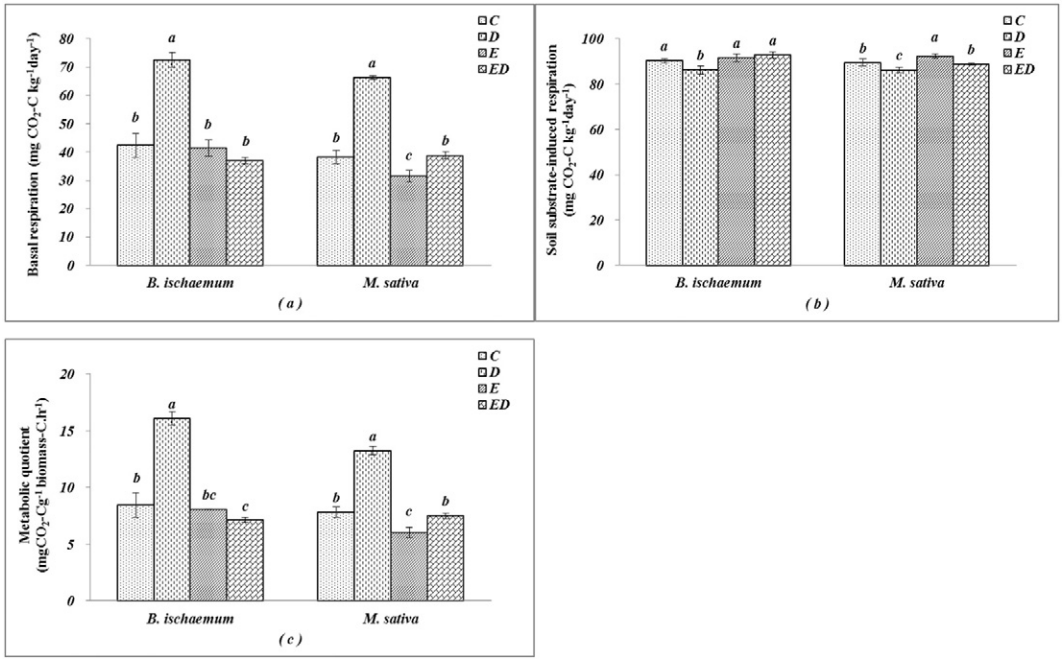

Fig. 3. Rhizosphere soil basal respiration, substrate-induced respiration and metabolic quotient of *B. ischaemum* and *M. sativa* under two CO_2 concentrations and two water regimes. Values with the same letter are not significantly different at $p < 0.05$. C: control; D: drought; E: elevated CO_2 and ED: elevated CO_2 and drought.

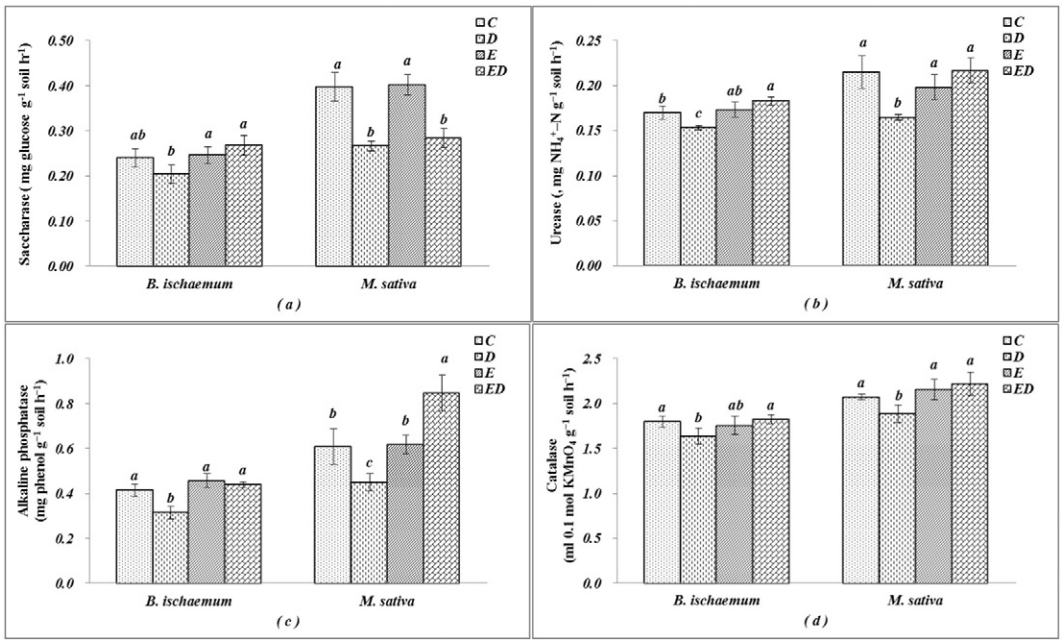

Fig. 4. Soil saccharase, urease, alkaline phosphatase and catalase activity of *B. ischaemum* and *M. sativa* under two CO_2 concentrations and two water regimes. Values with the same letter are not significantly different at $p < 0.05$. C: control; D: drought; E: elevated CO_2 and ED: elevated CO_2 and drought.

arrows represents the correlation between each variable and the canonical axes as well as the relationships among the variables. The length of the arrows represents the relative contribution of the variables to the axes and the index–treatment relationship. The Monte Carlo permutation tests indicated significant differences among all canonical axes ($p < 0.01$). In particular, the first canonical axis represents approximately 60.68% of the variation in the index–treatment relationship, and the first three axes represent 78.3%. The direction of the arrow for drought stress was opposite to that of the arrow for elevated CO_2 level, with a nearly 180° cross angle. The arrow for plant species was between these two factors, indicating that they affected the determination indexes conversely but influenced the plant species similarly. Most of the determination indexes clustered near the arrow for elevated CO_2 level, implying that the CO_2 concentration played a vital role in the determination indexes, which was confirmed by the comparative results reported above. The CO_2 concentration could affect the microenvironment of the soil; however, drought stress could also limit the microbial activities that were significantly influenced or determined by qCO_2 and BR. Therefore, as a result of the impacts of CO_2 concentration and drought stress, as well as plant species, the determination indexes along the canonical axis showed identical correlation eigenvalues and responses for these treatments.

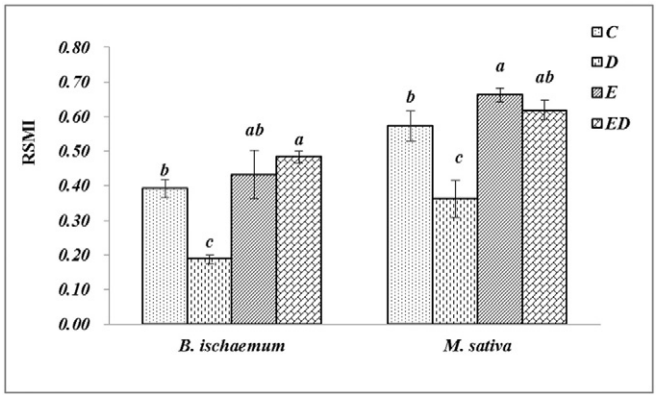

Fig. 5. The rhizosphere soil microbial index of *B. ischaemum* and *M. sativa* under two CO_2 concentrations and two water regimes. Values with the same letter are not significantly different at $p < 0.05$. C: control; D: drought; E: elevated CO_2 and ED: elevated CO_2 and drought; RSMI: the rhizosphere soil microbial index.

4. Discussion

4.1. Effects of drought and elevated CO_2 on plant growth

The present study showed that drought significantly decreased the shoot biomass and increased the root/shoot ratio in the two plant species evaluated. Drought stress often changes root biomass depending on plant species and drought frequency, duration, intensity, and other environmental stresses (Jaleel et al., 2009; Sanaullah et al., 2011). In

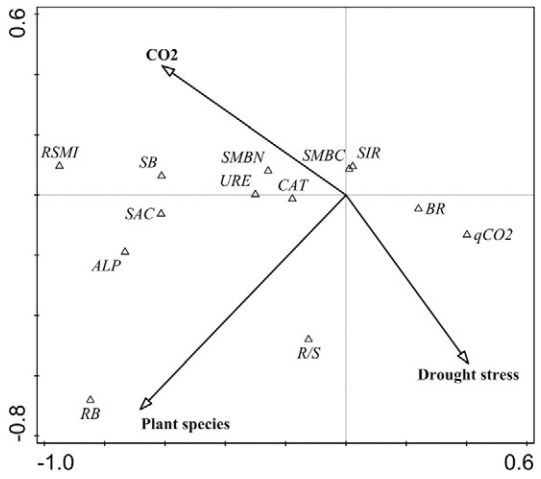

Fig. 6. Canonical correspondence analysis (CCA) ordination diagram of plant biomass, rhizosphere soil microbial properties with three treatment factors (plant species, drought stress, CO_2 concentrations) as arrows. Δ represents the different parameters. SB: shoot biomass; RB: root biomass; R/S: shoot/root ratio; SBMC: soil microbial biomass carbon; SMBN: soil microbial biomass nitrogen; BR: basal respiration; SIR: substrate-induced respiration; qCO_2: metabolic quotient; SAC: soil saccharase; URE: urease; ALP: alkaline phosphatase; CAT: catalase; RSMI: the rhizosphere soil microbial index.

general, drought induces biomass reallocation and C translocation from sources to sinks (Konôpka and Lukac, 2013), thereby increasing the allocation of C to the roots to enhance water and nutrient uptake (Liu and Li, 2005). Moreover, drought inhibits N_2 fixation in legumes (Coleto et al., 2014), which impedes plant growth. In the present study, drought affected root growth more in *B. ischaemum* than in *M. sativa*; this result may be attributed to the different drought tolerances of the two species. The results are consistent with previous findings that the advantages of C4 photosynthesis can be lost under drought than C3 plants (Taylor et al., 2011). In addition, *M. sativa* enhanced N availability through biological N_2 fixation, which weakened the influence of drought (Markelz et al., 2011).

With increasing CO_2 concentrations, the "CO_2 fertilization effect" results in elevated CO_2 levels enhancing photosynthesis and plant growth, decreasing stomatal conductance, and increasing the water use efficiency of leaves, which consequently increase biomass, in various species and ecosystems (Iversen et al., 2008). However, the responses of plants differ among species and growth conditions, such as temperature and nutrient and water availability (Song et al., 2015; Temperton et al., 2003). In the present study, elevated CO_2 increased shoot biomass only in *B. ischaemum* and caused no significant change in the root biomass of either species. Weak responses to elevated CO_2 are caused by the acclimation of photosynthesis to nutrient limitation. The loessial soil in our experiment had a low nutrient content, and no fertilizer was added during the experiment. This nutrient limitation possibly weakened the responses to elevated CO_2, as observed in previous studies (Koerner, 2006). In addition, the extended treatment of elevated CO_2 level after germination influenced photosynthesis, thus reducing plant growth (Aranjuelo et al., 2009b). Our finding that elevated CO_2 exerted a more significant effect on *M. sativa* than on *B. ischaemum* was in accordance with the general tendency of elevated CO_2 to have a more significant influence on C3 plants than on C4 plants (Kimball et al., 2002).

Understanding the responses of plants to the interaction between elevated CO_2 and drought is important, but information is still lacking. Elevated CO_2 may alleviate or delay the impact of drought on plant growth by increasing tolerance to and resisting the effects of drought. These processes involve lowering stomatal conductance and the transpiration rate, delaying the effects on photosynthesis, and increasing the acclimation of tissues and water use efficiency (Robredo et al., 2011). By contrast, a previous study suggested that elevated CO_2 levels do not exert a positive effect on the growth of drought-stressed plants (Vaz et al., 2012). These differences have been attributed to several factors, including plant (e.g., growth period or drought tolerance) and environmental (e.g., nutrient limitation or drought duration or intensity) factors. The present study indicated that elevated CO_2 significantly reduced the effect of drought on the growth of *B. ischaemum* and tended to reduce that of *M. sativa*. This result supported previous findings that elevated CO_2 levels exert definite compensatory effects on the photosynthetic physiological functions of *M. sativa* and *B. ischaemum* under drought stress, enhance the capacity for drought resistance, improve water use efficiency and alleviate the negative effects of drought stress (Fan et al., 2014; Zhang et al., 2012). Moreover, a lower shoot biomass and higher root biomass and root/shoot ratio were observed under elevated CO_2 and drought combined than under elevated CO_2 alone, indicating that drought caused the allocation of more biomass to the roots than to the shoots. These results supported the balanced growth hypothesis of preferential allocation of biomass to leaves and roots when aboveground and belowground resources are limited, respectively (Shipley and Meziane, 2002).

4.2. Effects of drought and elevated CO_2 on microbial activity

4.2.1. Effect of drought

Drought significantly affected the RSMI, but the effect was both property- and species-specific. Both the SMBC and SMBN significantly decreased under drought in the rhizosphere of *B. ischaemum* but not in that of *M. sativa*. Dry climatic conditions and deficiencies in the availability of soil water are generally thought to inhibit the accumulation of soil microbial biomass (Zhang and Zak, 1998) for two reasons. First, drought directly affects soil microorganisms by creating osmotic stress, which leads to cell lysis and microbial death (Turner et al., 2003). Second, changes in belowground inputs influence the functional structure and activities of the microbial community in the rhizosphere because of plant adaptation to drought stress (Milcu et al., 2011). In addition, plant species plays an important role in the effect of drought on microbial biomass because the responses of root biomass production and rhizospheric processes to drought are differentially altered by various plant species (Sanaullah et al., 2011). The root biomass of *M. sativa* was not affected by drought, which explained the maintenance of SMBC and SMBN levels. Drought stress also significantly affects the quantity and composition of root exudates and induces the release of increased amounts of mucilaginous material around drought-stressed roots (Dijkstra and Cheng, 2007). These compounds are important sources of labile C in soil and are rapidly consumed by microorganisms (Jones et al., 2009); they also stimulate the production of microbial biomass (Benizri et al., 2007). These effects were supported by our study and indicated that belowground processes were less affected by drought in the rhizosphere of the leguminous species *M. sativa* than in that of the non-leguminous species *B. ischaemum* (Sanaullah et al., 2012).

In the present study, drought significantly increased BR but decreased SIR. SIR is a basis for quantitatively estimating the total microbial biomass in soil; thus, the decrease in SIR can be explained by the same reasons as those for the decrease in SMBC in the rhizosphere of *B. ischaemum* discussed above. Interestingly, BR under drought conditions was significantly higher than that under control conditions, which is contradictory to results showing that drying soil reduces C mineralization (Smolander et al., 2005). Four possible factors might explain the high BR. First, drought may increase the content of labile compounds, such as dissolved organic C, which is a substrate accessible for respiration in microorganisms (Sanaullah et al., 2012). Second, the adjustment of soil moisture in the measurement of BR may induce a slight "Birch effect," which is an increase in the rates of mineralization in rewetted soil and litter immediately after dry periods (Borken and Matzner, 2009). Third, the soil used in the study has a high pH and carbonate content. BR may be influenced by CO_2 evolution from carbonate. Fourth, the microbial community may modify its microbial ecophysiology to adapt to stress (see below). The metabolic quotient qCO_2, also known as the respiratory quotient, provides an integrated measure of the ecophysiological state of the soil microbial community (Anderson and Domsch, 1985) and is a measure of substrate quality and availability (Dilly et al., 1997). We found a significantly high qCO_2 under drought stress; this observation is in accordance with the conclusion of Anderson and Domsch (1993) that a high qCO_2 may be attributed to stress responses. Moreover, qCO_2 is used as a sensitive indicator of the relative efficiency of C use by soil microorganisms; C is lost through respiration rather than being converted to microbial biomass and humus when microbial populations are increasingly stressed (Anderson and Domsch, 1993). Our data indicated that C use efficiency decreased and that C was further lost by respiration.

We observed significant decreases in SAC, URE, ALP, and CAT activities under drought conditions, which are consistent with the positive correlations between enzymatic activities and soil moisture reported in many studies (Baldrian et al., 2010; Sardans and Penuelas, 2005). Enzymatic activities decrease because of low microbial biomass and physiology (Baldrian et al., 2010) or because of enzymatic production and turnover (Steinweg et al., 2012). Drought affected plant growth, rhizodeposition and, ultimately, substrate availability. Changes in the microenvironment under drought stress restricted the diffusion of enzymes and substrates and decreased the contact between enzymes and insoluble organic matter, thereby decreasing enzymatic activity.

4.2.2. Effect of elevated CO_2

Numerous studies have failed to generalize the effect of elevated CO_2 levels on the biological properties of soil (Zak et al., 2000). In the present study, elevated CO_2 significantly affected SMBC, SMBN, BR, SIR, and qCO_2 in the rhizosphere of *M. sativa* but not in that of *B. ischaemum*. Previous studies have reported many conflicting results, i.e., positive, negative, or no effect, of elevated CO_2 on soil microbial biomass and respiration (Freeman et al., 2004; Zak et al., 2000). Freeman et al. (2004) concluded that plant type, nutrient status, soil type, analytical method, experimental system, and microbial diversity all influence the responses of plants to elevated CO_2. Niklaus and Korner (1996) suggested that microbial biomass is unlikely to respond to elevated CO_2 levels in nutrient-poor ecosystems. In the present study, nutrient limitation largely contributed to the lack of response of *B. ischaemum*. In addition, the unchanged root biomass under elevated CO_2 did not change the availability of substrates for microbes to alter microbial metabolism and growth. The symbiotic fixation of N by legumes accounted for the positive response of *M. sativa*. Symbiotic N fixation increased the N content in soil, which can alleviate nutrient limitation, improve substrate availability, and ultimately increase soil microbial biomass and SIR. Moreover, the significant decreases in BR and qCO_2 in the *M. sativa* rhizosphere indicated that the C use efficiency of soil microorganisms improved with low respiration and high microbial synthesis.

Previous studies have reached no consensus regarding the response of enzymatic activities to elevated CO_2 (Freeman et al., 2004; Zak et al., 2000). Our study found no significant changes for three possible reasons. First, the negligible changes in root biomass cannot significantly affect enzymatic production and turnover. Second, elevated CO_2 increases the microbial biomass associated with *M. sativa*, but the microbes must compete with actively growing vegetation for nutrients; as a result, microbial activity is generally decreased (Freeman et al., 1998), and the effect of high microbial biomass on enzymatic activities is offset. Third, no additional substrate was added during the experiment, which changed the substrate availability. Notably, the RSMI generally increased under elevated CO_2 despite the different properties of the two species, indicating that elevated CO_2 exerted a positive effect on the microbial system in the rhizosphere, but species also played an important role in the effect.

4.2.3. Interactive effects of elevated CO_2 and drought stress

As discussed above, many studies have addressed the effects of individual factors, such as elevated CO_2 levels or drought, but few of them have investigated the interactive effects of elevated CO_2 and drought on the underground ecosystem, particularly on the microbial properties of the rhizosphere (Kassem et al., 2008). The present study showed that elevated CO_2 could alleviate or offset the negative impacts of drought on soil microbial biomass, respiration, enzymatic activities, and the RSMI, except not on the activity of SAC in the rhizosphere of *M. sativa*. Kassem et al. (2008) similarly concluded that elevated CO_2 mitigates the negative impacts of drought on biomass, soil microbial activity, and certain plant properties. Elevated CO_2 alleviates or offsets the negative impacts of drought on the biomass and growth of plant roots, which in turn reduces the effect of drought on root exudation and substrate availability and subsequently maintains the activity and biomass of microbes. Furthermore, rhizodeposition and changes in the allocation of biomass and C accounted for this interactive effect. Schulze and Merbach (2008) found that elevated CO_2 levels increase belowground N transport and rhizodeposition, which are stimulated by drought stress. Xu et al. (2007) concluded that elevated CO_2 may partially offset the negative effects of enhanced drought by regulating the partitioning of C and N. These factors provided sufficient substrate for microbial survival and growth. Conversely, the interaction of elevated CO_2 and drought moderated the degree and diversity of substrate use; consequently, the direct effect of drought on microbes decreased. For example, the stomatal conductance of the two species in the present study decreased under the combined effects of elevated CO_2 and drought but decreased less under drought alone (Fan et al., 2014; Zhang et al., 2012); subsequently, the availability of water to the microorganisms was increased (Kassem et al., 2008). Changes in the physiological metabolism of soil microorganisms are a mechanism for the alleviation of the negative effects of drought. Our study noted that BR and qCO_2 decreased more under the combination of elevated CO_2 level and drought than under drought alone; thus, the microorganisms adapted to drought stress by decreasing microbial mineralization, increasing microbial synthesis, and/or enhancing C use efficiency.

4.2.4. Effects of plant species

The current study demonstrated that plant species significantly influenced the microbial properties of the rhizosphere and was responsible for 32.9%–63.41% of the total variability in the SMBN and RSMI and the activities of SAC, URE, ALP, and CAT (Table 1). The species × CO_2 and species × drought interactions exerted minimal effects on microbial properties, but the species × CO_2 × drought interaction exerted an important effect on these properties, except not on SAC, CAT, or the RSMI. The rhizosphere is subjected to specific processes arising from the interaction between roots and root-associated microorganisms (Griffiths et al., 1999). The responses of different plants to environmental stresses control rhizodeposition, which further affects the main processes and microbial properties of rhizospheres (Jones et al., 2004). Many studies have recently reported the effects of various stresses on plants that indicated different responses to and mechanisms of the stresses (Jaleel et al., 2009; Kimball et al., 2002; Sanaullah et al., 2011). Consequently, the structure and function of the rhizospheric ecosystem, including rhizodeposition, nutrient cycles, and microbial diversity, are altered (Grayston et al., 1998). In the present study, *B. ischaemum* and *M. sativa* showed different biological N_2 fixation and physiological characteristics, which resulted in species-specific soil biological properties.

5. Conclusions

Drought stress exerted a significant negative effect on plant growth and the microbial properties except for the soil basal respiration in the rhizospheres of *B. ischaemum* and *M. sativa*, but the effects were property- and species-specific. However, our results did not support the hypothesis that elevated CO_2 significantly changes soil microbial biomass and enzymatic activities but enhances the RSMI more significantly in the *B. ischaemum* rhizosphere than in that of *M. sativa*. Elevated CO_2 also significantly alleviated the negative effects of drought on plant growth and the microbial properties of the rhizosphere. The interaction between elevated CO_2 and drought exerted considerable effects on most of the microbial properties. Moreover, the effects of these two environmental factors on plant growth and microbial properties of the rhizosphere were species dependent. Further research is necessary to determine how the interactive effects and timescales of different elements of global change affect the composition and mechanisms of microbial communities.

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