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Stimulation of soil microbial functioning by elevated CO_2 may surpass effects mediated by irrigation in a semiarid grassland

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ABSTRACT

The functioning of microbial communities in response to elevated atmospheric carbon dioxide (eCO_2) is one of the most important, but poorly understood, contributors to climate change feedbacks. The effect of soil moisture caused by eCO_2 on soil microbial communities and functioning may be of high importance in water limited ecosystems. To address this knowledge gap, we measured the functional composition, structure, and functional processes of soil microbial communities under eCO_2 and an irrigation treatment simulating water availability with eCO_2 at two soil depths (0–5 cm and 5–15 cm) in a mixed-grass semiarid prairie. The overall functional diversity increased and the microbial composition and structure were significantly ($P < 0.01$) altered in response to eCO_2 and irrigation treatments. A greater number of key functional genes involved in soil, i.e. carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) cycling were stimulated under eCO_2 than under irrigation treatment. Structural equation modeling (SEM) suggested that eCO_2 significantly impacted soil microbial community structure by mediating both soil moisture and plant biomass, while irrigation influenced soil microbial community structure merely via regulation of soil moisture. Moreover, in this water-limited system, soil moisture mediated eCO_2 effects on soil microbial functional structure were relatively more important than those mediated by plant activity. The present study further improves our understanding of how global climate change regulates soil microbial functional processes in a semiarid grassland ecosystem.

1. Introduction

Global atmospheric carbon dioxide (CO_2) concentration has sharply increased by 44% since the industrial revolution (Le Quéré et al., 2012), leading to elevated global mean temperature. Together with the rising concentration of atmospheric CO_2 and temperature, soil water availability may change (increase or decrease) as well (Morgan et al., 2004). These changes could significantly impact terrestrial ecosystem functioning by altering biogeochemical cycling and ecosystem productivity (IPCC, 2007; Ryan et al., 2017). Grassland ecosystems store approximately 12% of global soil organic carbon and are located in regions where the timing and size of precipitation events could be affected by global climate change (Tiemann and Billings, 2011). Thus, impacts of atmospheric CO_2 and water availability can have significant feedback impacts on the global C balance. In arid and semiarid ecosystems, water availability is a constraint on most of biological activity (Collins et al., 2008; Nielsen and Ball, 2015). Soil microorganisms are sensitive to alterations of global climate change and soil water availability. The

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changes in microbial functional processes, including carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) cycling, largely determine the responses and feedbacks of an ecosystem to climate change. Therefore, evaluating microbial functional processes and the environmental factors driving them is crucial for improving the predictive understanding of arid and semiarid ecosystems.

Previous studies have shown inconsistent effects of eCO_2 on microbial communities. In some cases, eCO_2 increased the microbial diversity, altered microbial composition and structure, and enhanced their functional processes (Jansson and Hofmockel, 2020; Tu et al., 2014; Yu et al., 2018), but in other case, no significant effects on microbial communities were observed (Austin et al., 2009). In water-limited systems such as semiarid grasslands, a primary effect of eCO_2 is to reduce leaf transpiration by decreasing stomatal conductance, and such effect could increase plant water use efficiency and conserve soil water, resulting in higher soil moisture content (Blumenthal et al., 2013; Morgan et al., 2011, 2004). Such changes may result in a strong microbial response that could differ from the responses to eCO_2 in wetter ecosystems. Similarly, irrigation treatment has also been documented as influencing plant primary productivity, litter quality, belowground plant biomass through increasing soil moisture (Knapp et al., 2017; Nguyen et al., 2018; Ryan et al., 2017), and such changes may also impact soil microbial communities (Dijkstra et al., 2012; Li et al., 2017; Nielsen and Ball, 2015; Tiemann and Billings, 2011). It remains unclear, however, whether in arid systems eCO_2 and irrigation have similar effects on soil microbial functional processes. The importance of soil moisture in driving the responses of soil microbial communities and functioning to eCO_2 remains less understood.

The Prairie Heating and CO_2 Enrichment (PHACE) field experiment of warming, eCO_2, and water supplementation (irrigation) was conducted in a native semiarid temperate mixed-grass prairie in Wyoming, USA (Morgan et al. 2011). The irrigation treatment in this experiment was designed to simulate the change in soil moisture observed to be induced by eCO_2, providing a unique opportunity to disentangle the effects of moisture from other impacts of eCO_2. Previous studies of this site demonstrated that gross primary production, plant biomass, ecosystem respiration, and plant community stability were enhanced in response to eCO_2 and irrigation treatments (Morgan et al., 2011; Ryan et al., 2017, 2015; Zelikova et al., 2014) and that such changes increased soil labile C and decreased soil nitrate (NO_3^-) (Carrillo et al., 2012, 2011). However, to date, there is limited research studying the divergent effects of eCO_2 and irrigation on soil microbial structure and, in particular, on functional processes.

Geochip, a high-throughput metagenomics tool for characterizing microbial functional structure and processes was applied for characterization of soil communities at the PHACE experiment. (He et al., 2010a; Tu et al., 2014). GeoChip 3.0 contains approximately 28,000 oligonucleotide probes to assess a range of microbial functional processes (e.g. C, N, S, and P cycling) (He et al., 2010a) and had been demonstrated as a powerful tool for analyzing the microbial communities from various environments (Lu et al., 2012; Yu et al., 2014a, 2014b; Zhou et al., 2011).

In this study, microbial functional processes were indicated by the changes in the functional genes involved in soil C, N, P, and S cycling. We hypothesized that (i) eCO_2 and irrigation would alter the overall functional composition and structure of microbial communities at two soil depths (0–5 cm and 5–15 cm); (ii) both eCO_2 and irrigation would have positive effects on soil microbial functional processes (e.g., C, N, P, and S cycling) by increasing plant inputs and reducing water stress; (iii) eCO_2 and irrigation may impact soil microbial communities differently through differential regulation of soil water contents, plant biomass, and soil nutrients.

2. Materials and methods

2.1. Site description and sampling

The experimental site was located in a mixed-grass prairie with a semiarid, temperate climate at the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) High Plains Grasslands Research Station in Cheyenne, WY, USA (latitude 41°11′N, longitude 104°54′W). The dominant perennial vegetation consists of two C3 grasses, western wheatgrass (*Pascopyrum smithii* (*Rydb.*) *A. Löver*) and needle-and-thread grass (*Hesperostipa comata Trin* and *Rupr*), and a C4 grass, blue grama (*Bouteloua gracilis* (*H.B.K.*) *Lag*) (Ryan et al., 2017). The mean air temperature in this grassland was −2.5 °C in winter and 17.5 °C in summer, with mean annual precipitation of 384 mm (Morgan et al., 2011). The soil in this grassland is a fine-loamy, mixed, mesic Aridic Argiustoll (Dijkstra et al., 2018).

The experiment manipulated CO_2, temperature, and water availability. For our study, we focused on three treatments with each treatment containing five replicate plots: (i) ct, ambient CO_2 (380 ~ 400 μmol mol^{-1}) and no irrigation; (ii) Ct, eCO_2 (600 μmol mol^{-1}) and no irrigation; (iii) ct-i, ambient CO_2 and irrigation. All experimental plots (3.4 m in diameter) were set up randomly and surrounded by a 60 cm deep plastic flange barrier (Morgan et al., 2011; Ryan et al., 2017). In 2006, Free Air CO_2 Enrichment (FACE) technology was implemented for the elevated CO_2 plots to increase the concentration of atmospheric CO_2 (Miglietta et al., 2001). The irrigation treatment began in 2007 to maintain soil water content close to that in the eCO_2 treatment plots (Ryan et al., 2017). Three 21-mm irrigation events were applied to these plots (totaling 63 mm water addition in 2008 and 90 mm in 2007) when soil water content (SWC) was<85% of eCO_2 treatment at the soil depth of 5–25 cm (EnviroSMART probe; Sentek Sensor Technologies, Stepney, Australia), (Ryan et al., 2017).

Soil samples were collected in July 2008 at the peak of the growing season (10 days after a 21 mm irrigation event) by combining three cores at two soil depths (0–5 cm and 5–15 cm) for each plot. A total of 30 soil samples were collected from 3 treatments with 5 replicate samples for each treatment at both depths (3 treatments × 5 replicate samples × 2 depths). After the removal of residual grass roots and rocks by 2 mm sieving, all soil samples were immediately divided into two parts and stored at −80 °C or 4 °C for DNA extraction or soil property analysis, respectively.

2.2. Soil property and plant biomass analysis

Aboveground plant biomass was collected within a week of soil sampling and weighed after drying at 60 °C as previously described (Carrillo et al., 2011). Soil total carbon (TC) and total nitrogen (TN) were analyzed by dry combustion method with a Leco TruSpec carbon and nitrogen analyzer. Soil NO_3-N and NH_4-N were extracted with 1 M KCl solution and quantified by a Lachat Quickchem 8500 series 2 instrument. Soil pH was analyzed by a glass electrode in a 1:2.5 (soil: water) solution (w/v). Seasonal soil moisture was monitored from May 1st to July 30th in 2008 for the 0–10 cm soil depth using soil moisture sensors as previously described (Carrillo et al., 2012; Dijkstra et al., 2010). Soil moisture of samples was measured after drying for 24 h at 105 °C using 10 g of fresh soil.

2.3. DNA extraction and GeoChip analysis

Soil microbial community DNA was extracted from 5 g of soil by freeze-grinding mechanical lysis using the method described previously (Zhou et al., 1996). Soil DNA was purified using a Promega Wizard DNA clean-up system (Madison, WI, USA). DNA quality was measured by the ratios of 260 nm/280 nm and 260 nm/230 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), only those with ratios of > 1.8 (260 nm/280 nm) and > 1.7 (260

nm/230 nm), respectively, were retained for further analysis. The final concentration of DNA was quantified by Quant-iT^{TM} PicoGreen (Invitrogen, Carlsbad, CA). For each sample, 3 μg purified DNA was labeled by fluorescent dye Cy-5 as described previously (He et al., 2010b; Wu et al., 2006).

GeoChip 3.0 was used to characterize the microbial communities of soil samples and contained approximately 28,000 probes for 292 functional gene families involved in C, N, P, and S cycling, key energy metabolism, antibiotic resistance, metal resistance, and organic contaminant degradation (He et al., 2010a). All labeled DNA was hybridized on a MAUI hybridization system (Biomicro Systems, Salt Lake City, UT) at 42 °C for 12 hrs. After hybridization, microarray slides were scanned with a laser power of 95% and a photomultiplier tube (PMT) gain of 75% by a ProScan array microarray scanner (PerkinElmer, Boston, MA), and the signal intensity of each gene was quantified using ImaGene 6.0 (Biodiscovery, El Segundo, CA). Poor-quality spots were removed before statistical analysis with a signal-to-noise ratio (SNR) < 2.0 as described previously (He et al., 2010a). The signal intensities of each probe were normalized within and across all samples on the Microarray Data Manager (http://ieg.ou.edu/microarray/) using the data analysis pipeline (He et al., 2010a; Liang et al., 2010). Genes that appeared in at least two samples within one treatment group were considered positive and used for further statistical analyses. Data can be found on our website (http://mem.rcees.ac.cn/download.html).

2.4. Statistical analysis

In this study, we used the response ratio (RR) to calculate the significant difference of functional genes among ambient, eCO_2, and irrigation treatments. A significant response was defined as 95% confidence interval of the signal intensity of the functional gene. The standard deviation (SD) was calculated with standard error (SE) for each treatment as

$$SD = SE\sqrt{n}$$

Where n is the sample size. The SD can also be calculated with a mean and a confidence interval (CI) as

$$SD = (CI_u - CI_l)\sqrt{n}/2Z_{\alpha/2}$$

The CI_u and CI_I are the upper and lower limits of CI, respectively, and $Z_{\alpha/2}$ is Z score for a given level of significance equal to 1.96 when $\alpha = 0.05$ (Luo et al., 2006). The significant changes of key functional genes between treatment and control were measured by response ratio.

$$RR = ln(\overline{X}_t/\overline{X}_c) = ln(\overline{X}_t) - ln(\overline{X}_c)$$

Where $\overline{X}_t$ and $\overline{X}_c$ are the mean of the treatment and control group, respectively. The variance (ν) of RR was measured by

$$v = \frac{s_t^2}{n_t\overline{X}_t^2} + \frac{s_c^2}{n_c\overline{X}_c^2}$$

where n_t and n_c are the sample sizes of the treatment and control groups, and s_t and s_c are the SDs for all comparisons in the treatment and control groups, respectively. The standard error of RR was calculated using the following equation

$$s(RR) = \sqrt{v}$$

The 95% CI was calculated by

95% CI = RR ± 1.96 s(RR)

The effect of climate change treatments (ambient, eCO_2, and irrigation), depths (0–5 cm and 5–15 cm), and their interactions on soil properties were calculated by a two-factor ANOVA. Soil microbial functional diversities were evaluated by two α-diversity indexes (Shannon index (H') and the Simpson's reciprocal index (1/D)). Overall microbial community functional structure and phylogenetic structure among treatments were analyzed by detrended correspondence analysis (DCA) and the differences in communities between soil depths (0–5 vs. 5–15 cm) or among treatments (ambient vs. eCO_2, ambient vs. irrigation, and eCO_2 vs irrigation) were analyzed by three complementary non-parametric multivariate analyses, non-parametric multivariate analysis of variance (PerMANOVA), analysis of similarity (ANOSIM), and the multi-response permutation procedure (MRPP)) using R v.3.2.1 (www.R-project.org) with the Vegan and Agricolae packages.

Structural equation modeling (SEM) was performed in this study to determine the effect of CO_2 and irrigation treatments on soil microbial functional communities based on whole GeoChip data. SEM is a multivariate analysis technique based on *a priori* model used in ecology that can synthesize path analysis, factor analysis, and maximum likelihood analysis together and address complex sets of hypotheses among particular variables (Beaumelle et al., 2016; Delgado-Baquerizo et al., 2013). Parameters in the models included soil moisture, plant (aboveground plant biomass), plant N (aboveground plant nitrogen), chemical properties (first principal coordinate of Principal Component Analysis (PCA) conducted for TC, TN, NO_3^--N and NH_4^+-N), and microbial functional structure for each treatment (the first principal coordinate of principal coordinates analysis (PCoA) conducted for GeoChip data based on Bray dissimilarity matrix). SEM analyses were performed using Amos 24.0 (AMOS IBM, USA).

3. Results

3.1. Effects of eCO_2 and irrigation on plant and soil properties

In this study, treatments (eCO_2 and irrigation) showed significant effects on plant and soil properties (Table 1). Several variables, including total aboveground plant biomass, aboveground plant nitrogen (N), soil ammonium (NH_4^+-N), nitrate (NO_3^--N), moisture, were significantly ($P < 0.05$, ANOVA) influenced by eCO_2 and irrigation treatments. Irrigation and eCO_2 increased aboveground plant biomass by 8.9% and 32.3%, respectively (Table 1). Compared to ambient, the concentrations of soil NH_4^+-N and NO_3^--N decreased, while soil moisture increased under eCO_2 and irrigation treatments at both depths. Similarly, the concentrations of soil NH_4^+-N and NO_3^--N were higher under eCO_2 than under irrigation treatment. Average seasonal soil moisture also showed that soil moisture (monitored from May to July of that year) increased in the eCO_2 plots (by 18.3%) and irrigation plots (by 8.9%) (Table S1). At the time of soil collection, the eCO_2 and irrigated plots held relatively similar water content (Table 1, S1), but it should be noted that the average soil water content in the irrigated plots were intermediate between ambient and eCO_2 plots. Moreover, soil depth significantly ($P < 0.05$, ANOVA) influenced soil total nitrogen (TN), total carbon (TC), NH_4^+-N, NO_3^--N, and moisture. Soil moisture increased with depth, while the other five soil properties decreased with depth.

3.2. Effects of eCO_2 and irrigation on soil microbial communities

A total of 3389 and 4628 probes of functional genes were detected by GeoChip 3.0 among three treatments at the soil depths of 0–5 cm and 5–15 cm, respectively. In comparison with ambient, the detected gene numbers, Shannon index (H'), and the Simpson's reciprocal index (1/D) were greatly increased under eCO_2 and irrigation in both soil layers (Table S2). These three indexes were also significantly higher under eCO_2 than under irrigation at the soil depth of 5–15 cm, while no significant differences were observed between eCO_2 and irrigation at the soil depth of 0–5 cm.

At both depths, the most common phylum among the 3 treatments was *Proteobacteria* (69.6%–66.1%), followed by *Actinobacteria* (18.9%–13.7%), based on GeoChip data analysis (Fig. S1). At 0–5 cm depth, the abundances of genes derived from *Actinobacteria* were significantly increased ($P < 0.05$) by both eCO_2 and irrigation treatments, while the abundances of genes from the other four phyla (*Proteobacteria*, *Firmicutes*, *Ascomycota*, *Euryarchaeota*) were significantly increased ($P <$

Table 1
Average soil properties under ambient, eCO_2, and irrigation treatments in summer, 2008, at two depths.

Depth	Treatment	Plant responses		Soil properties					
		Plant (g/m^2)	Plant N (g/m^2)	TN (%)	TC (%)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Moisture (%)	pH
0–5 cm	ct	87.9 ± 1.5	1.19 ± 0.04	0.19 ± 0.021	2.02 ± 0.2	1.87 ± 0.22	3.62 ± 0.43	9.81 ± 0.35	7.05 ± 0.1
	Ct	95.7 ± 7.3	1.06 ± 0.1	0.17 ± 0.004	1.82 ± 0.03	1.11 ± 0.14	1.49 ± 0.18	11.79 ± 0.58	6.87 ± 0.14
	ct-i	116.2 ± 5.8	1.6 ± 0.05	0.18 ± 0.013	1.87 ± 0.11	1.22 ± 0.15	2.41 ± 0.45	10.8 ± 0.36	6.81 ± 0.18
5–15 cm	ct			0.15 ± 0.006	1.49 ± 0.06	1.24 ± 0.18	1.44 ± 0.13	12.57 ± 0.37	7.07 ± 0.12
	Ct			0.14 ± 0.013	1.35 ± 0.1	0.93 ± 0.13	0.86 ± 0.09	15.13 ± 0.45	7.02 ± 0.08
	ct-i			0.15 ± 0.007	1.49 ± 0.05	1.07 ± 0.08	1.23 ± 0.22	14.69 ± 0.41	6.96 ± 0.08
ANOVA (*P*-value)									
Treatment		**0.018**	**0.0008**	0.417	0.315	**0.006**	**0.0004**	**<0.0001**	0.392
Depth				**0.005**	**<0.0001**	**0.019**	**<0.0001**	**<0.0001**	0.304
Treatment × Depth				0.857	0.786	0.253	**0.037**	0.424	0.835

Plant, total aboveground plant biomass; Plant N, aboveground plant N; TN, total nitrogen content; TC, total carbon content.

0.05) only by eCO_2 (Table S3). At 5–15 cm depth, functional gene abundance for all groups was lowest in the ambient treatment, intermediate in the irrigated treatment, and highest in the eCO_2 treatment.

Overall functional structure of soil microbial communities was analyzed with detrended correspondence analysis (DCA). The first axis for both soil layers (0–5 cm and 5–15 cm) indicated that the samples from ambient, eCO_2, and irrigation treatments harbored distinct functional gene assemblages, with the irrigation treatment falling in the middle (Fig. 1a and 1b). To further address the microbial phylogenetic structure, a phylogenetic marker, the *gyrB* gene encoding DNA gyrase β-subunit gene, was selected for DCA analysis (He et al., 2010a). Similarly, the samples from ambient, eCO_2, and irrigation treatments were analyzed for each soil layer (Fig. S2a and S2b). In addition, three different non-parametric multivariate statistical tests, analysis of similarities (ANOSIM), multi-response permutation procedure (MRPP), and PerMANOVA (Table 2) showed that microbial communities under ambient were significantly different from the treatments for both soil layers.

3.3. Effects of eCO_2 and irrigation on key functional genes and processes

The effects of eCO_2 and irrigation on key functional genes involved in critical biogeochemical processes were further examined. A total of 193, 354, and 225 probes of functional genes involved in carbon cycling were detected under ambient, eCO_2, irrigation treatments at the soil depth of 0–5 cm, respectively, and 255, 513, and 412 probes of functional genes at the soil depth of 5–15 cm, respectively (Table S4).

The carbon degradation process was greatly impacted in both soil layers by eCO_2 and, to a lesser extent, irrigation. For example, in the soil depth of 0–5 cm, a high percentage of genes (43.3% of carbon degradation genes) for degradation of labile and recalcitrant C were significantly increased under eCO_2 and a relatively low percentage of (13.3%) genes were significantly ($P < 0.05$) increased under irrigation (*ara*, endoglucanase, *vanA*, and *lip*) (Fig. 2). Moreover, several genes (30%) were significantly higher ($P < 0.05$) under eCO_2 than under irrigation treatments, including *amyA*, *pulA*, *xylA*, acetylglucosaminidase, endochitinase, exochitinase, *limEH*, *vdh*, and phenol_oxidase (Table S5). Similarly, at 5–15 cm, 66.7% (20 genes) of detected functional genes and 43.3% (13 genes) of detected functional genes (*amyA*, *pulA*, xylA, xylanase, cellobiase, endoglucanase, acetylglucosaminidase, endochitinase, *limEH*, *vanA*, *vdh*, *mnp,* and phenol_oxidase) significantly increased ($P < 0.05$) under eCO_2 and irrigation treatments, respectively, compared with ambient. Among these genes, a relatively small portion (26.7%) of genes (*amyA*, glucoamylase, xylA, cellobiase, exoglucanase, endochitinase, *vanA*, and phenol_oxidase) showed a significant difference ($P < 0.05$) between eCO_2 and irrigation. The results indicated that both eCO_2 and irrigation had positive effects on both labile and recalcitrant C degradation, and these effects were stronger under eCO_2 than under irrigation.

The abundance of functional genes involved in methane production and oxidation also changed in response to eCO_2 and irrigation treatments. A total of 3 (ambient), 8 (eCO_2), and 2 (irrigation) probes of functional genes involved in CH_4 production were detected at 0–5 cm, and 4 (ambient), 6 (eCO_2), and 7 (irrigation) probes of functional genes at the soil depth of 5–15 cm (Table S4). Elevated CO_2 significantly ($P < 0.05$) increased the abundance of *mcrA* for CH_4 production and *pmoA* for CH_4 oxidation, while irrigation did not affect these two genes in the upper soil layer (0–5 cm) (Fig. S3). Additionally, the differences of *mcrA* and *pmoA* genes between eCO_2 and irrigation were also significant ($P < 0.05$) in this soil layer. At 5–15 cm, the abundance of *pmoA* gene was significantly ($P < 0.05$) increased under both eCO_2 and irrigation treatment, but no significant differences between eCO_2 and irrigation

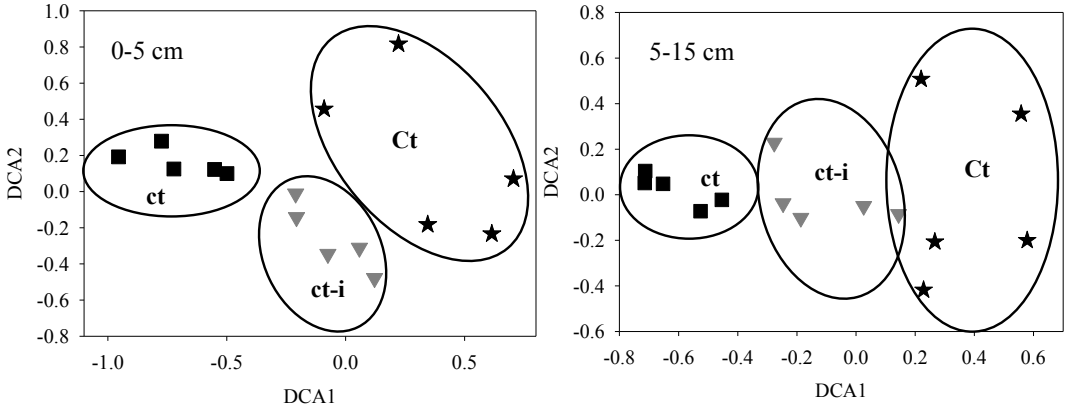

Fig. 1. Detrended correspondence analysis (DCA) of all detected functional genes under ambient, eCO_2, and irrigation treatments at soil depths of 0–5 cm and 5–15 cm.

Table 2
Significance tests of the effect of eCO_2, irrigation, and depths on overall microbial community structures with three different statistical approaches.

Depth		ANONISM[a]		MRPP[b]		PERMANOVA[c]	
		R	*P*	δ	*P*	F	*P*
ct vs. Ct	0–5 cm	0.856	**0.011***	0.399	**0.012***	5.263	**0.009****
	5–15 cm	1.000	**0.006****	0.284	**0.008****	11.053	**0.013***
ct vs. ct-i	0–5 cm	0.888	**0.014***	0.34	**0.008****	5.864	**0.007****
	5–15 cm	0.660	**0.008****	0.308	**0.007****	4.278	**0.007****
Ct vs. ct-i	0–5 cm	0.436	**0.01****	0.355	**0.017***	2.808	**0.032***
	5–15 cm	0.436	**0.008****	0.308	**0.009***	3.099	**0.006****
0–5 vs. 5–15 cm	Ambient	0.924	**0.009****	0.334	**0.008****	5.870	**0.006****
	eCO_2	0.612	**0.005****	0.348	**0.005****	5.019	**0.009****
	Irrigation	0.888	**0.010****	0.314	**0.007****	5.671	**0.004****

[a] Analysis of similarities (ANOSIM); [b]Multi-response permutation procedure (MRPP). [c]Non-parametric permutational multivariate analysis of variance (PERMANOVA) with the PerMANOVA function. Bold *P* values indicate statistical significance ($P < 0.05$) between two treatments. **:$P < 0.01$, *:$P < 0.05$. R represents the value of ANOSIM statistic R. δ represents the overall weighted mean of group mean distances. F represents *F* statistics.

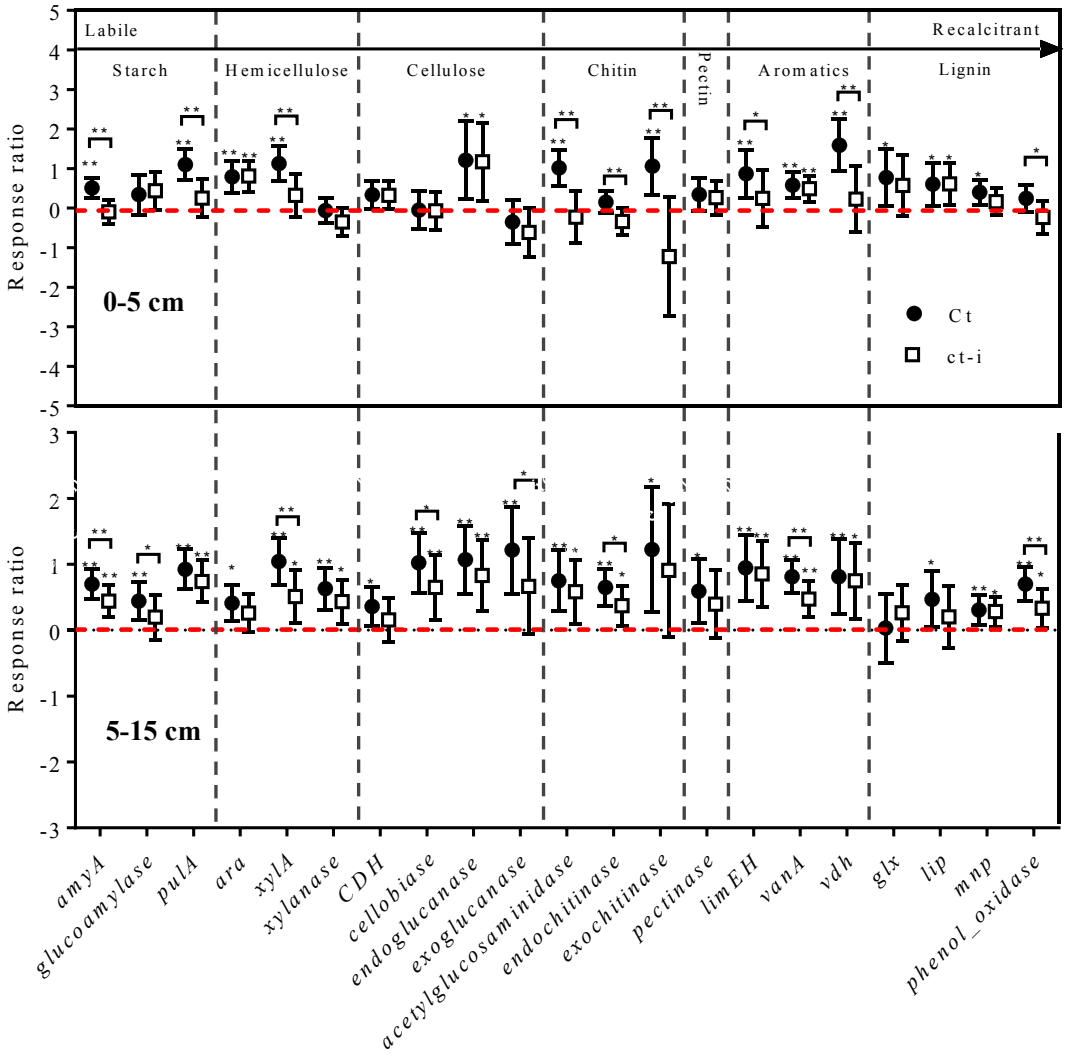

Fig. 2. Effect of eCO_2 and irrigation on functional genes involved in carbon degradation processes at soil depths of 0 to 5 cm (top) and 5 to 15 cm (bottom). Significant differences were calculated by *meta*-analysis of response ratio. Error bars indicate 95% confidence interval. Asterisks indicate the significance level: * at 95% confidence interval, ** at 99% confidence interval.

were detected for either *mcrA* or *pmoA* genes (Fig. S3).

A total of 171, 299, and 202 probes of functional genes involved in N cycling were detected from ambient, eCO_2, and irrigation treatments at the soil depth of 0–5 cm, respectively, and 222, 471, and 369, respectively, at 5–15 cm (Table S4). Relative to the ambient treatment, eCO_2 significantly ($P < 0.05$) increased the abundance of 12 genes involved in N_2 fixation (*nifH*), nitrification (*amoA*), denitrification (*narG*, *nirS/K*, *norB* and *nosZ*), dissimilatory N reduction to ammonium (*napA* and *nrfA*), ammonification (*gdh* and *ureC*) and assimilatory N reduction (*nasA*) at 5–15 cm, and 9 genes (*nifH*, *narG*, *nirS/K*, *nosZ*, *nrfA*, *gdh*, *ureC* and *nasA*) at 0–5 cm (Fig. 3). For irrigation treatment, the abundance of 10 genes (*nifH*, *narG*, *nirK*, *nirS*, *norB*, *nosZ*, *nrfA*, *gdh*, *ureC* and *nasA*)

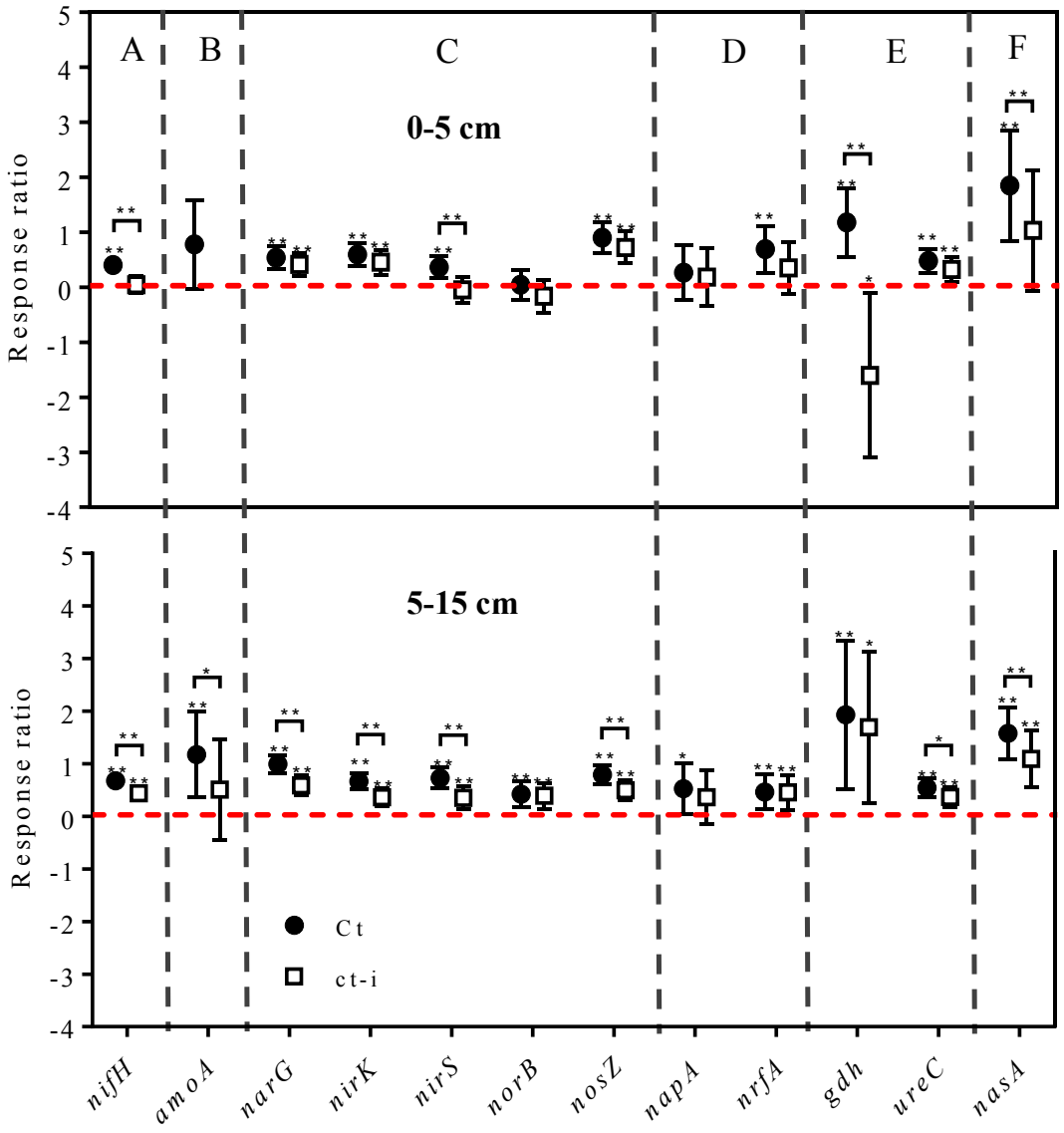

Fig. 3. Effect of eCO_2 and irrigation on detected functional genes involved in N cycling at soil depths of 0 to 5 cm (top) and 5 to 15 cm (bottom). (A) N_2 fixation; (B) Nitrification; (C) Denitrification; (D) Dissimilatory N reduction to ammonium; (E) Ammonification; (F) Assimilatory N reduction. Significant differences were calculated by *meta*-analysis of response ratio. Error bars indicate 95% confidence interval. Asterisks indicate the significance level: * at 95% confidence interval; ** at 99% confidence interval.

were significantly ($P < 0.05$) increased in the lower soil layer (5–15 cm), and 4 genes (*narG*, *nirK*, *nosZ*, and *ureC*) were significantly ($P < 0.05$) increased in the upper soil layer (0–5 cm), relative to the ambient treatment. Moreover, the abundance of 4 (*nifH*, *nirS*, *gdh*, and *nasA*) genes showed significant ($P < 0.05$) differences between eCO_2 and irrigation at 0–5 cm, and 8 (*nifH*, *amoA*, *narG*, *nirS/K*, *nosZ*, *ureC* and *nasA*) genes at 5–15 cm.

For P cycling, there were 51, 58, and 43 probes of functional genes detected from ambient, eCO_2, and irrigation treatments at the soil depth of 0–5 cm, respectively, and 50, 88, and 71 at 5–15 cm, respectively (Table S4). The abundance of exopolyphosphatase (Ppx) and polyphosphate kinase (Ppk) genes significantly ($P < 0.05$) increased under eCO_2 treatment in both soil layers. Under irrigation treatment, these two genes only displayed a significant ($P < 0.05$) increase at a depth of 5–15 cm (Fig. S4). Similarly, this pattern was also observed in S cycling. A total of 78, 104, and 76 probes of functional genes involved in sulfur cycling were detected under ambient, eCO_2, and irrigation treatments at the soil depth of 0–5 cm, respectively, and 82, 172 and 131 at the soil depth of 5–15 cm, respectively (Table S4). A significant ($P < 0.05$) increase in the abundance of *dsrA* and *sox* were detected under eCO_2 treatment at 0–5 cm, and significant ($P < 0.05$) increase in the abundance of *dsrA*, *dsrB* and *sox* were detected both under eCO_2 and irrigation treatments at 5–15 cm.

3.4. Linkages between microbial functional communities and soil and plant variables

To determine the impact of plant and soil potential drivers on individual functional genes, a Mantel test was performed with GeoChip data and eight variables (TN, TC, NH_4^+-N, NO_3^--N, moisture, pH, aboveground plant biomass, and aboveground plant nitrogen (N)). Three variables, including NO_3^--N, moisture, and aboveground plant biomass, showed significant ($P < 0.05$) correlations with functional microbial communities (Table S6). In addition, the Mantel test results also showed that correlations between individual functional genes involved in C, N, P, and S cycling and soil properties and plant variables indicated that many functional genes were significantly correlated ($P < 0.05$) with TN (2 genes), TC (5 genes), NH_4^+-N (9 genes), NO_3^--N (15 genes), moisture (34),

aboveground plant biomass (19 genes) and aboveground plant N (13 genes) (Table S6).

Finally, we constructed structural equation models (one model for each condition) to further investigate the pathways of impact of eCO_2 and irrigation on microbial functional structure. The model explained 92% and 87% of the variation under eCO_2 and irrigation treatments, respectively (Fig. 4). Under eCO_2, soil moisture and aboveground plant biomass were identified as the most significant factors ($P < 0.001$) with a positive relationship to the microbial functional structure (represented by the first component of the PCoA), while under irrigation, only soil moisture was determined to have a significant positive relationship with microbial functional structure (Fig. 4a and 4b). Standardized direct effects from SEM revealed that under eCO_2 the direct effect of moisture was larger than that of plant biomass (Fig. 4a and S6a). In comparison with irrigation, the effects of plant biomass and plant N were strong and significant under eCO_2 treatment.

4. Discussion

As soil microbial communities regulate many important biogeochemical processes (e.g., C, N, P, and S cycling), understanding the mechanisms of impact of eCO_2 and changes in water availability on soil microbial communities in arid ecosystems is crucial for ecologists to predict the ecosystem feedbacks to future global change (He et al., 2014). In this study, we comprehensively investigated the effects of eCO_2 and irrigation on microbial communities using GeoChip technology. Our results indicated that functional diversity, composition, and structure were substantially altered under eCO_2 and irrigation treatments across two soil depths (0–5 cm and 5–15 cm). Both elevated CO_2 and irrigation had positive effects on functional genes involved in C, N, P, and S cycling, with relatively weaker impacts by irrigation.

4.1. Elevated CO_2 and irrigation altered composition and structure of soil microbial community

One of our hypotheses was that microbial community composition and structure are altered under eCO_2 and irrigation treatments. In this study, eCO_2 and irrigation treatments substantially shifted the functional diversity, composition, and both the phylogenetic and functional structure of soil microbial communities in this semiarid grassland ecosystem within both soil layers (0–5 cm and 5–15 cm). At the phylum level, *Proteobacteria* and *Actinobacteria* had relatively higher abundance among different treatments, which was consistent with previous reports from grasslands (Bastida et al., 2017; Xia et al., 2017). Moreover, several previous studies have shown that soil microbial community composition and structure change under eCO_2 (Drigo et al., 2013; Yu et al., 2018) and irrigation treatments (Bastida et al., 2017; Engelhardt et al., 2018). In agreement with those findings, we found that a greater number of functional genes derived from most of the dominant phyla were detected under eCO_2 and irrigation than under ambient treatment at two soil layers. Our results suggested that the composition of communities could be altered under eCO_2 and irrigation treatments in similar ways by stimulating some dominant phyla.

However, dramatic differences in soil microbial composition and structure were observed between the two treatments (Fig. 1, Table 2, and S2), revealing that the effects of eCO_2 and irrigation on microbial communities' structure could be different. These findings are in accordance with a previous study of plant community responses, which also showed divergent shifts in response to eCO_2 and irrigation, indicating that the biotic responses in this semi-arid ecosystem were not mediated by effects of soil water content alone (Zelikova et al. 2014).

4.2. Elevated CO_2 and irrigation had positive effects on soil microbial functional processes

As microbial community structure was significantly altered under eCO_2 and irrigation treatments, another important question is how these treatments affected specific microbial functional processes. In this study, eCO_2 increased the abundance of many functional genes involved in both labile C and recalcitrant C degradation at 0–5 cm and 5–15 cm, indicating that the entire C cycle was enhanced. Similarly, the abundance of functional genes involved in starch, hemicelluloses, cellulose, and simple aromatics degradation processes significantly increased in BioCON (Biodiversity, CO_2 and Nitrogen deposition) grassland under eCO_2 treatment was shown using the same GeoChip technology (He et al., 2010b). In the semiarid grassland, soil N cycling was improved under eCO_2 by increasing most functional gene abundance involved in N_2 fixation, denitrification, dissimilatory N reduction to ammonium, ammonification, assimilatory N reduction processes. Several FACE studies also found that eCO_2 could enhance the soil microbial C and N cycling through increased plant C input (He et al., 2014, 2010b; Yu et al., 2018). This is generally consistent with the current study, where it was observed that most detected functional genes involved in C and N cycling had been stimulated under eCO_2 in both soil layers (0–5 cm and

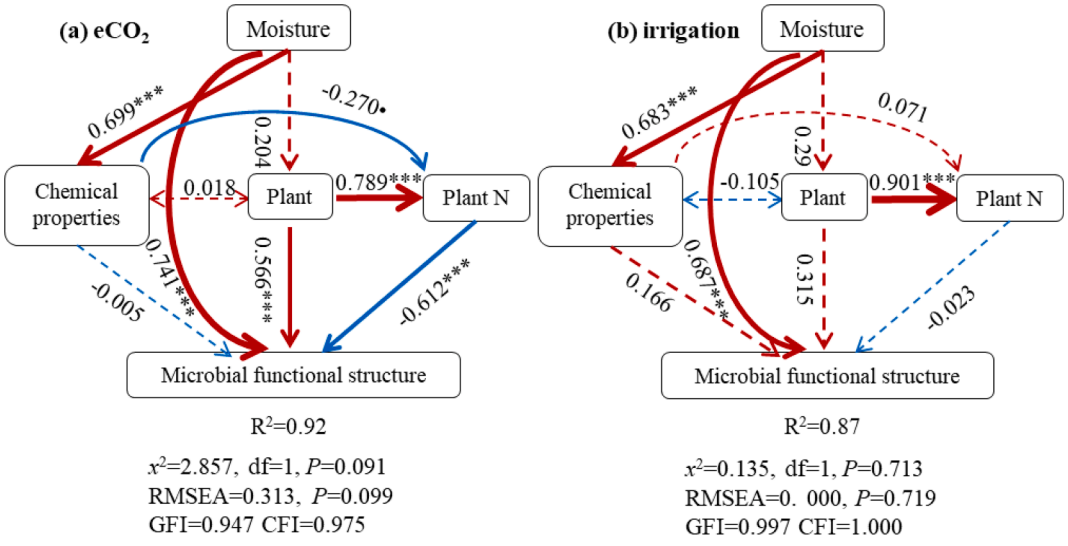

Fig. 4. Effects of eCO_2 (a) and irrigation (b) on microbial functional community in semiarid grassland. Solid lines indicate positive effects, and dashed lines indicate negative effects. Dashed lines indicate that the effects were not at significant level. Thickness of the arrows is proportional to the strength of path coefficients. R^2 represents the proportion of variance explained. Asterisks indicate the significance level: •: $P < 0.1$, *:$P < 0.05$, **:$P < 0.01$, ***:$P < 0.001$.

5–15 cm) (Fig. 2). However, in this semiarid grassland ecosystem, eCO_2 may impact soil functional processes by increasing not only plant C and nutrient input to the soil through root exudation and plant litter (Carrillo et al., 2018), but also soil water content. This deduction is strongly supported by our data that many of the functional genes involved in C and N cycling significantly correlated with both soil water content and aboveground plant biomass (Table S6). Soil microbial S and P cycling may also be stimulated under eCO_2 treatment by the significant increase in abundance of functional genes involved in P utilization, sulfate reduction, and sulfur oxidation. The increase of soil microbial C and N cycling may be accompanied by microbial S and P demand. Those findings are generally in accordance with previous studies for FACE sites (Xiong et al., 2015; Yu et al., 2018).

Another critical issue for this study is how microbial communities regulate their functional processes under irrigation treatment. Some previous studies focused on the effect of irrigation on the responses of soil microbial community, which was mainly measured by PLFA analysis (Huang et al., 2015; Xu et al., 2020). However, detailed studies on soil microbial community composition, specific functional groups, and key microbial processes are required to improve predictions of future ecosystem functioning under altered precipitation regimes (Xu et al., 2020). In the present study, a majority of genes involved in C degradation processes increased under irrigation treatment. This is in agreement with a recent study, using the same GeoChip technology, on a similar water-limited grassland in Inner Mongolia, which indicated that water addition could significantly impact functional genes involved in starch, hemicellulose, cellulose, chitin, simple aromatic compounds, and lignin degradation processes (Li et al., 2017). In addition, functional genes involved in N_2 fixation, ammonification, nitrification, denitrification, assimilatory and dissimilatory N reduction processes were significantly enhanced under irrigation (Li et al., 2017). These results are consistent with the current study showing that the signal intensity of genes involved in those specific N cycling process also significantly increased under irrigation treatment at 0–5 cm or/and 5–15 cm in this study. Furthermore, irrigation enhanced the abundance for the majority of genes involved in P utilization, sulfite reductase, and sulfur oxidation processes. Together, these results suggested that irrigation may have a positive effect on microbial C, N, P, and S cycling. Based on our analyses, the impacts of irrigation were mainly and directly driven by soil water availability. This finding revealed that soil water availability was the key factor in driving microbial functional processes with irrigation, rather than indirect impacts by plant inputs to the soil. Moreover, compared with 0–5 cm, more significant changes in genes were detected under eCO_2 and irrigation at 5–15 cm, revealing that both treatments' effects on microbial functional process were more evident at 5–15 cm than at 0–5 cm. This could be explained by the fact that eCO_2 and irrigation may conserve more soil water at 5–15 cm than at 0–5 cm. For instance, eCO_2 increased soil moisture by 1.98% at 0–5 cm and by 2.56% at 5–15 cm, and irrigation increased soil moisture by 0.99% at 0–5 cm and by 2.12% at 5–15 cm (Table 1).

4.3. *Different mechanisms regulate the effects of eCO_2 and irrigation on soil microbial communities*

Although both eCO_2 and irrigation treatments had a positive effect on soil microbial communities, stronger impacts on soil microbial functional processes (C, N, P, and S) were observed under eCO_2 treatment. For instance, C degradation genes were stimulated by eCO_2 more frequently than by irrigation (Fig. 2), which is consistent with observations of significantly higher root decomposition rates under eCO_2 than irrigation (Carrillo et al. 2014). Periodic irrigation events, however, could not maintain soil moisture as consistently as eCO_2 treatment (Dijkstra et al., 2012), leading to greater variability in soil water content in irrigation treatments which could have had an impact on the effects of the irrigation treatment. Consistently higher soil moisture under eCO_2 may help microorganisms alleviate the physiological stress and constraints of enzymatic activities caused by water limitation in semiarid regions (Tiemann and Billings, 2011), thus causing greater enhancement of functions.

Previous research has shown that methane uptake rates increased with both eCO_2 and irrigation at this experimental site in 2008, but only when the antecedent soil moisture content was below the optimum (Dijkstra et al., 2011). In our study, the upregulation of *mcrA* and *pmoA* genes associated with methane metabolism was associated with eCO_2 to a greater extent than irrigation (Fig. S3). Together, these findings would suggest that methanotroph activity may have responded to both moisture content and plant input availability.

Several previous studies have found that eCO_2 mediated microbial functional structure and diversity via altered water availability and plant biomass (Feng et al., 2010; He et al., 2012, 2014; Yu et al., 2018). Although we found that both factors had significantly positive effects on soil microbial functional structure, the SEM analysis showed that eCO_2 significantly influenced soil microbial communities mainly through changing soil moisture and secondarily by affecting above-ground plant biomass, whereas the irrigation treatment impacted soil microbial communities mainly by regulating soil moisture. We note that soil moisture in the irrigated treatment was on average slightly lower than that in the eCO_2 treatment, and thus may not have had as strong a stimulatory effect on plant and microbial activity as might be expected. Nevertheless, we further revealed that under eCO_2 the effect of moisture was higher than that of plant biomass which suggests that soil moisture was more important than plant biomass in mediating the eCO_2 effect on microbial functioning in this grassland ecosystem. Several previous studies of this site demonstrated that both total root biomass and gross primary production were significantly higher under eCO_2 than under ambient, while they were slightly higher or unchanged under irrigation (Carrillo et al., 2014; Dijkstra et al., 2010; Ryan et al., 2017).

5. Conclusions

Our results demonstrated that in response to eCO_2 and irrigation treatments, the functional diversity, composition, and structure of soil microbial communities were shifted in a semiarid grassland ecosystem across two soil depths (0–5 cm and 5–15 cm). While the impacts of eCO_2 were generally stronger than those of irrigation, both eCO_2 and irrigation treatments had positive effects on microbial processes by causing a significant increase in the abundance of key functional genes involved in C, N, P, and S cycling. The functional gene responses provide clear evidence of microbial mechanisms underlying previous observations of increased rates of litter decomposition, methane uptake, and N cycling at the site. These results demonstrate the over-arching influence of soil moisture as the primary regulator of microbial functions in this semiarid grassland ecosystem. In addition, since the irrigation treatment did not increase the soil moisture content to the same level as eCO_2, more accurate simulation experiments are needed to comprehensively investigate the microbial functional processes due to water savings under eCO_2.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115162.

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