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Influence of rice cultivars on soil bacterial microbiome under elevated carbon dioxide

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Abstract

Purpose Elevated CO_2 concentration (eCO_2) may stimulate plant growth and influence the soil microbial community, but questions remain for whether microbial responses to elevated CO_2 would vary by different CO_2-responsive plants. We thus attempted to elaborate the changes of soil microbiome to different rice cultivars under the eCO_2 condition.

Materials and methods Two rice cultivars, i.e., the CO_2-tolerant cultivar, Wuyunjing23 (WYJ23), and the CO_2-sensitive one, Yandao 6 (YD6), were grown under eCO_2 and ambient CO_2 (aCO_2) conditions. The contents of dissolved organic carbon (DOC) and nitrogen (DON) in soil were measured. Real-time qualitative PCR (qPCR) and high-throughput sequencing techniques were employed to characterize the bacterial community. Furthermore, co-occurrence network analysis was applied to reveal the ecological interactions among bacterial taxa.

Results and discussion No significant differences were found among all treatments in terms of bacterial population, alpha-diversity indices, and bacterial community structure. However, the topological parameters of ecological networks highlighted the distinct co-occurrence patterns among treatments. YD6 under eCO_2 led to more links, lower modularity, and greater centralization degree compared to that under aCO_2. Opposite trends of those parameters were observed for WYJ23 under eCO_2 compared to that under aCO_2. Besides, more Proteobacteria and Acidobacteria served as keystone taxa in the CO_2-sensitive cultivar treatments, compared to those in WYJ23, implying the different influences of rice cultivars on the microbial ecological network.

Conclusions Different rice cultivars under eCO_2 did not influence the alpha- and beta-diversity of the soil bacterial community, but changed the co-occurrence network of the community. More attention should be paid to the assembly mechanisms of the soil microbial microbiome when evaluating the impacts of productive crops on the soil-plant ecosystem under the eCO_2 condition.

Keywords 16S rRNA gene · Co-occurrence network · Free air carbon dioxide enrichment · Rice cultivar

1 Introduction

Atmospheric CO_2 concentration is expected to rise during the next several decades (Okubo et al. 2015), from its present 370 to 550 μmol mol^{-1} by the middle of this century (IPCC 2014). Increasing evidence has demonstrated that the global ecosystems, such as grassland, forest, and agricultural systems (Calfapietra et al. 2010; Norby and Zak 2011), are being influenced under elevated CO_2 (eCO_2). A consensus has been reached that eCO_2 generally stimulates plant growth and primary productivity (Yang et al. 2006; Shimono et al. 2009). Rice is the world's most important staple food crop, covering 9% of the earth's arable land (Johnson et al. 2016). The existing data show that eCO_2 generally increases the rice productivity by 9–15% (Kim et al. 2003; Yang et al. 2006), which is, however, much lower than the expected value of 30% grain

Jiangbing Xu and Jianwei Zhang contributed equally to this work.

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yield (Long et al. 2006). Hence, efforts have been made to select/breed rice varieties with high CO_2 responsiveness. A recent research based on the China free air carbon dioxide enrichment (FACE) platform has proven that an indica rice genotype, Yangdao6 (YD6), could reach the expected rice yield, suggesting the promising selection for future food productivity (Zhu et al. 2015).

In contrast to the consensus on the increased plant yields under eCO_2 condition, distinct effects have been shown in regard to the soil microbial community (Austin et al. 2009; He et al. 2012; Liu et al. 2014; Lee and Kang 2016; Liu et al. 2017). The underlying reason is that soil microorganisms are intimately related to the plant-derived substrates (Chung et al. 2007; Qin et al. 2017), and eCO_2 mainly influences the soil microorganisms through indirect ways such as plant root exudates (Bhattacharyya et al. 2013; Okubo et al. 2015). In other words, the responses of soil microorganisms to eCO_2 may vary with the plant species/cultivars. It is thus reasonable to speculate that the CO_2-sensitive cultivar, i.e., YD6, might impact the soil microbial properties in a different manner from the regular cultivar, especially under the eCO_2 condition, which, however, has never been documented before.

It is noteworthy that most of the current studies related to the soil microbial community under the eCO_2 condition are focusing on the total numbers of taxa or unique lineages (that is, taxonomic or phylogenetic alpha-diversity) and the relative abundances of overlap (or turnover) between communities (that is, beta-diversity) (Barberan et al. 2012). Such analyses are effective in deciphering how microbial communities are structured in soil. However, there is a lack of knowledge about the interspecies co-occurrences between microbial taxa due to the complexity of soil ecological processes (Gu et al. 2017). Molecular ecological network analysis, an approach that has been widely used to study the impacts of global changes on plant and animal diversity, may provide comprehensive perspective into the microbial association patterns (Wood et al. 2017). Based on molecular ecological network analysis, deeper insight could be inferred in respect to the positive (commensalism and mutualisms) or negative (predation and completion) co-occurrence between different microbial taxa, and the keystone taxa important for network stabilization, which may help to ascertain the functional roles of certain microorganisms. With the ever-increasing data from high-throughput sequencing, it is possible to elaborate the co-occurrence network of the taxa beyond the alpha- and beta-diversity patterns. In fact, such analyses have been applied to evaluate the microbial responses to eCO_2 in earlier investigations (Zhou et al. 2011; Drigo et al. 2017). Results from those literatures suggest that the topological parameters derived from network analyses would be indicative of the network stability, such as modularity (Wood et al. 2017) and decentralization degree (Ling et al. 2016). Nevertheless, questions remain unclear as yet whether the plant cultivars grown under the eCO_2 condition could lead to the shifts in soil bacterial community network, as well as in the bacterial alpha- and beta-diversity.

Considering the distinct responses of rice cultivars to eCO_2 in grain yields, we hypothesized that such variations could as well be reflected in the underground properties, especially in the soil microorganisms. However, the responses of the alpha- and beta-diversity and co-occurrence network pattern of the soil microbial community varied in their magnitudes. To this end, two types of rice cultivars responsive distinctly to eCO_2 were planted in the rings of the China FACE platform (Yangzhou, Jiangsu, China) in 2014. In the third year of the growing season, soil samples were collected from both the FACE and the ambient rings at the anthesis stage of rice. Real-time quantitative PCR and high-throughput sequencing techniques were employed to examine the changes in soil bacterial community. Furthermore, network analysis was used to unravel the co-occurrence patterns among the bacterial taxa. This work was expected to provide a previously undocumented dimension of the combining effects of rice cultivars and eCO_2 on soil microbial properties, and would be instructive to build up a sustainable agricultural ecosystem.

2 Materials and methods

2.1 Site description

The FACE platform was established in Jiangdu County, Jiangsu Province, China (119° 42′ 0″ E, 32° 35′ 5″ E). The relevant soil properties are as follows: 9.2% sand (1–0.05 mm), 65.7% silt (0.05–0.001 mm), 25.1% clay (< 0.001 mm), 1.2 g/cm^3 bulk density, 15.0 g/kg soil organic C (SOC), 1.59 g/kg total N, 1.23 g/kg total P, 10.4 mg/kg available P, and pH 6.8. This site is located in the subtropical climatic zone with a mean annual precipitation of 900–1000 mm, mean annual temperature of 16 °C, an average daily integral radiation of 12.3 MJ/m^2, a total annual sunshine time of more than 2000 h, and a frost-free period of more than 230 days.

2.2 FACE system and cropping system

Detailed information about the FACE system was described in our previous studies (Okada et al. 2001; Zhu et al. 2008). Briefly, three octagonal rings were arranged with a target CO_2 concentration of 550 μmol mol^{-1} at the center of the rings (hereinafter referred to as eCO_2), and three ambient rings without CO_2 enrichment (hereinafter referred to as the aCO_2) served as control. All eCO_2 rings, with a diameter of 12.5 m, were kept away from aCO_2 rings more than 90 m. Towards the center of the eCO_2 rings, pure CO_2 at high pressure was

released about 50 cm above the crop canopy all day long from tubes surrounding crops.

In 2014, two rice cultivars distinct in their response to eCO_2 were planted in all eCO_2 and aCO_2 rings: one was the CO_2-tolerant inbred indica cultivar, "Wuyunjing23" (WYJ23), and the other was CO_2-sensitive hybrid indica cultivar, "Yangdao6" (YD6). In our previous study, WYJ23 and YD6 have been proven to increase grain yield by 12.8% and 32.9% under eCO2, respectively (Zhu et al. 2015). Due to their different tillering ability, the seedlings of WYJ23 and YD6 were transplanted by hand into the ambient and FACE plots at a density of 2 plants/hill and 1 plant/hill, respectively. The spacing of the hills was 16.7 cm × 25 cm (equivalent to 24 hills/m^2). For all treatments, N was applied as a basal dressing (40% of the total) 1 day prior to transplanting and as a top dressing at early tillering (30% of the total) and at the panicle initiation stage (30% of the total) at 22.5 g N/m^2. Phosphorous (P) and potassium (K) were applied as a compound fertilizer at 9 g P_2O_5/m^2 and 9 g K_2O/m^2; both P and K were applied as a basal dressing 1 day before transplanting.

2.3 Soil sampling and soil characteristics analysis

In 2016, the third year of the growing season, soil samples were collected from each cultivar subplot at the anthesis stage. To satisfy the demand of statistical and ecological network analysis, 12 parallel samples were collected for each treatment or each cultivar. That is, 48 soil samples were collected in total. Before sampling, the field was drained for 5 days to facilitate sample collection, and samples were taken with 3.5-cm-diameter cores (0~15 cm depth). Five soil cores were randomly collected from each subplot and then mixed together to make one composite sample. Then, samples were divided into two portions: one was stored at 4 °C for biochemical assay and the other was kept at − 80 °C for molecular analysis.

Dissolved organic carbon (DOC) and nitrogen (DON) were exacted from 10.0 g fresh soil using 50 ml 0.5 mol/l K_2SO_4 ultrapure water by centrifugation (8000 rpm, 10 min). The filtrate that passed through a 0.45-mm filter membrane was analyzed with a total C analyzer (Elementar, Germany) and a continuous-flow analyzer (Skalar, Holland), respectively.

2.4 Soil DNA extraction

Soil DNA was extracted from the moist soil (0.5 g) by using FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. Cell lysis was performed by vigorous shaking in a FastPrep® 24 bead-beating instrument at a speed setting of 6.0 for 40 s. The extracted DNA was dissolved in 70 μl of the DNA elution solution and stored at − 20 °C. DNA quantity and purity were determined using a Nanodrop® ND-1000 UV-Vis spectrophotometer.

2.5 Quantitative real-time PCR

The abundances of bacterial 16S rRNA gene fragments were measured by quantitative real-time PCR (qPCR) on a CFX96^{Tm} Real-Time system (Bio-Rad, USA) with primers 519F/907R (Biddle et al. 2008). The PCR mix contained 10 μl SYBR Green I PCR master mix (Applied Biosystems, USA), 0.2 μl each primer (10 μ mol/l), and 2.5 μl template DNA (sample DNA or plasmid DNA for standard curves), and finally was filled with sterile deionized water to 20 μl. The qPCR conditions contained an initial denaturation at 95 °C for 5 min, followed by 37 cycles of denaturation at 95 °C for 45 s, annealing at 56 °C for 45 s. Standard curves were obtained using tenfold serial dilutions of plasmid DNA. The R^2 value for each standard curve exceeded 0.99, indicating linear relationships over the concentration ranges used in this study. All of the amplifications were run in triplicate with the DNA extracted from each soil sample.

2.6 High-throughput sequencing procedure

Soil bacterial 16S rRNA gene was amplified using universal primers 519F/907R targeting the V4-V5 hypervariable region (Biddle et al. 2008). To perform sequencing with the MiSeq sequencing system (Illumina Inc., USA), primers were tagged with unique 5-bp barcodes for each replicate DNA sample. PCRs were carried out in 50-μl reaction mixtures containing each deoxynucleoside triphosphate at a concentration of 1.25 μM, 2 μl (15 μM) forward and reverse primers, 2 U of Taq DNA polymerase (TaKaRa, Japan), and each reaction mix received 1 μl (50 ng) of genomic community DNA as a template. The thermal cycling was as follows: 35 cycles of 95 °C for 45 s, 58 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min. Negative controls using sterilized water instead of soil DNA were included to check for primer or sample DNA contamination. Each DNA sample was amplified in three technical replicates. Reaction products were then pooled, purified using the QIAquick PCR Purification Kit (QIAGEN, Germany), and quantified using NanoDrop ND-1000 (Thermo Scientific, USA). The bar-coded PCR products from all samples were normalized in equimolar amounts then prepared using a TruSeq™ DNA Sample Prep LT Kit and sequenced using a MiSeq Reagent Kit (500-cycles-PE) on a MiSeq platform following the manufacturer's protocols.

2.7 Processing of high-throughput sequencing data

After sequencing was completed, 16S rRNA gene data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1 pipeline using default parameters unless otherwise noted (Caporaso et al. 2010). Briefly, the sequences were binned into operational taxonomic units (OTUs) using a sequence similarity cutoff of 97% (USEARCH V8)

(Edgar 2017). Taxonomy was assigned to OTUs against a subset of the Silva 119 database. UPARSE was used to align, trim quality, and remove chimera OUT. There were totally 676,550 16S rRNA gene sequences that passed QIIME's quality filtering, within a range of 7036 and 36,850 sequences per sample. All samples were then rarefied to 7000 sequences per sample to evaluate the alpha- and beta-diversity of bacterial phylotypes, since an even depth of sampling is required for alpha- and beta-diversity comparison (Shaw et al. 2008).

2.8 Co-occurrence network construction and visualization

To identify OTUs that may be involved in the maintenance of community structure and function, phylogenetic molecular ecological networks (pMEN) were constructed for each treatment using a random matrix theory (Deng et al. 2012; Zhou et al. 2011). The framework for network construction can be divided into four key steps: (1) metagenomic sequence collection, (2) data standardization, (3) pairwise similarity estimation using Bray-Curtis, and (4) adjacent matrix determination. Five hundred top abundant OTUs were applied to construct for the network analysis. The cutoff value for the similarity matrix was automatically generated using default settings, and the P value was set at the 0.01 level. Global network properties such as density, average centralization of degree, transitivity, average degree, average path distance, and geodesic efficiency of pMEN were characterized using a web-based pipeline (http://ieg2.ou.edu/MENA/). The networks were visualized using Cytoscape V2.6.2 software.

Based on the concepts of within-module connectivity (Z_i) and between-module connectivity (P_i) (Guimerà and Nunes Amaral 2005), node topologies were organized into four categories: (1) module hubs (highly connected nodes within modules, $Z_i > 2.5$), (2) network hubs (highly connected nodes within the entire network, $Z_i > 2.5$ and $P_i > 0.62$), (3) connectors (nodes connecting modules, $P_i > 0.62$), and (4) peripherals (interconnected nodes in modules with few outside connections, $Z_i < 2.5$ and $P_i < 0.62$) (Olesen et al. 2007; Deng et al. 2012). It is proposed that module hubs, connectors, and network hubs are critical in maintaining community structure and function, and thus serve as keystone taxa (Shi et al. 2016; Oberholster et al. 2018).

2.9 Statistical analysis

All statistical analyses were carried out in R (Version 3.3.3) (R Core Team 2017) and SPSS 19.0 for Windows (IBM Corporation, NY, USA). The differences among treatments were analyzed with two-way analysis of variances (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at $P < 0.05$. The complexity of species diversity (alpha-diversity) was calculated with QIIME 1.9.1 (Caporaso et al. 2010), using Chao1 and Faith's phylogenetic diversity (PD), Observed OTUs, Simpson, and Shannon as indices. To visualize the similarity of community composition (OTUs) between the different samples, nonmetric multidimensional scaling (NMDS) analyses were carried out for ordination based on Bray-Curtis distance metric using the "metaMDS" function in R's vegan package (Oksanen et al. 2013). Permutation multivariate analysis of variance (PERMANOVA, 9999 permutations; Bray-Curtis distances) was conducted for a group difference test between community groups.

3 Results

3.1 Soil DOC and DON contents

The contents of DOC and DON are shown in Table 1. Generally, the YD6 treatments had higher DOC contents than the WYJ23 treatments. WYJ23 and YD6 under eCO_2 significantly increased the DOC content by 18.6% and 8.7%, respectively, compared to their counterparts under aCO_2. Through two-way ANOVA, significant effects of CO_2, rice cultivar, and their interactions were observed for DOC ($P < 0.05$, Tukey's test).

By contrast, the DON contents varied with the atmospheric CO_2 concentrations and the rice cultivars. Specifically, compared to the counterparts under aCO_2, WYJ23 under eCO_2 increased DON content, while YD6 under eCO_2 decreased the value. However, two-way ANOVA results showed that no significant effects of CO_2 and rice cultivar were observed, but significant interactions between CO_2 and rice cultivar were detected (Table 2).

Table 1 Contents of dissolved organic C and N in soil

Treatment		DOC	DON
WYJ23	aCO_2	4.95 ± 0.11c	16.48 ± 0.78b
	eCO_2	5.87 ± 0.09b	17.93 ± 0.39ab
YD6	aCO_2	5.88 ± 0.10b	18.55 ± 0.37a
	eCO_2	6.40 ± 0.12a	16.65 ± 0.61b
2-way ANOVA			
CO_2 effect		*	ns
Rice cultivar effect		*	ns
CO_2 * rice cultivar		*	*

Results are shown as means ± standard deviations. Different letters in the same column indicate the significant difference at the 0.05 level (Tukey's post hoc test). The effects of CO_2, rice cultivar, and their interactions were assessed using two-way ANOVA

* significance at $P < 0.05$, *ns* not significant

Table 2 The alpha-diversity indices of the soil bacterial community

Treatment		Chao1	PD	Observed OTUs	Simpson	Shannon
WYJ23	aCO_2	3946.27 ± 213.45a	74.43 ± 5.05a	2103.42 ± 85.13a	9.91 ± 0.11a	0.9974 ± 0.0003a
	eCO_2	3958.57 ± 197.72a	72.95 ± 5.44a	2077.83 ± 49.74a	9.87 ± 0.08a	0.9972 ± 0.0003a
YD6	aCO_2	3882.68 ± 218.55a	72.28 ± 2.99a	2083.50 ± 86.81a	9.90 ± 0.12a	0.9973 ± 0.0004a
	eCO_2	3925.30 ± 146.11a	73.25 ± 5.52a	2085.75 ± 62.76a	9.90 ± 0.07a	0.9974 ± 0.0002a
2-way ANOVA						
CO_2 effect	ns	ns	ns	ns	ns	ns
Rice cultivar effect	ns	ns	ns	ns	ns	ns
CO_2 * rice cultivar	ns	ns	ns	ns	ns	ns

Results are shown as means ± standard deviations. The effects of CO_2, rice cultivar, and their interactions were assessed using two-way ANOVA
* significance at $P < 0.05$, *ns* not significant

3.2 Quantitative analysis of bacterial 16S rRNA genes

The bacterial 16S rRNA gene copy number varied from 3.69×10^{11} to 4.82×10^{11} copies/g soil dry weight (Fig. 1) for all samples. Nevertheless, no significant effects of CO_2, rice cultivar, and their interactions were found among all treatments ($P > 0.05$).

3.3 Soil bacterial community diversity and structure

Based on the high-throughput sequencing results, it was found that no significant effects of CO_2, rice cultivar, and their interactions were found for the alpha-diversity indices, i.e., Chao1, observed OTUs, phylogenetic diversity (PD), Shannon, and Simpson ($P > 0.05$, Table 2).

The NMDS plot was used to visualize the beta-diversity among all samples. As showed in Fig. 2, irrespective of the rice cultivars and eCO_2, the replicates of each treatment were not situated closely. In addition, there were no obvious distinctions among all treatments, suggesting the similarities of the bacterial communities. In support, the differences between treatments were not significant at the 0.05 level, proved by the PERMANOVA test (Table S1, Electronic Supplementary Material, ESM).

3.4 Co-occurrence network patterns

The ecological network was generated individually for each treatment at the OTU level to illustrate the co-occurrence patterns of the soil bacterial community (Fig. 3), and the derived topological properties of networks are summarized in Table 3. Compared to those under the aCO_2 condition, WYJ23 and YD6 under eCO_2 consistently increased the values of some parameters, such as total/negative links and average degree. However, for some other parameters, their responses to eCO_2 varied with rice cultivar treatments. For example, YD6 under eCO_2 had higher centralization of degree, but lower values of positive links, total nodes, modularity, module number, and averaged path distance compared to those under aCO_2; nevertheless, opposite trends were observed for those parameters in WYJ23 under eCO_2 compared to that under aCO_2. Such features demonstrated the distinct impacts of rice cultivars on the soil bacterial co-occurrence patterns.

3.5 Key bacterial taxa

To assess the possible topological roles of taxa in the networks, we classified nodes into four categories based on their within-module degree Z_i and the among-module degree P_i:

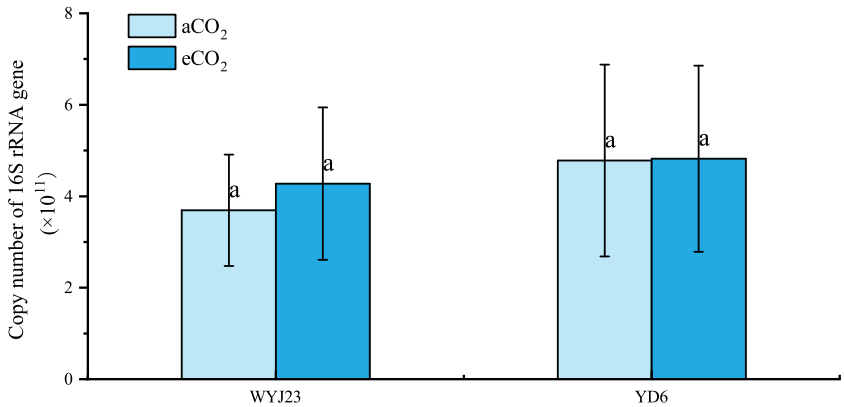

Fig. 1 Gene copy number of bacterial 16S rRNA genes in different treatments

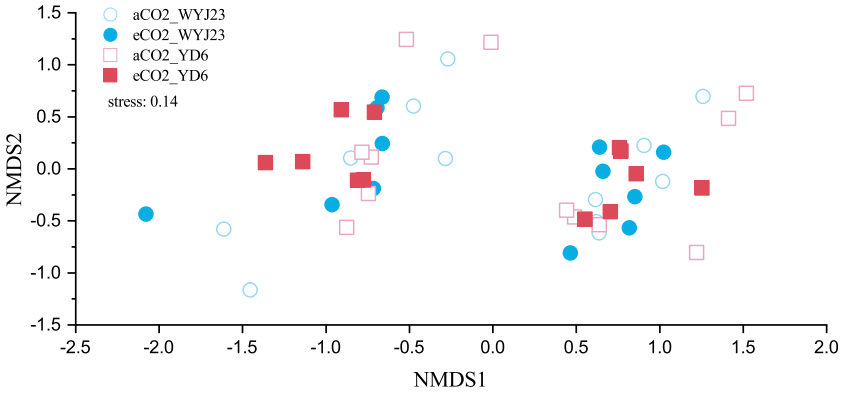

Fig. 2 Nonmetric dimensional scaling (NMDS) ordination derived from Bray-Curtis dissimilarity matrices showing the community dissimilarities (OTU level) within different treatments

peripherals, connectors, module hubs, and network hubs (Fig. 4).

In this study, the networks of all treatments contained taxa with module hub properties (high Z_i, low P_i; 27 OTUs), i.e., highly connected OTUs linked to many OTUs within their own module, and taxa classified as connectors (low Z_i, high P_i; 29 OTUs), which link several modules. However, no network hubs were present for all treatments (with both a high Z_i and a high P_i). Most of the taxa belonged to peripherals (specialists), accounting for 94.2% of the total OTUs (Fig. 4). Among all treatments, 12 keystone taxa were annotated as unclassified bacteria, reflecting the limited knowledge on soil microbial community. For WYJ23 under eCO_2, more module hubs but fewer connectors were found compared to that under aCO_2, although their numbers of keystone taxa (module hubs and connectors) were pretty similar. In particular, WYJ23 under eCO_2 harbored more Acidobacteria and Beta-proteobacteria acting as module hubs (Fig. 4a), and more Actinobacteria serving as connectors. In the YD6 treatments, eCO_2 appeared to influence the keystone taxa to a greater extent than aCO_2, with more Proteobacteria, including Alpha- and Beta-proteobacteria, and Acidobacteria serving as connectors under eCO_2 (Fig. 4b).

4 Discussion

4.1 Rice cultivars lead to variations in soil labile components

Plants play important roles in C flow in a terrestrial ecosystem. Atmospheric CO_2 fixed by plants is assimilated into plant biomass and, on the other hand, might be transferred into soil via root exudation, plant tissue breakdown, etc. (Drigo et al. 2010). In this respect, plant species or cultivars are crucial factors for evaluating the effects of eCO_2 on soil biochemical properties and heterotrophic microbiota (Calvo et al. 2017; Eisenhauer et al. 2017). Our prior study on the same FACE platform has proven that YD6 has a much higher grain yield (> 30% increase under eCO_2 compared to under aCO_2) than the inbred one (~ 13% increase) (Zhu et al. 2015). In addition, the

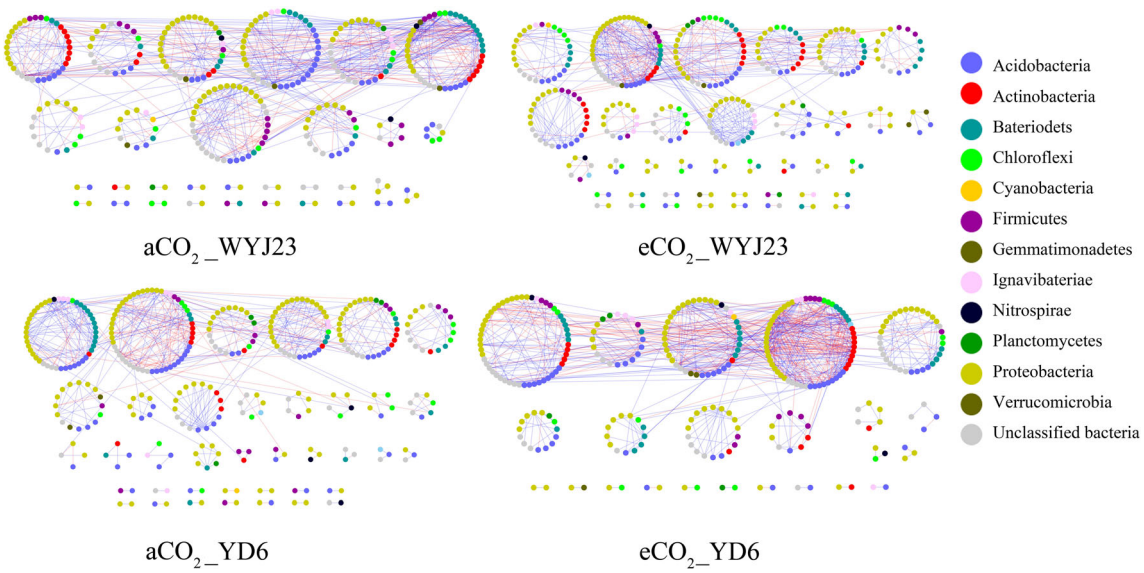

Fig. 3 Soil bacterial co-occurrence networks from different treatments based on RMT analysis from OTU profiles

Table 3 Topological properties of co-occurring bacterial networks obtained from different treatments

Topological properties	WYJ23		YD6	
	aCO_2 (cutoff value 0.83)	eCO_2 (cutoff value 0.83)	aCO_2 (cutoff value 0.82)	eCO_2 (cutoff value 0.85)
Total nodes	302	344	314	267
Total links (positive/negative)	519 (376/143)	608 (443/165)	471 (341/130)	481 (304/177)
Average degree	3.437	3.535	3.000	3.603
Average clustering coefficient	0.150	0.165	0.110	0.131
Average path distance	5.609	6.139	5.710	4.699
Geodesic efficiency	0.223	0.209	0.212	0.262
Harmonic geodesic distance	4.487	4.789	4.712	3.81
Centralization of degree	0.069	0.054	0.032	0.130
Maximal betweenness	6359.7	5910.1	7053.4	1056.0
Centralization of betweenness	0.130	0.091	0.136	0.292
Centralization of stress centrality	0.605	1.13	0.479	0.883
Density	0.011	0.01	0.010	0.014
Transitivity	0.220	0.294	0.200	0.228
Modularity	0.682	0.693	0.761	0.593
Module number	32	39	39	32

amount and the quality of straw biomass in the CO_2-sensitive treatment are enhanced under the eCO_2 condition (Zhu et al. 2016). Such process might potentially bring more DOC into soil, and as for our study, the highest value of DOC was observed for the CO_2-sensitve cultivar under eCO_2 (Table 1). However, higher plant biomass/yield is accompanied by more N uptake to coordinate the C/N balance and avoid photosynthetic acclimation in plants (Cheng et al. 2012). That is, more N nutrient in soil will be assimilated by plants under eCO_2, resulting in the decreased DON content (Table 1). Given the important roles of DOC and DON in the soil ecosystem, it is pertinent to further investigate the microbial responses to rice cultivars and eCO_2.

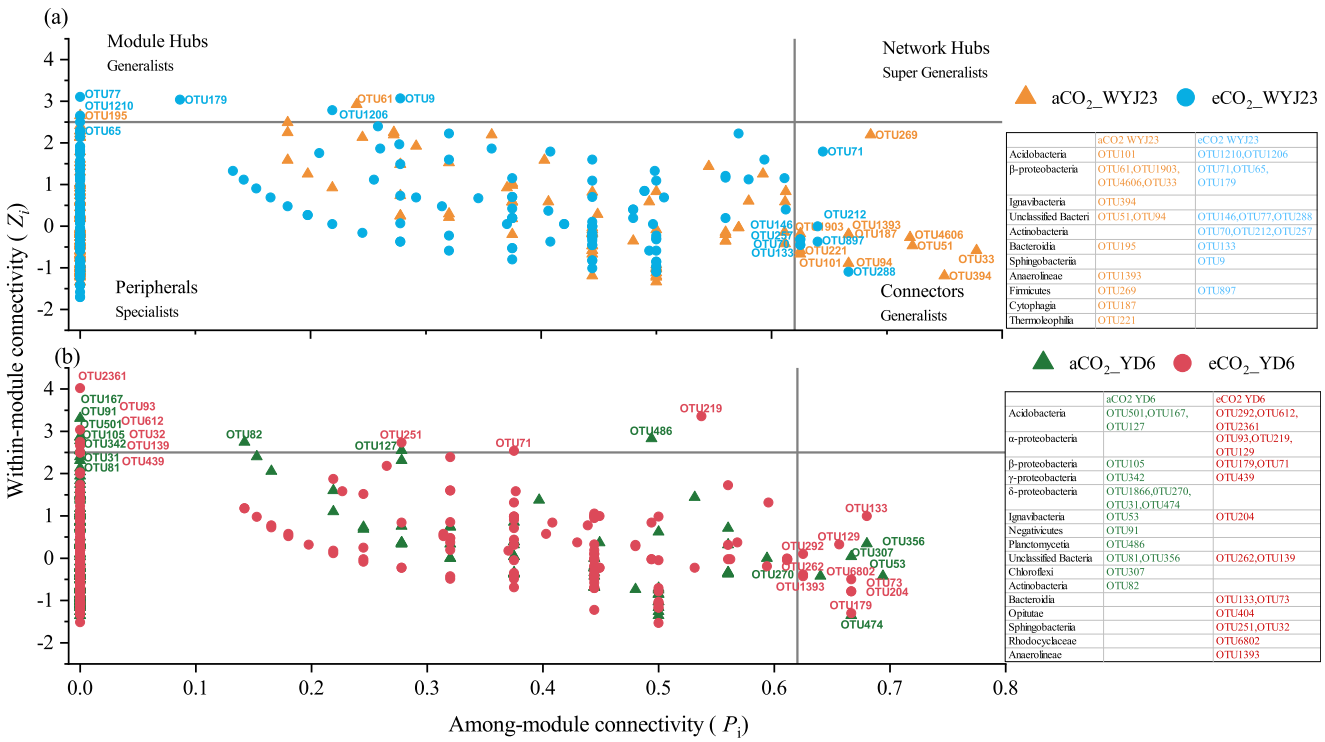

Fig. 4 Distributions of OTUs based on their module-based topological roles according to among-module (P_i) and within-module (Z_i) connectivity

4.2 Rice cultivars and eCO_2 do not influence soil bacterial alpha- and beta-diversity

No significant differences were found among all treatments for the bacterial population, the taxonomic alpha-diversity indices (i.e., Chao1, PD, and Shannon), and the community structure (Fig. 1 and Table 2). In literatures, the effects of eCO_2 on soil bacterial structure/components were not consistent. For example, the changes of soil bacterial alpha-diversity or beta-diversity under eCO_2 were shown in the grassland/forest ecosystem (Ebersberger et al. 2004; Simonin et al. 2017; Xia et al. 2017), but not observed in the agricultural ecosystem, i.e., in a wheat field (Liu et al. 2017). Those features implied that the response of soil microbiota to eCO_2 might be plant-specified. Besides, the direct impact of eCO_2 on soil microbiota might be minor, since CO_2 concentration in the pore space of active soils (2000~38,000 ppm) is much higher than that in the atmosphere (Drigo et al. 2008). In other words, eCO_2 influences the soil microbial properties basically through indirect approaches, such as root exudation or plant tissue breakdown (Bhattacharyya et al. 2013; Okubo et al. 2015), which makes the eCO_2 effect unapparent.

It was interesting that eCO_2 changes the diazotrophic community in soil, as shown in our previous study (Yu et al. 2018). The possible reason might be that soil microorganisms related to N cycling are susceptible to the N-limited environment caused by eCO_2. By contrast, the functional redundancy of soil microorganisms masks the responses of some sensitive lineages to eCO_2, resulting in the nonsignificant changes of the whole bacterial community in this study.

4.3 Co-occurrence patterns of soil bacterial taxa influenced by eCO_2 and rice cultivars

In natural habitats, microorganisms live together within complicated networks through various types of direct and indirect interactions, which could be positive, neutral, or negative (Faust and Raes 2012; Wang et al. 2017). Different agricultural managements, e.g., land utilization patterns (Wood et al. 2017) or fertilization (Ling et al. 2016), have been proven to modify the soil microbial processes and subsequently the co-occurrence network patterns. In the FACE ecosystems, the co-occurrence network based on a random matrix theory (RMT) has revealed that eCO_2 impacts the topological characteristics of grassland soil (Zhou et al. 2011). Using the same approach, we found that not only eCO_2, but also the plant cultivar, influenced the co-occurrence pattern of the soil bacterial community. For example, eCO_2 led to more total/negative links in both cultivar treatments, while rice cultivars exhibited contrasting impacts on positive links, i.e., fewer positive links for YD6 and more positive links for WYJ23 under eCO_2 compared with those under aCO_2 (Table 3 and Fig. 3). Links, connecting two nodes, represent the potential relationship between two functional species (nodes). Positive links/interactions signify complementation or cooperation, while negative links/interactions may indicate competition or predation between the taxa (Yang et al. 2017). More links led by eCO_2 suggested the higher-level species-species interactions within the ecological network happened. This might be intimately related to the available sources in soil. Specifically, more C and N substrates in the WYJ23 under eCO_2 would bring about more cooperative and competitive events in soil. By contrast, more C source but less N substrate in the treatment of YD6 under eCO_2 condition might strengthen the competition, but weaken the cooperation among species due to the limitation in the N source in soil.

Similarly, other topological parameters, such as module number, modularity, centralization degree, and transitivity, varied to different extents in different treatments. CO_2-sensitive cultivar under eCO_2 enhanced the centralization degree and transitivity, but decreased the modularity module compared with that under aCO_2. By contrast, opposite trends were found for the CO_2-tolerant cultivar treatments. Generally, ecological networks can be naturally divided into different submodules considered as functional units, which respectively perform identifiable tasks in the ecological networks (Deng et al. 2012; Yang et al. 2017). Modularity measures the compartmentalization of a network into sub-networks (Wood et al. 2017). A lower modularity value indicates that taxa within a network tend to co-occur more with a wider range of other taxa, while the highly modularized structure was characterized by numerous clusters of multiple interacted species (Jiang et al. 2017). In this respect, the lowered modularity by eCO_2 for the CO_2-sensitive cultivar treatment meant several submodules might be integrated into a much larger one to carry out similar functions. Transitivity represents the likelihood of linked neighboring vertices/keystone species (Barrat et al. 2004; Wood et al. 2017), whose removal can have a disproportionate effect on overall community structure (Berry and Widder 2014). In the CO_2-sensitive cultivar treatments, the enhanced transitivity by eCO_2 suggested the stronger interactions of keystone species within the network (also see the keystone taxa in Fig. 4). According to some studies, such a process might cause the ecological network to be less stable (Liang et al. 2016; Yu et al. 2018). However, in our study, it is still too risky to reach such a conclusion because in an agricultural ecosystem the anthropogenic factors are important in influencing the soil microbiota and the results from one growing season might not be representative. Therefore, more samples from long-term investigation are required in order to confirm such effect of the CO_2-sensitive cultivar on ecological network stabilization, which should be done in future work.

4.4 Keystone taxa in the co-occurrence network

Taxa identified as module hubs and connectors are thought to be keystone taxa due to their role in maintaining network structures (Oberholster et al. 2018). Their loss would lead to the deterioration of the entire network, while the loss of peripherals does not affect the functions of ecological networks (Guimerà and Nunes Amaral 2005; Jiang et al. 2017). Regardless of rice cultivars, the keystone taxa changed under different CO_2 concentrations. In particular, no single taxon acted as a hub or connector in both eCO_2 and aCO_2 conditions, suggesting that both eCO_2 and rice cultivars would change the roles of bacterial taxa in soil. This feature was in agreement with the context dependency theory that keystone species play critical roles only under certain conditions (Power et al. 1996). More keystone taxa mostly affiliated to Acidobacteria and Proteobacteria (especially Alpha- and Beta-proteobacteria) served as keystone taxa in CO_2-sensitive treatments compared to those in CO_2-tolerant treatments. Proteobacteria have been suggested to respond positively to labile C compound (r-strategists) (Fierer et al. 2007), which are abundant in plant root exudates (Bais et al. 2006). It was strange that more Acidobacteria, the K-strategists, also served as keystone taxa in the CO_2-sensitive cultivar under eCO_2. As root exudates are a key factor in shaping the microbiome in soil (He et al. 2017), the exudate components, including amino acids and other low-molecular organic acids, promote the development of niches preferred by Acidobacteria and subsequently result in their keystone role in the co-occurrence networks.

In contrast, for the CO_2-tolerant cultivar treatments, shifts in the distribution of keystone taxa were detected though their total numbers were pretty similar between the aCO_2 and eCO_2 conditions. Specifically, some Bacteroidetes and Actinobacteria play important roles under the eCO_2 condition, while Bacilli (OTU269) Cytophagia (OTU187, Bacteroidetes) served as connectors under the aCO_2 condition. That is, the putative keystone species changed as conditions changed. Bacteroidetes and Actinobacteria were recognized as copiotrophic taxa (taxa that thrived in conditions of elevated C and N availability and exhibited relatively rapid growth rates) (Fierer et al. 2007; Sun et al. 2016), and the increased contents of DOC and DON in soil under the eCO_2 condition ameliorated the niche for their proliferation. Firmicutes, including Bacilli, are actively involved in degrading recalcitrant C sources such as cellulose and chitin in soils (Shange et al. 2012; Sapp et al. 2015). Less input of ready-to-consume C source might account for their keystone role under aCO_2 condition.

5 Conclusions

In this study, the influences of two different responsive rice cultivars on soil bacterial microbiome were investigated under aCO_2 and eCO_2 conditions. Though no significant variations among treatments were detected for bacterial alpha-diversity and beta-diversity, shifts in the co-occurrence patterns of the bacterial community were detected. Under the eCO_2 condition, the CO_2-sensitive and the CO_2-tolerant cultivars exhibited distinct influences on some topological parameters of the co-occurrence network, such as nodes, links, modularity, and transitivity. Moreover, the keystone species in the network were also changed in different rice cultivar treatments. Collectively, the high yield of the CO_2-sensitive rice cultivar under the eCO_2 condition is accompanied by the shifts in the microbial ecological network. More attention should be paid to the assembly mechanisms of the soil microbial microbiome when taking advantage of its productivity in agriculture.

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