Agriculture, Ecosystems and Environment 264 (2018) 94–98

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Elevated CO_2 increases the abundance but simplifies networks of soybean rhizosphere fungal community in Mollisol soils

Zhenhua Yu[a,1], Yansheng Li[a,1], Xiaojing Hu[a], Jian Jin[a,b,*], Guanghua Wang[a], Caixian Tang[b], Junjie Liu[a], Xiaobing Liu[a], Ashley Franks[c,d], Elenora Egidi[c,d], Zhihuang Xie[a]

[a] Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, 150081, China
[b] Centre for AgriBioscience, La Trobe University, Melbourne Campus, Bundoora, VIC, 3086, Australia
[c] Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne Campus, VIC, 3086, Australia
[d] Centre for Future Landscapes, La Trobe University, Bundoora, VIC, 3086, Australia

ARTICLE INFO

Keywords:
Illumina MiSeq sequencing
Black soil
High atmosphere CO_2
Genotypes
Fungal communities

ABSTRACT

Elevated atmosphere CO_2 (eCO_2) levels lead to changes in the quantity and composition of rhizodeposition of soybeans. Previously, a majority of studies have focused on the bacterial community response to the eCO_2 in the rhizosphere of soybean with little information regarding the quantitative and compositional changes in the fungal community available. To provide insight into the fungal community response, next generation sequencing of the internal transcribed spacer (ITS) region was conducted for in-depth analysis of changes in fungal abundance and diversity in response to eCO_2. Four soybean cultivars (i.e. Xiaohuangjin, Suinong 8, Suinong 14 and Heinong 45) were grown for 65 days under ambient CO_2 (aCO_2) (390 ppm) and eCO_2 (550 ppm) in Mollisol soils. Elevated CO_2 significantly increased ITS copy numbers in the rhizosphere of the soybean cultivars except Xiaohuangjin and Suinong 14. Principal coordinate analysis (PCoA) revealed that eCO_2, rather than soybean cultivars, altered the composition of soil fungal communities. Network analysis indicated that eCO_2 simplified the network structure by changing topological roles of operational taxonomic units (OTUs) and key fungal members, which were likely regulated by concentrations of NH_4^+-N, NO_3^--N and available K and microbial biomass C under eCO_2.

1. Introduction

Soil fungi perform a wide range of important ecological functions including carbon (C) and nutrient cycling, plant-growth promotion, pathogenesis, and parasitism in terrestrial ecosystems (Buee et al., 2009; Christensen, 1989). Plant-derived rhizodeposition is a major source of C input into the soil supporting the growth of fungi. Elevated CO_2 (eCO_2) alters rhizodeposition due to the changes in plant carbohydrate status (Barron-Gafford et al., 2005). Changes in the quantity and quality of plant-derived C compounds can significantly affect soil fungal communities.

Only a few studies regarding the responses of soil fungal communities to eCO_2 have been reported with inconsistent findings in their results. An increase, decrease and no changes has been reported in soil fugal diversity in response to eCO_2 (Curlevski et al., 2014; Liu et al., 2017; Nguyen et al., 2011). The different findings are likely attributed to the differences in plant species and soil types between studies.

A richer abundance of phylotypes in the fungal community of the rhizosphere of *Larrea tridentate* were found when grown under eCO_2 compared to aCO_2 using gene clone libraries (Nguyen et al. (2011)), While a recent NextGen sequencing amplicon base sequencing study reported that there was no significant change in the richness of fungal community in the rhizosphere of wheat in response to eCO_2 (Liu et al., 2017). Terminal restriction fragment length polymorphism indicated a significant community composition CO_2 effect on the arbuscular mycorrhizal fungal community at SoyFACE (Illinois USA) (Cotton et al. (2015)).

Elevated CO_2 shifted fungal taxa but not the community β diversity in the surface mineral soil (0–5 cm depth) associated with aspen (*Populus tremuloides*) (Dunbar et al., 2014) and altered the succession of soil fungal communities in temperate forest soil (Zheng et al., 2009). The fungal communities in the rhizosphere of *Carex arenaria* (a non-mycorrhizal plant species) and *Festuca rubra* (a mycorrhizal plant species) grown in three dune soils were affected at a genus specific level (Drigo et al., 2009). However, to our knowledge, little information is available about the impact of eCO_2 on fungal communities in the

* Corresponding author at: 138 Haping Road, Harbin, 150081, China.
E-mail address: jinjian@iga.ac.cn (J. Jin).
[1] These authors make an equal contribution to this paper.

https://doi.org/10.1016/j.agee.2018.05.006
Received 4 November 2017; Received in revised form 5 May 2018; Accepted 9 May 2018

Table 1
Summary of numbers of OTUs, Chao and Shannon indices, and ITS gene copies in the rhizosphere of soybean cultivars grown for 65 days under ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2). The values are means of three replicates with standard deviation in brackets.

Cultivar	Number of OTUs		Chao		Shannon		ITS gene copies ($\times 10^9$ copies g^{-1} dry soil)	
	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
Xiaohuangjin	597 (21)	529 (43)^{ns}	672 (28)	672 (87)^{ns}	4.17 (0.13)	4.00 (0.21)^{ns}	1.87 (0.77)	2.44 (0.26)^{ns}
Suinong 8	512 (31)	517 (17)^{ns}	628 (71)	663 (34)^{ns}	3.23 (0.17)	3.98 (0.07)*	2.36 (0.17)	4.70 (0.81)*
Suinong 14	526 (34)	487 (81)^{ns}	632 (12)	623 (51)^{ns}	3.60 (0.45)	3.59 (0.43)^{ns}	1.92 (0.22)	1.66 (0.30)^{ns}
Heinong 45	519 (62)	464 (43)^{ns}	644 (54)	644 (41)^{ns}	3.82 (0.49)	3.37 (0.22)^{ns}	1.86 (0.86)	3.73 (0.84)*

*and ns indicate significant ($P \leq 0.05$) and non-significant difference between aCO_2 and eCO_2 (*t*-test), respectively, for each cultivar.

cropping soils. Such information is important to shaping the soil management strategies in agricultural systems in adaption to the climate change.

Soybean [*Glycine max* (L.) Merr.] is the major legume crop in Northeast China with the sown acreage accounting for 33% of the nation's total (Liu and Herbert, 2002). Understanding the responses of soil fungal community to eCO_2 is important due to the role of rhizospheric fungi in the mediation of soil C and nutrient cycling, and disease suppression. While eCO_2 significantly changes bacterial diversity in the soybean rhizosphere and the extent of change depends on soybean cultivars (Yu et al., 2016), it is unknown whether eCO_2 has a similar effect on the fungal community structure and/or abundance in the rhizosphere of soybean.

This study examined the effect of eCO_2 on fungal community in the rhizosphere soil of four commercial soybean cultivars. It employed network analysis to reveal interactions among community members, community organization and keystone organisms, and the key members of fungi responses to environmental factors. We hypothesized that all fungal communities in the rhizosphere would exhibit similar responses to eCO_2, but the level of response would differ between soybean cultivars.

2. Materials and methods

This study used four soybean cultivars (Xiaohuangjin, Suinong 8, Suinong 14 and Heinong 45) which were widely grown in Northeast China. Plants (two per pot) were grown in pots, each containing 14 kg of a silty clay Mollisol collected from cropland. Basal nutrients were applied in the following composition (mg kg^{-1}): 218 urea, 219 KH_2PO_4, 167 $CaCl_2{\cdot}2H_2O$, 43 $MgSO_4{\cdot}7H_2O$, 9 Fe-EDTA, 6 $ZnSO_4$, 5 $CuSO_4$, 0.7 H_3BO_3, 6.7 $MnSO_4{\cdot}H_2O$, 0.3 $CoSO_4{\cdot}7H_2O$, and 0.2 $Na_2MoO_4{\cdot}2H_2O$ (Jin et al., 2010), and were thoroughly mixed with the soil before sowing. All pots were placed in open-top chambers (OTCs) in triplicate under the conditions of ambient CO_2 (aCO_2 = 390 ppm), or elevated CO_2 (eCO_2 = 550 ppm) that is predicted to reach by 2050 (Houghton et al., 2001). Soil water content was maintained at 80 ± 5% of field water capacity. The experiment was harvested at the initial pod filling (R5) stage (65 days after sowing). Rhizosphere soils were collected by shaking the roots and a portion of the soil samples were immediately placed into sterile microcentrifuge tubes (2 mL), and stored at −80 °C for the investigations of microbial molecular ecology. A proportion of fresh soil samples were used for the measurements of the microbial biomass C (MBC) (Vance et al., 1987), ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations (Wang et al., 2017). The rest of soil samples were air-dried for determining Olsen phosphorus (P) (Olsen et al., 1954), available potassium (K) and pH. Detailed sampling procedures and methodology were described in Yu et al. (2016). DNA of frozen soils was extracted using Fast DNA SPIN Kit for Soil (Qbiogene Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. To examine the structure of the soil fungal community, Illumina MiSeq sequencing was performed by creating the amplicon libraries of the internal transcribed spacer (ITS) region using fungus-specific primers of ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC) (Gardes and Bruns, 1993; White et al., 1990). Quantitative PCR (qPCR) was also conducted by targeting the fungal ITS region of ribosomal RNA gene, using the same primers. The phylogenetic molecular ecological networks (pMENs) were used to evaluate the differences in fungal community structures between aCO_2 and eCO_2 based on the Random Matrix Theory (RMT) (Zhou et al., 2010). The ITS gene sequencing data were uploaded into the molecular ecological network analyses pipeline (MENAP) according to the instruction (http://129.15.40.240/mena/). More information on the network theories, algorithms, pipeline structure and operational procedures refer to Zhou et al. (2010, 2011) and Deng et al. (2012). The functional groups (guild) of the operational taxonomic units (OTU) were inferred using FUNGuild v 1.0 (Nguyen et al., 2016). Using the program R version 3.1.2 for Windows (R Development Core Team 2010), principal coordinate analysis (PCoA) was processed to assess the patterns of similarity (Bray-Curtis similarity) in the composition of the microbial community between treatments. To link the fungal community composition with soil properties, Mantel test was performed to examine the relationship between the whole fungal community dissimilarities and soil properties. In addition, the relationship between the 30 most abundant genera and soil properties were also examined by performing a Spearman Correlation Heatmap; the data from all treatments were pooled. Sequence data have been deposited in the Genbank short-read archive SRP 109148.

3. Results and discussion

3.1. Abundance of fungal community

The number of fungal ITS gene copies ranged between 1.86×10^9 and 2.36×10^9 copies g^{-1} dry soil under aCO_2 and 1.66×10^9 to 4.70×10^9 copies g^{-1} dry soil in the eCO_2 treatments (Table 1). Elevated CO_2 significantly increased the abundance of fungi in the rhizosphere of cultivars Suinong 8 and Heinong 45. Similarly, eCO_2 enhanced fungal ITS gene copy abundance in a Chinese paddy field across the tillering, heading and ripening stages of rice (Liu et al., 2014). The main effects of eCO_2 and soybean cultivar, and their interactions on the fungal abundance were significant ($P < 0.001$). The cultivar difference may be due to the different root growth and amounts of organic C efflux from roots in response to eCO_2 (Yu et al., 2016; Li et al., 2011), but this assumption needs further experimental test. Under 540 ppm CO_2, Blagodatskaya et al. (2010) observed a higher proportion of fast-growing *r*-strategists, a grouping that includes several fungal species, such as *Neurospora* sp., *Geomyces* sp., *Madurella* sp., *Capronia* sp., *Cladophialophora* sp. and *Neurospora* sp. (Bastian et al., 2009). Furthermore, we found that the fungal abundance highly correlated with MBC ($r = 0.643$, $P < 0.05$), in line with previous studies that showed an increase of MBC under eCO_2 compared to aCO_2 (Yu et al., 2016).

3.2. Fungal community composition

After quality filtering, a total of 1,750,052 qualified reads were obtained from 24 samples with 30,062–43,802 sequences per sample.