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Structural inflexibility of the rhizosphere microbiome in mangrove plant *Kandelia obovata* under elevated CO_2

Panqing Yin[a,b,1], Mengqing Yin[a,1], Zhonghua Cai[a], Guoqiang Wu[b], Guanghui Lin[a], Jin Zhou[a,*]

[a] Shenzhen Public Platform for Screening and Application of Marine Microbial Resources, Graduate School at Shenzhen, Tsinghua University, Shenzhen, 518055, Guangdong Province, PR China

[b] The School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou, 730050, Gansu Province, PR China

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ABSTRACT

Rhizosphere microbial communities play an important role in mediating the decomposition of soil organic matter. Increased CO_2 concentration may increase plant growth by stimulating photosynthesis or improving water use efficiency. However, possible eco-physiological influences of this greenhouse gas in mangrove plants are not well understood, especially how rhizosphere microbial communities respond to CO_2 increase. We characterized the effect of elevated CO_2 (eCO_2) on rhizospheric microbial communities associated with the mangrove plant *Kandelia candel* for 20 weeks, eCO_2 increased plant chlorophyll *a* levels and root microbial biomass. Operational taxonomic unit analysis revealed no significant effects of eCO_2 on rhizospheric bacterial communities; however, some influence on archaeal community structure was observed, especially on the ammonia-oxidizing archaea. Principal component analysis showed that microbial biomass C, total nitrogen, C/N ratio, nitrate nitrogen, and salinity were the main factors structuring the microbial community. The relative contribution of environmental parameters to variability among samples was 31.0%. In addition, functional analysis by average well color development showed that carbon source utilization under eCO_2 occurred in the order amino acids > carbohydrates > polymers > carboxylic acids > amines > phenolic acids; whereas, sugars, amino acids, and carboxylic acids were the preferred carbon sources in control groups. Differences in utilization ability of carbohydrates and amino acids resulted in changes in carbon metabolism between the two groups. Rhizosphere microbial communities appear to have some buffering ability in response to short-term (20 weeks) CO_2 increase, during which the metabolic efficiency of carbon sources is changed. The results will help better understand the structural inflexibility and functional plasticity of the rhizosphere microbiome in mangrove plants facing a changing environment (such as global climate change).

1. Introduction

Carbon dioxide concentration in the atmosphere has increased about 21% from 280 parts per million (ppm) in preindustrial times to approximately 390 ppm today and is predicted by some models to double within the next century (Black et al., 2011). Current estimates suggest that the atmospheric CO_2 concentration range will lie between 450 ppm and 600 ppm by the year 2150 (Barrios et al., 2012). Increases in CO_2 can contribute to global warming, which may have a direct impact on plant growth and development by stimulating photosynthesis or improving water use efficiency. As elevated CO_2 (eCO_2) would increase carbon supply to soil sediment (Loiseau and Soussana, 1999), studies on the effects of increased atmospheric CO_2 in a land ecosystem may yield valuable information to better understand its feedback to global climate change. In particular, as plants are essential for the recycling of materials in natural ecosystems, it is important to study the impacts of eCO_2 on various kinds of plant ecosystems, including the wetland niche.

Mangroves are among some of the most diverse and highly productive coastal ecosystems in tropical and subtropical regions (Bhattacharyya et al., 2015). Wetland plants contain dense and abundant microbial communities within the thin layer of root-adherent soil known as the rhizosphere environment (Bulgarelli et al., 2013). Rhizospheric bacteria live in the direct vicinity of the roots and play important roles in mangrove ecosystems, including nitrogen fixation (Alfaro et al., 2015), nutrient acquisition (Holguin et al., 2001; Bakker,

* Corresponding author. Room 902, Marine-Building, Graduate School at Shenzhen, Tsinghua University, Shenzhen University Town, Xili Town, Shenzhen, 518055, Guangdong Province, PR China.
E-mail address: zhou.jin@sz.tsinghua.edu.cn (J. Zhou).

[1] These two authors contributed equally to this work.

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2012; Jayaprakash et al., 2015), abiotic stress tolerance (Rout and Southworth, 2013) as well as production of regulators of plant growth and development such as auxins, cytokinins, and gibberellins (Ahmad et al., 2013). Additionally, the microbial communities associated with roots also play an essential role in the cycling of matter (for instance phosphorus, organic acids, and siderophores) and maintenance of the health of wetland ecosystems (Gomes et al., 2011; Zeng et al., 2014).

Several factors have been identified that influence microbial communities, including global climate changes and eCO_2. Based on the important relationship between plants and microbes, therefore, it is necessary to examine the diversity, composition, and structure of microbial communities and their links with environmental factors for improving our understanding of mangrove ecosystem functioning. In past decade(s), many studies have made attempts to observe how rhizosphere microorganisms respond to changes in CO_2. However, the results have been inconsistent. Due to the complex interactions with biotic and abiotic factors, the responses of microbial communities to eCO_2 are still poorly understood and contradictory. Some studies have revealed changes in microbial community composition under elevated CO_2 (Hodge et al., 1998; Hayden et al., 2012), while others have shown no significant differences (Klamer et al., 2002; Grüter et al., 2006; Kanerva et al., 2008). Studies on the effects of eCO_2 on microbial biomass, metabolic activity, and total community patterns have documented a variety of responses from significant changes to no response (Grüter et al., 2006; Hayden et al., 2012; Terrer et al., 2018). These differences may be related to plant type, niche conditions, and the sensitivity of methodologies (Luna-Vega et al., 2012; Wang et al., 2014). This has led to the suggestion that soil microbial communities still hold many mysteries due to their extraordinary complexity. Our understanding of the effects of elevated atmospheric CO_2 on plant-soil interactions is still incomplete. Thus, it is necessary to determine how the bacterial community structures and related functions in wetland ecosystem will respond to eCO_2.

To address these issues, we took advantage of a short-term (20 weeks) CO_2 enrichment experiment under laboratory conditions. We hypothesized that increasing CO_2 would alter the composition and function of rhizosphere microbes. To test the hypothesis, the effect of eCO_2 on the activity and structure of the microbial community associated with *Kandelia obovata*, a dominant, important local species in the south of China, was investigated. The aim was to better understand the potential effects that global climate change might have on soil microbial communities. In addition, the habitat of *K. obovata* lies in the southern coastal area of China, which is influenced by the dual factors of human activities and climate change. Improved understanding of the microbial response to eCO_2 perturbations may potentially enable predictions to be made regarding how complex wetland microbial communities may be affected by future anthropogenic changes.

2. Materials and methods

2.1. Experimental design

Six independent chambers were constructed (Fig. 1). Young mangrove individuals (plant height 33.1 ± 2.4 cm, and diameter at breast height 0.96 ± 0.14 cm) were transferred to chambers containing soil collected from a field in the Leizhuo Peninsula (south coastal region of China) that was dominated by *K. obovata*. Prior to use, the soil was homogenized by thorough hand mixing while wearing sterile gloves and sieved. The plants were arranged into six chambers (three treatment groups and three control groups) each containing seven individual pots. The plants were cultured under laboratory conditions for 20 weeks: (i) control (aCO_2; incubation at 350 ppm ambient CO_2); (ii) eCO_2 (incubation at 700 ppm elevated CO_2).

In cubation parameters were automated by continuous monitoring during the experimental process. No fertilizer was added throughout the experiment, and all groups were treated with the same temperature and humidity. The chambers were maintained at 28 °C and 500 μE/m^2/s of light on a 12 h light/dark cycle. Natural brackish seawater with a salinity of 14.1‰ was used every day for plant submergence (Chaudhary et al., 1974). The water depth was kept 5 cm above the soil surface, and water levels in the containers were checked weekly and were adjusted with distilled water to compensate for evapotranspiration losses. Each aquarium was covered with a custom clear acrylic lid with neoprene seals that was secured to the top of the tank with bungee cords. The lid contained two gas port fittings and one temperature probe. Automatic monitoring and control of CO_2 flux within the chambers were accomplished using electronic input/output hardware and programmable software. To monitor CO_2 levels, an infrared CO_2 gas analyzer was used in each chamber. Each of the chambers was connected with two 1/8 internal diameter Teflon tubes to the gas solenoids. CO_2 and temperature readings for each chamber were saved to a local data server. When the CO_2 level was less than the set point, the CO_2 gas solenoid opened for 1 s with 99% CO_2 gas at 20 kPa for each chamber independently. This “round robin” sampling method was continuous throughout the 20-week exposure period. Fig. 1A shows the schematic diagram of the system; and Fig. 1B shows the CO_2 concentration curve during the whole experimental process.

2.2. Plant parameters

Sampling and index measurement were conducted according to time series. Samples were taken at seven different time-point (i.e. 0^{th}, 1st, 3rd, 5th, 9th, 13th, and 20th week) for twenty weeks, and three plant individual were collected at each time. Collection of roots and leaves was made according to described methods (Paula-Freire et al., 2013). Leaf area was detected by a scanner for scanning and conversion. Chlorophyll *a* (ChII *a*) was extracted by using 95% acetone-ethanol. The absorption of the extract was measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Briefly, leaves were washed with water, and absorbent paper was used to blot dry surface moisture; the main vein was removed, and the leaf was cut into strips (about 1–2 mm); 0.5 g leaf (weighed with an accuracy of 0.1 mg) was put into a 50 mL colorimetric tube, and 25 mL extract was added (leaf direct immersion method). Absorbance at 645 nm and 663 nm was measured after 48 h using Arnon's formula (Almeselmani et al., 2011): ChII *a* concentration (μg/L): $C_a = 2.7A_{663}\text{-}2.69A_{645}$; ChII *b* concentration (μg/L): $C_b = 22.9A_{645}\text{-}4.68A_{663}$; total ChII concentration: $C_{a+b} = C_a + C_b$. The ChII concentration of the extract was calculated and converted to ChII content per gram of fresh leaves (μg/g fresh leave weight) (Lin et al., 2015).

2.3. Soil physicochemical parameters

At each sampling time-point, the rhizosphere soil was collected from three representative pots using a sterile blade. We manually removed soil adhering to the roots using spatulas. The rhizosphere soil was homogenized, frozen at −80 °C, and lyophilized prior to analysis. Homogenized subsamples were collected for determination of total C (TC), total N (TN), microbial biomass C (MBC), and dissolved inorganic N (NH_4^+ and NO_3^-). The methods were as described previously (Adam et al., 2000). Briefly, soil pH was determined using a fresh soil-to-water ratio of 1:5 (pH meter). The total soil C and N content were determined by combustion using a TC-TN analyzer (CNS-2000; LECO, MI, USA). The soil nitrate (NO_3^-) and ammonium (NH_4^+) were prepared by adding deionized water (9 mL) to soil (1 g) and shaking for 10 min. After centrifuging at 10,000 rpm for 5 min, the samples were passed through a 0.45-μm filter and frozen until analysis. Soil nitrate was measured using a nitrate electrode (Zhen et al., 1991). NH_4^+ concentrations were measured using the indophenol blue method (Mantoura and Woodward, 1983) with a DR 2000 Autoanalyzer at 425 nm. MBC was determined using the chloroform fumigation direct extraction technique (Brookes et al., 1985). Briefly, 5 g of homogenized,