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Impact of elevated atmospheric CO_2 on soil bacteria community in a grazed pasture after 12-year enrichment

Weiwei Xia [a,b], Zhongjun Jia [b,*], Saman Bowatte [c], Paul C.D. Newton [d]

[a] Jiangsu Key Laboratory of Agricultural Meteorology, College of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, PR China
[b] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, Jiangsu Province, PR China
[c] State Key Laboratory of Grassland Agro-Ecosystems, College of Pastoral, Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, Gansu, PR China
[d] AgResearch, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand

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ABSTRACT

This study was designed to compare soil bacterial communities under ambient (aCO_2) and elevated (eCO_2) carbon dioxide after 12 years of enrichment using Free Air Carbon Dioxide Enrichment (FACE) in a grazed grassland. Grazing animals can have profound effects on nutrient cycling through the return of nutrient in excreta and by their influence on plant community composition through diet selection. The abundance and composition of bacterial communities were evaluated by real-time quantitative Polymerase Chain Reaction (qPCR) and pyrosequencing based on the analysis of bacterial 16S rRNA genes. The results showed the overall bacterial community structure was not altered by the eCO_2 treatment despite the substantial changes in soil functions, pools and fluxes under eCO_2 documented at this site in previous studies. The dominant phyla in both treatments were Actinobacteria, Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Planctomycetes, accounting for 87% of the total microbial 16S rRNA sequence reads. At the phylum level, Planctomycetes and Bacteria incertae sedis increased and BRC, Cyanobateria and TM7 decreased significantly at eCO_2. Most changes were observed at lower taxonomic levels where the abundance of 30 of the 200 most abundant OTUs were responsive to eCO_2 however these changes were not sufficient to differentiate the overall communities. It remains uncertain whether these changes in the lower order taxa could be responsible for the observed changes in soil properties. These first data for a grazed ecosystem are broadly consistent with those from a range of other ecosystems where CO_2 effects are confined to relatively few taxa.

1. Introduction

The CO_2 concentration in the atmosphere has increased by over 30% since the industrial revolution due to anthropogenic interference and is predicted to reach 500 ppm by 2050 (Collins et al., 2013). Elevated CO_2 (eCO_2) not only leads to climate change but also has a direct impact on biological systems. While there is a consensus that eCO_2 has a generally stimulatory effect on plant growth and primary productivity (Ainsworth and Long, 2005; Lukac et al., 2009; Luo et al., 2006; Rogers et al., 1994) there are varied reports of the effects of eCO_2 on belowground microbial communities (Austin et al., 2009; Carney et al., 2007; Gruber and Galloway, 2008; Lesaulnier et al., 2008). For example, studies have reported that soil bacterial diversity increases (Janus et al., 2005; Jossi et al., 2006; Lesaulnier et al., 2008; Sonneman and Wolters, 2005; Liu et al., 2014; Lee et al., 2015), decreases (He et al., 2012; Chen et al., 2014) or remains unchanged (Austin et al., 2009; Ebersberger et al., 2004; Ge et al., 2010; Grüter et al., 2006; Lipson et al., 2006) under eCO_2. This variation may reflect genuine differences among ecosystems or, perhaps, differences in methodology (He et al., 2012).

Recent studies in long-term Free Air Carbon Dioxide Enrichment (FACE) experiments have found changes in microbial communities in a sown biodiversity experiment (Deng et al., 2012; He et al., 2010, 2014) but few changes after 10 years of eCO_2 in a grass/clover pasture (Staddon et al., 2014) or after 11 years enrichment of an aspen plantation (Dunbar et al., 2014). An omission in research on soil microbial responses to eCO_2 is any data from grazed grasslands. This is a land use that covers 37% of the land surface, makes a major contribution to food production (O'Mara, 2012), is a potential source/sink for carbon (C) (McSherry and Ritchie, 2013), a source of emissions of nitrous oxide (N_2O) (Oenema et al., 2005) and both a sink (in the soil) and source (from ruminants) of methane. The impacts of eCO_2 on grazed grassland are thus of considerable importance. In this paper we present data on soil microbial communities gathered from the New Zealand FACE (NZ-FACE) experiment which is the only FACE experiment to consider eCO_2 effects on grazed grassland (Newton et al., 2006, 2014).

* Corresponding author.
E-mail address: jia@issas.ac.cn (Z. Jia).

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Grazed pastures have characteristics that distinguish them from other ecosystems; in particular, nutrients are re-cycled through the animals and the return of these nutrients in dung and urine results in marked heterogeneity in nutrient availability; in addition, animals may prefer some plant species over other and this selection can result in changes in botanical composition. Both of these processes are likely to influence bacterial communities (Anderson et al., 2011; Garbeva et al., 2006; Thomson et al., 2010) and be influenced by eCO_2 (Newton et al., 2001). In the NZ-FACE, Ross et al. (2013) have found significantly greater pools of soil C and N after 10-years continuous exposure to eCO_2 and Rütting et al. (2010) have identified altered N transformations in the soil suggesting changes in microbial activity were occurring.

One g of soil contains an estimated 4×10^7 to 2×10^9 prokaryotic cells (Daniel, 2005) and our ability to culture these bacteria is generally considered to be poor (Curtis et al., 2002; Rappé and Giovannoni, 2003; Schloss and Handelsman, 2004; Zinder and Salyers, 2001). However, recent development of high-throughput sequencing technology has markedly advanced our ability to characterize soil microbial communities (Petrosino et al., 2009; Xia and Jia, 2014). Studies using this new technique have demonstrated how land use (Acosta-Martínez et al., 2008; Nacke et al., 2011), soil management history (Sugiyama et al., 2010), geography (Chu et al., 2010) and environment (Lauber et al., 2009; Yu et al., 2012) can lead to shift in microbial communities. In this study we use pyrosequencing-based soil metagenomics analysis of bacterial 16S rRNA gene to investigate soil microbial communities in the NZ-FACE experiment and provide the first data on the response of grazed grassland to long-term eCO_2.

2. Materials and methods

2.1. NZ-FACE experiment

The NZ-FACE experiment is on a pasture grazed by sheep on the west coast of the North Island of New Zealand (40°14′S, 175°16′E). The pasture contains about 25 species of C3 and C4 grasses, forbs and legumes. The experimental design pairs ambient CO_2 (aCO_2) and eCO_2 rings into three blocks; each ring is 12 m in diameter and is fenced to contain sheep during the grazing periods. Enrichment, to 475 ppm, started in October 1997 and is continuous during the photoperiod. Until 2012 the rings were grazed by adult sheep when the herbage mass reached 180–200 g m^{-2} dry weight and removed when the residual herbage was 50–70 g m^{-2} (Newton et al., 2010; Watanabe et al., 2013). On average there were six grazing per year with 3–4 days duration each time giving an annual average stocking rate of 16.3 sheep ha^{-1} (Newton et al., 2014). The average annual temperature and rainfall at the site is 12.9 °C and 870 mm.

The soil is a Pukepuke black sand (Mollic Psammaquent) with 0.25 m black loamy fine-sand topsoil (Cowie and Hall, 1965). Fertilizer was applied from 1997 to 2009 (Newton et al., 2010) to maintain adequate levels of phosphorus (P), potassium (K) and sulfur (S) based on annual soil sampling and established guidelines (Cornforth and Sinclair, 1984). No N fertilizer was used – the N inputs coming from the legume component of which *Trifolium repens* was the dominant species (Newton et al., 2006). Initial soil properties of the NZ-FACE site are given in Ross et al. (2004) and changes in properties over time in Ross et al. (2013).

2.2. Soil sampling and soil characteristics analysis

Six soil cores (25 mm diameter × 75 mm depth) were collected from random locations in each ring in June 2010. The cores were mixed to form one composite sample for each ring and sieved through a 2 mm mesh. Subsamples were then stored at 4 °C for 4 weeks. In total, there were 6 composite soil samples (2 CO_2 treatments × 3 replicates).

Soil pH was determined using a soil-to-water ratio of 1:2.5 with a glass electrode. Soil moisture was determined by overnight drying at 105 °C. Soil total C (TC) and total N (TN) were determined by dichromate oxidation (Mebius, 1960) and Kjeldahl digestion (Nelson and Sommers, 1982), respectively. Extractable C (Ext C), extractable organic N (Ext ON), microbial C (Mic C) and N (Mic N) and mineral N (Min N) were determined by the methods described in Ross et al. (2013). The soil properties are shown in Table S2.

2.3. DNA extraction

Total DNA from each soil sample was extracted from 0.5 g of soil using a FastDNA® spin kit for soil (MP Biomedicals, Santa Ana, CA) õaccording to the manufacturer's instructions. Cell lysis was performed by vigorous shaking in a FastPrep® 24 bead-beating instrument at an intensity of 6 m s^{-1} for 45 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.

2.4. Quantitative real time PCR

The absolute abundance of bacteria was measured by quantitative real time PCR (qPCR) on a CFX96 thermal cycler (Bio-Rad Laboratories, Hercules, CA). Quantification was performed in a 20 uL 1 × SYBR® Premix Ex Taq™ reaction mixture (TaKaRa Biotech, Dalian, China) in triplicate using the primers 515F/907R (Lane, 1991; Stubner, 2002) for bacterial 16S rRNA genes. Standards were generated by a 10-fold dilution of plasmid DNA. Specific amplification was verified by melting curve analysis, which always resulted in a single peak. PCR amplification efficiencies of 94.6% were obtained with R^2 values of 0.993.

2.5. Bar-coded pyrosequencing

The primers of 515F and 907R were used to amplify 16S rRNA gene amplicons (Lane, 1991; Stubner, 2002; Xia et al., 2011) which targets the hyper variable V4 regions of bacterial 16S rRNA genes. The 515F primer was modified by adding the Roche 454 'A' pyrosequencing adapter, 4-bp key sequence (TCAG) and a unique 11-bp Tag sequence, while the 907R primer was modified by adding the Roche 454 'B' adapter at the 5′ end only (Table 1). Tag sequences were used to barcode PCR amplicons from different samples. PCR reactions were prepared in a 50 μL reaction mixture containing 1 × PCR buffer, 0.2 mM dNTP, 0.5 μM of each primer, 2 μL of DNA template and 2.5 U of TaKaRa Ex Taq HS (TaKaRa Biotech, Dalian, China). The thermal protocol for amplification is shown in Table S1. PCR products were purified using an Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa Biotech, Dalian, China) and visualized on 2.0% agarose gels. All the PCR amplicons from different soils were then mixed in equal amount into a single tube and run on a Roche 454 GS FLX Titanium sequencer (Roche Diagnostics Corporation, Branford, CT, USA).

2.6. Processing of pyrosequencing data

Pyrosequencing data were processed using the Quantitative Insights Into Microbial Ecology (QIIME version: 1.3.0) pipeline (http://qiime.sourceforge.net) (Fierer et al., 2008; Hamady et al., 2008; Lauber et al., 2009). First, we assigned the multiplexed reads (sequences obtained from DNA fragments) to samples based on their barcodes and performed quality filtering based on the characteristics of each sequence (parameters: minimum quality score = 25, minimum/maximum length = 200/1000, no ambiguous bases or mismatches were included in the primer sequence). Next, all of the sequences were clustered into Operational Taxonomic Units (OTUs) at 97% sequence identity with the UCLUST algorithm (Edgar, 2010) and a representative sequence from each OTU was picked for taxonomic identification using a Ribosomal Database Project (RDP) classifier (http://rdp.cme.msu.edu/). Good's coverage is an estimator of sampling completeness (Claesson