CHANGES IN SOIL MICROBIAL COMMUNITY STRUCTURE UNDER ELEVATED ATMOSPHERIC CO_2

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Abstract
The response of soil microbes to elevated atmospheric CO_2 is fundamental to understanding how ecosystems will respond to global change. Here, we investigated soils that had been exposed to the ambient atmospheric CO_2 concentration or to 12 years of elevated atmospheric CO_2 in a Free Air Carbon Dioxide Enrichment (FACE) experiment in New Zealand. Through pyrosequencing, we obtained an average of 3,458 sequences per soil. We then assigned the sequencing data of 16S rRNA genes to RDP taxonomical hierarchy based on nomenclatural taxonomy and Bergey's Manual with a minimum support threshold of 80%. The result of classification at different levels (such as phylum-, class-, subclass-, order-, suborder-, family- and genus) showed that the most taxonomic groupings were present in both treatments but there were marked changes in relative abundance within the groupings. For example, under elevated CO_2 there was an increase in the abundance of *Actinobacteria* and *Planctomycetes* , no change in *Proteobacteria* and a decrease of F*irmicutes*. We expect these changes have functional consequences and this is an area for future research.

Introduction

The CO_2 concentration in the atmosphere has increased by >30% since the industrial revolution due to anthropogenic interference, and is predicted to be 450-600 ppm in 2050 (Houghton et al., 2001; Keeling & Whorf, 2004). Elevated CO_2 not only leads to climate change and global warming, but also has a direct impact on biological systems. While much is known about the stimulating effects of increasing CO_2 on aboveground plant growth and primary productivity (Rogers et al., 1994; Edwards et al., 2003; Ainsworth & Long, 2005; Luo et al., 2006) its influence on belowground microbial communities is poorly understood (Gruber & Galloway, 2008, Carney et al., 2007; Lesaulnier et al., 2008; Austin et al., 2009). Microorganisms play an important role in soil biogeochemical processes, such as C, N, P and S cycling, so how soil microbes respond to elevated atmospheric CO_2 is fundamental to understanding ecosystem responses to global change.

In this paper, we used 16S rRNA gene-based pyrosequencing technique to investigate changes in soil microbial community structure in a Free Air CO_2 Enrichment (FACE) experiment which was established in New Zealand in 1997.

Materials and Methods

FACE experiment
The New Zealand FACE experiment was established on a pasture in the west of the North Island which had received infrequent application of phosphate based fertilizer and grazing by a mixture of sheep, goats and cattle. The study began in 1997, and continues today, with continuous, year-round CO_2 enrichment to 475 ppm in three 12 m diameter FACE rings and

ambient CO_2 in three control rings. Sheep graze the experiment, and excrete back onto the plots. Biomass cuts are taken pre- and post-grazing. Fertilizer, typically 30 g m^{-2} superphosphate and 5 g m^2 potassium, is applied during spring each year (Morgan et al. 2004).

Sampling
Six soil cores (25 mm diameter and 10 mm depth) were collected from each ring in February 2010. The fresh soil was sieved through a 2 mm mesh and plant debris and stones were removed. Subsamples were stored at 4°C.

DNA extraction
Soil total DNA was extracted using a FastDNA® spin kit for soil (MP Biomedicals, LLC) according to the manufacturer's instruction manual. 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. DNA was finally dissolved in 70 μl of the DNA elution solution. DNA quantity and purity were determined using a Nanodrop® ND-1000 UV-Vis Spectrophotometer.

Pyrosequencing
Pyrosequencing was carried out on a Roche 454 GS FLX Titanium sequencer (Roche Diagnostics Corporation, Branford, CT, USA) by analyzing the V4 regions of bacterial 16S rRNA genes(Xia et al., 2011). The primers of 515F and 907R were used to amplify 16S rRNA gene amplicons (Lane, 1991; Stubner, 2002). 515F was modified by adding Adapter A and Taq sequence at 5' end, and 907R was modified by adding Adapter B at 3' end only. Taq sequence was used to barcode PCR amplicons from different soils. PCR reactions were prepared in a 50-μL reaction mixture containing 1 X PCR buffer, 0.2mM 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 was as follows: 5 min at 94 °C; 33 cycles of 30 s at 94 °C, 30 s at 54 °C, and 45 s at 72 °C; and 5 min at 72 °C. The products were purified and visualized on 1.2% agarose gels. All the PCR amplicons from different soils were then combined in equimolar ratios into a single tube in preparation for pyrosequencing analysis. Taxonomy of the sequence reads was assigned using the RDP classifier, based on nomenclatural taxonomy (Cole et al., 2009) and Bergey's Manual with a minimum support threshold of 80%.

Results
After removing low quality sequences (length < 150bp, ambiguous and homologous), on an average, 3,458 sequences per treatment were obtained with a range from 2844 to 3853. The average read length was about 387 bp. Of these sequences, more than 88% were able to be classified. After classification, the sequences were affiliated to different groups at different taxonomic levels. A small amount of archaea (<0.2%) were detected. For each treatment, the relative abundance was represented by the percentage of each taxonomic group in the total sequence reads. The 12 groups with the most sequences were selected to observe the microbial changes caused by elevated CO_2.

At the phylum level, the prominent phyla in both FACE and ambient were similar as shown in Figure 1. The most abundant phylum was *Actinobacteria,* the second was *Proteobacteria,* and other major phyla were *Firmicutes, Actinobacteria* and *Bacteroidetes*. These five phyla accounted for more than 85% of the sequences in each of the soils examined. However, the relative abundance of the phyla was different under the two CO_2 treatments. Compared to ambient, the relative abundance of *Actinobacteria* increased and the abundance of *Firmicutes, Bacteroidetes*, *Nitrospira* and *Crenarchaeota* phyla decreased (Figure 1).

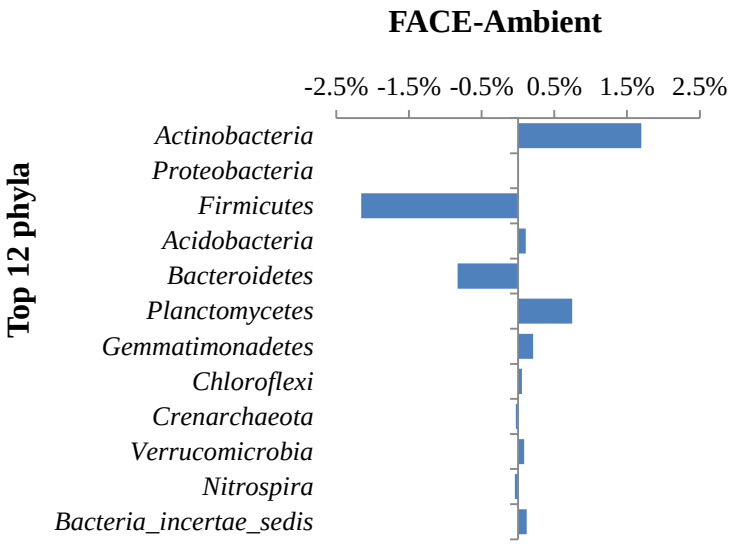

Figure 1. Changes in the relative abundance under elevated CO_2 of the top12 phyla. Phyla (from upper to lower) were arranged from the most to the least abundant under ambient CO_2.

Sequences in different phyla were further divided into classes. At the class level, 24 classes were identified, and the top 12 groups are shown in Figure 2. The dominant classes at ambient CO_2 were *Actinobacteria, Bacilli*, *Alphaproteobacteria*, *Gammaproteobacteria and Betaproteobacteria*, representing approximately 28.8%, 14.3%, 11.5%, 6.3%, and 5.4% of the total sequences. The *Proteobacteria* phylum comprised approximately 26% of the bacterial soil population in this study in both ambient and elevated CO_2 with no difference between the treatments (Figure 1); however, at the lower taxonomic level differences were apparent (Figure 2); the *Betaproteobacteria* class increased and the *Gammaproteobacteria* and *Deltaproteobacteria* classes decreased under elevated CO_2. Compared with ambient, the *Acidobacteria* phylum was dominated by members of groups 1, 3, 6 and 7 which all increased. The *Sphingobacteria* class dominated the *Bacteroidetes* phylum and the *Bacilli* class dominated the *Firmicutes*; these both became less abundant at elevated CO_2.

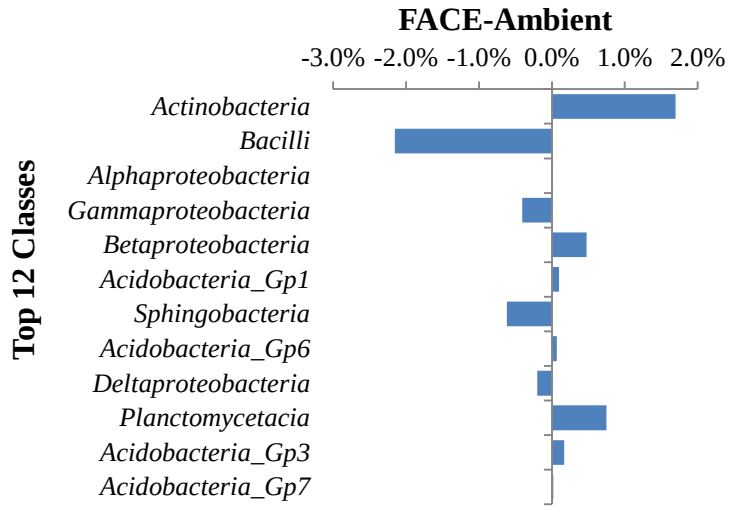

Figure 2. Changes in the relative abundance under elevated CO_2 of the top 12 classes. Classes (from upper to lower) were arranged from the most to the least abundant under ambient CO_2.

There were only 5 groups detected at the subclass level as shown in Figure 3. *Acidimicroidae*, *Rubrobacteridae* and *Actinobacteridae* all belong to the *Actinobacteria* class with the relative abundance in total reads about 15.8%, 7.2% and 1.1% respectively. The increase in these subclasses (Figure 3) led to the difference observed between ambient and elevated CO_2 in the *Actinobacteria* class (Figure 2). *Coriobacteridae* was dectected in the ambient, but *Sphaetobacteridae* only appeared in the elevated CO_2.

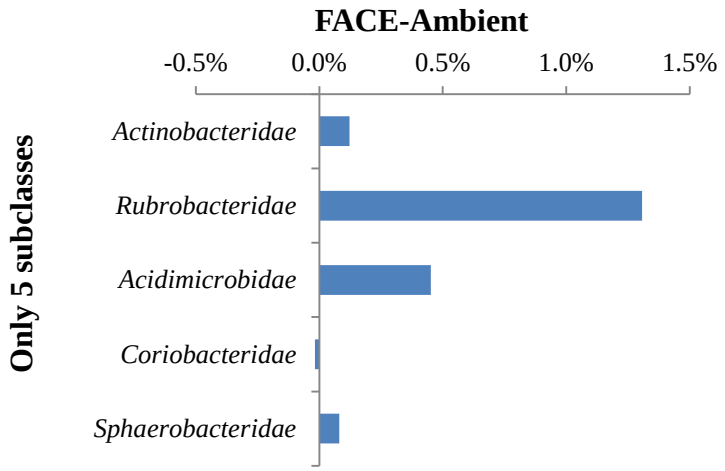

Figure 3. Changes in the relative abundance under elevated CO_2 of the top 5 subclasses. Subclasses (from upper to lower) were arranged from the most to the least under ambient CO_2.

At the order level (Figure 4), *Actinomycetales* and *Solirubrobacterales* were the most abundant groups within the *Actinobacteridae a*nd *Rubrobacteriadae* subclasses, respectively. *Rhizobiales, Burkholderiales* and *Xanthomonadales* dominated in *Alpha-, Beta-* and *Gamma-proteobacteria*, respectively. *Bacillales* contributed to the largest decrease and *Planctomycetales* contributed to the largest increase under elevated CO_2.

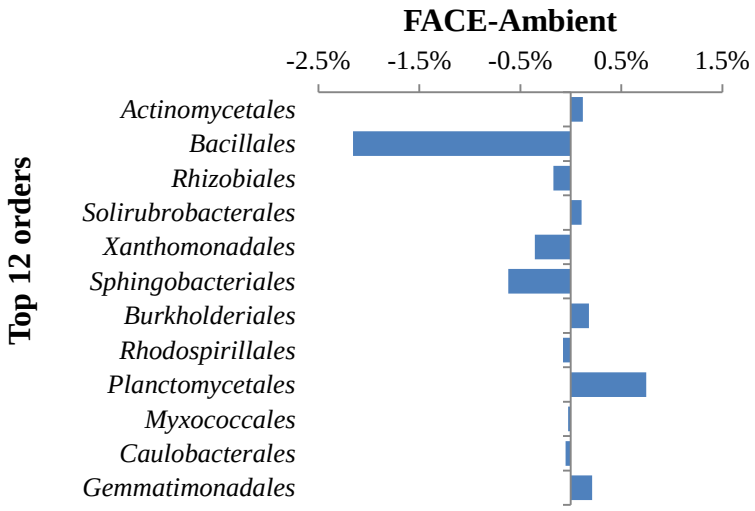

Figure 4. Changes in the relative abundance under elevated CO_2 of the top12 orders. Orders (from upper to lower) were arranged from the most to the least abundant under ambient CO_2.

At the suborder level (Figure 5), the top 12 suborders all belonged to *Actinobacteria* and *Proteobacteria* phyla. The top 9 sequences for suborders all came from the *Actinobacteria* phylum, of these, two suborders, *Micrococcineae* and *Frankineae*, decreased under elevated CO_2 (Figure 5) although there was a general increase in the *Actinobacteria* phylum in this treatment (Figure 1). The top 3 suborders in *Proteobacteria* phylum, *Cystobacterineae Sorangiineae*, *Nannocystineae* all decreased under elevated CO_2 (Figure 5).

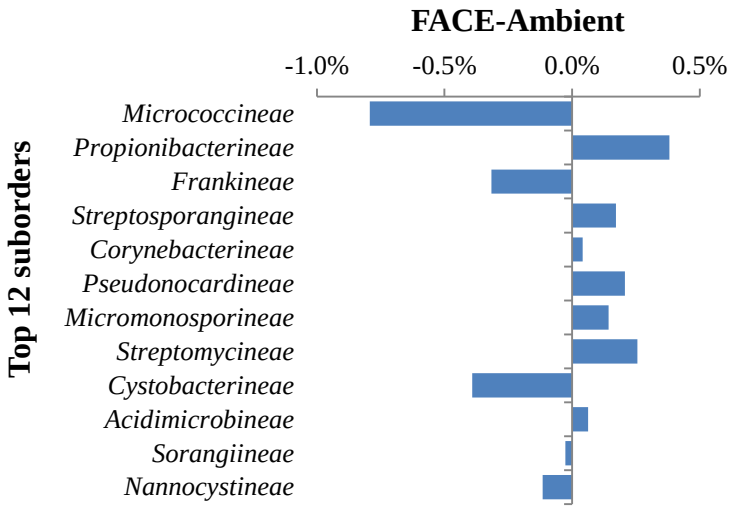

Figure 5. Changes in the relative abundance under elevated CO_2 of the top 12 suborders. Suborders (from upper to lower) were arranged from the most to the least abundant under ambient CO_2.

At the family and genera levels (Figures 6 and 7) there were many differences in relative abundance between elevated and ambient CO_2.

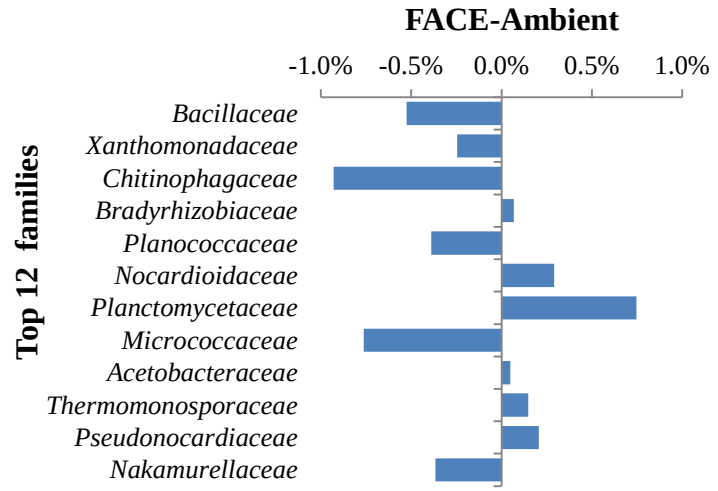

Figure 6. Changes in the relative abundance under elevated CO_2 of the top 12 families. Families (from upper to lower) were arranged from the most to the least abundant under ambient CO_2.

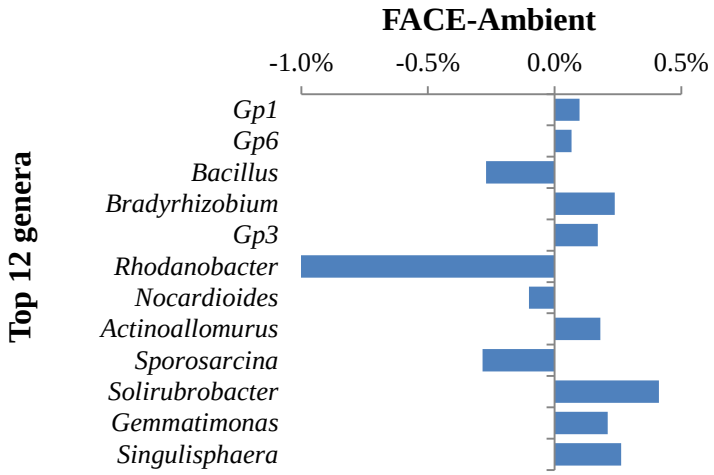

Figure 7. Changes in the relative abundance under elevated CO_2 of the top 12 genera. Genera (from upper to lower) were arranged from the most to the least abundant under ambient CO_2.

Discussion

Soil bacterial diversity is high and our ability to culture these bacteria is generally considered to be poor (Curtis et al., 2002; Rappe´ & Giovannoni, 2003; Schloss et al., 2004; Zinder & Salyers, 2001). However, high-throughput pyrosequencing allows us to study all soil microbes and even unculturable bacteria can be detected. Though the sequencing depth (<4000 sequences per soil) in this study is not enough to survey the full extent of bacterial diversity in the samples, our results suggest that soils are dominated by a small number of phyla. The most abundant 12 phyla comprised 88% of the total bacteria. Similar results were reported by Lauber et al. (2009) who surveyed 88 different soils from different ecosystems across North and South America and found all of soils were dominated by five major groups (*Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Firmicutes*), with these five groups accounting for more than 90% of the sequences in each of the soils examined.

The soils in our study were exposed to different CO_2 concentration for 12 years. With few exceptions, the same taxonomic groups were present in both treatments however there were marked changes in relative abundance. We might expect that these changes would result in, or reflect, differences in decomposition and nutrient cycling. For example, *Actinobacteria*, which are linked to the decomposition of less labile compounds, such as chitin, cellulose, and hemicelluloses, increased at elevated CO_2 as did *Planctomycetes* which can perform anaerobic ammonium oxidation. Our intention now is to use these data to study potential changes in function and microbial networks; the new methods developed by Zhou et al. (2011) to describe phylogenetic molecular ecological networks will be particularly useful for this purpose.

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