# Title: Arid land ecosystem responses to long-term elevated atmospheric $CO_2$ in a large manipulated field experiment.

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Project Goals: Atmospheric $CO_2$ levels are expected to double within the next century. Arid land ecosystems, which comprise over 40% of the earth terrestrial surface, are predicted to be particularly vulnerable to changes in climate. Processes mediated by soil microorganisms responsible for carbon and nitrogen turnover are critical in arid land ecosystems where plants are sparse. We conducted highly replicated surveys of soil microbial communities associated with the dominant shrub species (creosote bush) and in interspace biological soil crusts (biocrusts) at the DOE Nevada Test Site Free Air $CO_2$ Enrichment (FACE) site to determine the effects of over ten years of elevated $CO_2$ conditions on the soil biota.

First, the ability of a targeted metagenomic approach (small subunit (SSU) rRNA pyrosequencing) and shotgun metagenome approaches were compared to identify known distinguishing features between the creosote root zone communities and biocrusts, and differences due to the more subtle elevated $CO_2$ treatment. The biocrust datasets were clearly differentiated from root zone datasets using either of the sequencing approaches. However, different compositional features were identified using the different approaches. The ability to detect possible treatment effects was largely approach-dependent, as the magnitude of resolved differences due to elevated $CO_2$ was smaller when shotgun metagenome reads were used, compared to pyrosequenced SSU datasets or even SSU reads recruited from the shotgun metagenomes. Based on prior knowledge of the biocrust communities, the SSU-based datasets more accurately identified the dominant biocrust cyanobacteria populations compared to the shotgun metagenome datasets.

Second, using quantitative PCR (qPCR) of cyanobacteria 16S rRNA genes, 16S rRNA pyrotag sequencing, and shotgun metagenome sequencing, we explored the response of biocrust cyanobacteria to elevated $CO_2$ in more detail. In years with sufficient moisture for growth, higher plants in this ecosystem have responded to elevated $CO_2$ with increased biomass. We hypothesized that the photosynthetic cyanobacteria that are dominant in the biocrusts would respond similarly. In contrast to our prediction, the relative abundance of cyanobacteria biomass (qPCR) was not significantly different between biocrusts under ambient or elevated $CO_2$ conditions, and trended toward a decrease in cyanobacteria biomass under elevated $CO_2$ conditions. Similarly, the proportion of cyanobacteria in 16S rRNA gene libraries or in shotgun metagenomes was either not significantly different or was reduced in biocrusts under elevated $CO_2$ conditions relative to the ambient controls. Comparison of the shotgun metagenomes provided information to suggest physiological and functional differences in the biocrust cyanobacteria under the elevated and ambient $CO_2$ conditions. Taken together these results indicate long-term elevated $CO_2$ produced shifts in both the structure and function of the biocrust cyanobacteria.

Employing highly replicated field sampling and DNA sequencing, we identified that a major effect of elevated atmospheric $CO_2$ on the biocrusts was a divergence in microbial community composition, resulting in increased spatial heterogeneity. The increased variability of the microbial communities in response to elevated $CO_2$ was robust using multiple sequencing approaches and was observed in both taxonomic and functional profiles. These results will inform future studies to quantify natural variability of soil microbial communities and for the design of future climate change experiments having predictive potential.

Funding statement: This project was supported by the U.S. Department of Energy Biological System Science Division, through a Science Focus Area Grant (2009LANLF260) to CRK. Sequencing was made possible through the DOE Joint Genome Institute and the Los Alamos National Laboratory LDRD program.