22. Soil carbon cycling communities and their response to climate and land use changes in patchy arid land ecosystems

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Project Goals: The goals of this project are to (a) explore the relative utility of multiple 'omic approaches to track soil community responses to environmental changes, (b) map the relative abundance and composition of soil bacterial and fungal communities in shallow soil strata of arid land ecosystems at local and regional scales, (c) determine community responses to combinations of climate and land use changes, (d) identify key responsive community members with relevance as indicators of community change and with utility for modeling soil processes. Achieving these goals will provide an understanding of the active and responsive components of arid land soils that contribute to carbon cycling, their collective responses to environmental change, and development of efficient molecular tools for broad-scale soil monitoring.

The majority of arid land soil biomass resides in shallow strata and this biomass contributes greatly to C and N cycling, both as plant root associates and as biological soil crusts (biocrusts) that colonize the large patches between the widely spaced plants. Targeted rRNA gene and shotgun metagenomic approaches were used to map the relative biomass and composition of soil bacterial and fungal communities at multiple scales in the landscape. Using bacterial and fungal rRNA-based quantitative PCR assays, rRNA gene sequencing, and shotgun metagenomes the communities inhabiting root zones of the dominant shrub, *Larrea tridentata* (creosote bush), and the interspace biocrusts in a Mojave desert shrubland within the Nevada Free Air CO_2 Enrichment (FACE) experiment were mapped (1). Most of the numerically abundant bacteria and fungi were present in both the biocrusts and root zones. However, the proportional abundance of those members differed significantly between root-zones or biocrusts. Functional gene abundances in metagenome sequence datasets reflected the taxonomic differences noted in the 16S rRNA datasets. For example, functional categories related to photosynthesis, circadian clock proteins, and heterocyst-associated genes were enriched in the biocrusts, where populations of Cyanobacteria were larger. Genes related to potassium metabolism were also more abundant in the biocrusts, suggesting differences in nutrient cycling between biocrusts and root zones.

To understand the influence of soil type and soil depth (0-1 cm, 2-5 cm) on community structure, we used spatially nested sampling and 16S rRNA gene sequencing to describe the soil bacterial/archaeal communities in three soils derived from different parent material (2). In all three soils, Cyanobacteria and Proteobacteria demonstrated significantly higher relative abundance in the biocrusts, while Chloroflexi and Archaea were significantly enriched in the below-crust soils. Biomass and diversity of the communities in biocrusts or below-crust soils did not differ significantly with soil type, but composition was affected by soil type. The uniformity with which small-scale vertical community differences were maintained across larger horizontal spatial scales (5 m to 10 km) is a feature of dryland ecosystems that should be considered when designing management plans and determining the response of these patchy ecosystems to environmental disturbances.

Using multiple long-term (5 to 15 yr duration) ecosystem experiments supported by the DOE, USGS, and National Park Service, we conducted replicated field surveys to determine the impacts of multiple climate change factors and land use changes on soil bacterial and fungal communities. Fungal and bacterial community responses to over 10 years of elevated CO_2 were minimal (2, 3), except for a

surprising negative impact on the photosynthetic cyanobacteria, which comprises the dominant biocrust biomass (4). Quantitative PCR, rRNA sequencing and shotgun metagenomes all supported this observation.

Multiple climate and land use changes are operating in concert in arid lands worldwide. Physical damage (e.g. foot traffic) and altered environmental conditions caused by changes in precipitation pattern and/or warming temperatures have been shown to cause dramatic changes in biocrust structure and function. To determine the potential for resilience and regrowth of biocrusts after multiple years of physical or physiological damage, we characterized the bacterial communities in biocrusts after multiple years of foot traffic, altered precipitation pattern and 2- 3°C warming. Targeted rRNA sequencing identified significant differences in community structure when biocrusts were subjected to different types of stress. Impacts of physical disturbance and altered precipitation pattern were the most noticeable and were visibly similar at the soil surface. However, the impacted soil communities were different in structure, suggesting legacy effects specific to the type of disturbance. Furthermore, combined soil warming and altered precipitation resulted in biocrust compositional changes that differed from precipitation alone, highlighting the importance of considering the combined influences of multiple disturbances. Analysis of shotgun metagenomes and soil transcriptomes is in progress.

Using combinations of targeted and shotgun metagenome approaches, we have shown the benefits and pitfalls of each approach for detecting soil microbial community shifts. Target gene approaches generally provide more sensitive detection of taxonomic differences among complex communities across the inherent variability of field-scale experiments and shotgun surveys provide clues about altered physiology that may be a consequence of community structure changes.

(1) Steven B, LV Gallegos-Graves, C Yeager, J Belnap, CR Kuske (2014) *Common and distinguishing features of the bacterial and fungal communities in biological soil crusts and shrub root zone soils.* Soil Biol Biochem 69:302-312.

(2) Steven B, LV Gallegos-Graves, J Belnap, CR Kuske (2013) *Dryland soil bacterial communities display spatial biogeographic patterns associated with soil depth and soil parent material*. FEMS Microbiol Ecol 86:101-113.

(3) Steven B, L Gallegos-Graves, SR Starkenburg, PS Chain, CR Kuske (2012) *Targeted and shotgun metagenomic approaches provide different descriptions of dryland soil microbial communities in a manipulated field study*. Environ Microbiol Rep 4:248-256

(4) Steven B, LV Gallegos-Graves, CM Yeager, J Belnap, RD Evans, CR Kuske (2012) *Dryland biological soil crust cyanobacteria show unexpected decreases in abundance under long-term elevated CO_2.* Environ Microbiol 14:3247-3528.

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