Title: Differential Response of Microdiversity to Simulated Global Change Within a Bacterial Genus

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Project Goals:

We sought to establish an amplicon sequencing method which would allow us to characterize within-genus diversity (microdiversity) of an ecologically important soil bacterium to simulated global change. *Curtobacterium* is one of the most abundant bacteria in the surface litter layer of soil at the Loma Ridge Global Change Experiment. We applied this method to determine the subclade-level response of the taxon to simulated drought and nitrogen addition. We did this within two ecosystems, a grassland and a shrubland, to allow us to elucidate differences in response to the global change treatments by ecosystem type. Lastly, we sought to explain these differences by analyzing the genomes of *Curtobacterium* strains to look for differences in carbohydrate-active enzyme (CAZyme) content.

Abstract text:

Global change experiments often observe shifts in soil bacterial composition based on 16S rRNA gene sequences, however, less is known about how global change might alter bacterial microdiversity, defined here as diversity below the genus level. In particular, it is not known whether the broad taxonomic shifts are consistent within a genus, or whether they represent the summation of divergent responses occurring at a finer scale, i.e., whether the 16S-level data masks the microdiversity response. To investigate the response of bacterial microdiversity to global change, we focused on *Curtobacterium*, a genus of gram-positive aerobic Actinobacteria highly abundant in leaf litter, the topmost layer of soil within the Loma Ridge Global Climate Experiment (LRGCE). Established in 2007, the LRGCE manipulates drought and nitrogen in two adjacent plant communities, grassland and coastal sage scrub (CSS), by intercepting approximately 50% of the rainfall, and adding soluble $CaNO_3$. To characterize fine scale diversity within the genus, we used an amplicon sequencing approach, designing *Curtobacterium*-specific primers for the *groEL* gene, a molecular chaperone found in all bacteria. This method revealed an enormous amount of diversity – more than 6,000 exact sequence variants (ESVs) that fall within at least 12 distinct phylogenetic lineages. The composition of *Curtobacterium* microdiversity varied significantly within the global change experiment, across the two ecosystems, and over time. Although added nitrogen did not alter ESV composition, the drought treatment did significantly alter ESV composition, explaining 9.79% of the variation (PERMANOVA: $p = 0.001$). Ecosystem (grassland or CSS) contributed

the largest amount of variation in *Curtobacterium* microdiversity, accounting for 19.6% (PERMANOVA: $p = 0.001$). Since most of the variation was explained by the two ecosystems, we further investigated the carbohydrate-active enzyme (CAZyme) content by subclade among sequenced isolates. The CAZyme genomic content of the strains differed significantly between subclades (ANOSIM: $R = 0.8044$; $p = 0.001$). These data support the idea that resolving fine-scale patterns of niche differentiation in microbes are key to understanding the response of microbial communities to global change.

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