

## Sequential Growth of Bacteria Following the Rewetting of a Seasonally Dried Grassland Soil

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**Project Goals:** Microorganisms play key roles in soil carbon turnover and stabilization of persistent organic matter via their metabolic activities, cellular biochemistry, and extracellular products. Microbial residues are the primary ingredients in soil organic matter (SOM), a pool critical to Earth's soil health and climate. We hypothesize that microbial cellular-chemistry, functional potential, and ecophysiology fundamentally shape soil carbon persistence, and we will characterize this via stable isotope probing (SIP) of genome-resolved metagenomes. We focus on soil moisture as a 'master controller' of microbial activity and mortality, since altered precipitation regimes are predicted across the temperate U.S. *Our SFA's ultimate goal is to determine how microbial soil ecophysiology, population dynamics, and microbe-mineral-organic matter interactions regulate the persistence of microbial residues under changing moisture regimes.*

Our SFA project objectives include:

- 1) Apply SIP-metagenomics to delineate how changing water regimes shape activity of individual microbial populations and expression of ecophysiological traits that affect the fate of microbial and plant C.
- 2) Identify and quantify mechanisms of mortality in the soil microbiome (focusing on phage lysis and water stress) and their contribution to C turnover and the biochemistry of microbial residues.
- 3) Measure how the soil microbiome and its products (cell envelope, extracellular polymeric substances, exo-enzymes) interact with contrasting mineral assemblages to control both short- and long-term soil C persistence.
- 4) Synthesize genome-scale ecophysiological trait data, population-specific growth and mortality, and SOM chemistry to build models of microbial functional guilds and SOM turnover, to predict the long-asked connection between soil microbiomes and fate of soil C.

Microbial activity is stimulated by the rewetting of dry soils, resulting in a pulse of carbon mineralization and nutrient availability. This phenomenon is of particular interest because predicted changes in precipitation patterns in semi-arid life zones could profoundly change soil C dynamics and nutrient availability. While there has been much interest in the response of indigenous communities to wet-up of dry soil, to date, no work has identified the specific microorganisms in semi-arid soils that grow in response to soil wet-up. We used heavy water (H<sub>2</sub><sup>18</sup>O) DNA stable isotope probing coupled with high throughput sequencing of bacterial 16S rRNA genes to characterize taxonomic and phylogenetic composition of bacteria following the rewetting of a seasonally dried California annual grassland soil.

Bacterial growth was detected at all time points throughout the incubation (3, 24, 72, 168 h), with patterns of sequential growth observable at the phylum and order levels. Of the 25 phyla detected in the pre-wet community, members of the Bacillales order (phylum Firmicutes) were the only detectable early responders – with an approximately 5% increase in relative abundance due to growth in the first 3 h after wet up. The second group of growing bacteria (detected at 24 h) included only Betaproteobacteria and Bacteroidetes. Members of the Burkholderiales order in the Betaproteobacteria phylum were the dominant growers during this period, with a 21% increase in relative abundance. A substantial amount of CO<sub>2</sub> is released within the first 24 hours after wet up, which suggests these few microbial groups may be predominantly responsible for a significant flux of CO<sub>2</sub> from this terrestrial ecosystem.

For actively growing bacteria, the highest richness was detected during the third time-period (between 24-72 h), with significant changes in relative abundance due to growth of 11 phyla. Nonmetric multidimensional ordination of community 16S rRNA sequences through time suggests a cyclical pattern for phylogenetic composition of growing bacteria, with the composition at 3 hours differing slightly from the pre-wet community, differing greatly at 24 h, and then becoming progressively more similar to the pre-wet community at 72 and finally 168 h. This suggests a degree of community resilience in response to this abrupt environmental change; the composition of new growth first diverges from the original community composition but eventually results in a partial return to the original composition. However, some net compositional changes were observed following wet-up. Actinobacteria were the most dominant pre-wet phylum, but Proteobacteria became the most dominant phylum by 168 h. This change in composition was likely driven by new growth, since Proteobacteria were found to increase in relative abundance across most time periods following wet-up, unlike Actinobacteria where comparatively small increases in growth were only observed during the latter two time points. Sequential growth patterns found at the phylum and order level suggest that an ecologically coherent response was observable at a high taxonomic level with members of certain groups employing different life strategies in response to rewetting of a dried soil.

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