

## Linking Microbial Growth to Edaphic Variation Across a Mediterranean-Grassland Precipitation Gradient Using Shotgun-Metagenomic qSIP

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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 are characterizing 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.***

Recent evidence suggests that persistent soil organic matter (SOM) is largely derived from microbial sources, but the microbial-community level processes that mediate SOM formation and stabilization—and the edaphic factors that control these processes—are largely unknown. To elucidate mechanistic links between microbial ecophysiology, soil variables, and SOM formation, our SFA team is using stable-isotope labeling techniques to synthesize microbial ecology through metagenome sequencing with chemical characterization of SOM under varying mineralogical and precipitation conditions.

Here we present our initial characterization of soils from three Mediterranean grassland sites which are the primary field locations for our SFA. In addition to soil chemistry and mineralogy, we assessed the microbial communities active during the California winter, when water is least limiting to microbial growth. Triplicate soil cores were collected during the 2018 wet season from three sites that span a natural precipitation and geographic gradient in CA, with overlapping mineralogical composition at differing degrees of weathering. X-ray diffraction (XRD) suggests these sites vary primarily in clay mineral content, particularly montmorillonite. We measured soil CO<sub>2</sub> respiration after a 6-day incubation and found the highest median respiration rates at the moderate-precipitation site. Radiocarbon dating indicates the age of soil carbon increases with precipitation, but that  $\Delta^{14}\text{C}$  of respired CO<sub>2</sub> is stable, and close to modern across the sites, suggesting microbial populations in all locations preferentially metabolize younger carbon sources. We applied <sup>13</sup>C nuclear magnetic resonance (NMR) spectroscopy to determine the composition of SOC from each site, and found high levels of aromatic compounds (likely plant-derived) at the highest-precipitation site, and higher lipid and carbohydrate signatures (likely microbial) at the moderate-precipitation site.

To link soil properties to microbial processes, we are developing an approach for shotgun-metagenomic quantitative stable isotope probing (qSIP). Triplicate soil samples from each site were incubated for 8 days with either natural abundance  $^{16}\text{O}\text{-H}_2\text{O}$  or 97% atom-fraction excess  $^{18}\text{O}\text{-H}_2\text{O}$ . DNA from each incubation was separated by ultracentrifugation into nine density fractions. Total community DNA from each density fraction for each isotope treatment and soil replicate, as well as unfractionated DNA from each incubation was used for metagenomic library preparation and sequenced on the Illumina NovaSeq platform to an average depth of 8 Gbp per fraction for each sample and treatment (180 metagenomes). We have implemented a novel approach to metagenomic assembly, separately assembling sliding-windows of adjacent density fractions and evaluating whether density fractionation improves metagenome-assembly contiguity and binning relative to assembling all fractions together, estimating that sliding windows result in approximately 40% more genome bins, with more bins at higher genome completeness. From these nine initial soil samples we predict recovery of upwards of 2,000 genome bins, with 800 at high completeness. We have quantified levels of microbial activity during incubation as shifts in density in  $^{18}\text{O}$ -treatment libraries for individual metagenome-assembled genome bins and taxonomically-defined markers in unbinned scaffolds. In this poster we describe a statistical framework for linking the functional potential of active and inactive genome bins to mineralogical, environmental and molecular attributes of sampled soils. This work details the geographic variation in active microbial communities across the three sites that underpin our SFA and explores the utility of shotgun-metagenome sequencing to qSIP, laying the groundwork for experiments that will mechanistically link microbial ecophysiology at each site to soil moisture variation, rhizosphere carbon utilization and SOM persistence.

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