Using Quantitative Stable Isotope Probing to Link Precipitation Regimes of Mediterranean-Grassland Ecosystems to Soil Microbial Ecophysiology

Megan Foley*[1] (mmf289@nau.edu), Alex Greenlon[2], Dinesh Adhikari[3], Karis McFarlane[3], **Steve Blazewicz[3]**, **Mary Firestone[2]**, **Jill Banfield[2]**, **Bruce Hungate[1]**, **Jennifer Pett-Ridge[3]**

[1]Center for Ecosystem Science and Society, Northern Arizona University; [2]Department of Environmental Science, Policy, and Management, University of California, Berkeley; [3]Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA

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 and viromes. 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.*

Given the primacy of microorganisms in the formation, stabilization, and breakdown of soil organic matter, identifying how soil moisture regimes shape microbial ecophysiology may be insightful for understanding the link between microbial communities and soil C in a changing climate system. We characterized soils from three Mediterranean grasslands along a precipitation gradient and used quantitative stable isotope probing (qSIP) to assess the active microbial communities at each site. We used qSIP with iTag sequencing to compare patterns of bacterial growth across sites, and we have also developed an approach to combine qSIP with genome-resolved metagenomes to explore variation in the expression of microbial traits that may be shaped by soil moisture and relevant for understanding the fate of soil C.

We characterized soils from our three primary SFA sites during the 2018 wet season, when water is least limiting to growth. Triplicate soil cores were collected from Hopland Research and Extension Center, Angelo Coast Range Reserve, and Sedgwick Reserve. To establish soil chemistry and minerology, we used solid state ^{13}C NMR and quantitative XRD on bulk soils, and performed ^{14}C analyses on bulk soil and respired soil C. Based on solid-state ^{13}C NMR there, was slight variation in bulk soil C composition. Angelo, the site with the highest precipitation (2160 mm yr^{-1}) has the highest aromatic content, while Sedgwick (383 mm yr^{-1}) has the highest carbohydrate content; Hopland (956 mm yr^{-1}) has the highest lipid content. Lipid was the most well represented fraction of bulk soil C for all sites, followed by carbohydrate and protein. Based on quantitative XRD analysis, Angelo has the highest fraction of clay minerals while Hopland has the lowest. In addition to muscovite and chlorite that were present in all soils, Sedgwick soil contained about 15% halloysite and Hopland soil contained about 10% kaolinite. Samples were also analyzed for their ^{14}C content using LLNL's accelerator mass spectrometer. The average age of soil C increased with mean annual precipitation ($p<0.01$) and total C content,

suggesting that higher precipitation supports accumulation of SOM across the three sites. The ^{14}C values of CO_2 produced during six-day lab incubations were similar across sites and younger than bulk SOM—reflecting a microbial preference for recently fixed C.

To assess the active microbial communities at our sites, soils were incubated for 8 days with either natural abundance ^{16}O-H_2O or 98 atom % ^{18}O-H_2O. DNA was then extracted, separated by ultracentrifugation, and density-gradient fractionated. Total community DNA from 9 fractions, as well as unfractionated DNA from each incubation, was used for sequencing of the 16S rRNA marker gene, and 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).

Using ^{18}O enrichment of bacterial DNA as a proxy for growth, our amplicon-based qSIP analysis highlights distinct patterns in growth between the three sites. The intermediate site, Hopland, has the highest proportion of actively growing to total taxa, as well as the highest average enrichment of the significantly enriched taxa. Growing communities are not only distinct between the three sites, but also diverge from the total communities at a given site identified by sequencing alone. The growing communities at each site included many taxa within the phyla Acidobacteria, Actinobacteria, and Proteobacteria, and the drier two sites additionally had growing taxa within the phyla Armatimonadetes and Bacteriodetes. Relatively few bacterial taxa show growth at both Sedgwick and Angelo, the driest and the wettest sites. Only 8 taxa, all of which belong to the phylum Proteobacteria or Actinobacteria, showed growth at these two sites, compared to 48 and 20 shared growing taxa between Angelo and Hopland, and Sedgwick and Hopland, respectively.

Genome-resolved metagenomic analyses of the ^{18}O-H_2O SIP experiment enable quantitative assessment of metabolic pathways and microbial functional traits corresponding to growth and stasis in our study soils. Genomes with pathways for metabolizing 1-carbon molecules (in particular methanol and carbon monoxide) are overrepresented in ^{18}O-enriched genomes, corroborating previous observations that C1 metabolism plays an important role in soil microbial community functioning. We have not observed variation in isotopic enrichment of genomes with the capacity for compatible-solute synthesis by site, likely reflecting high moisture levels during the incubation. Isotopic enrichment values calculated on metagenome-assembled genome bins average 0.09, 0.15, and 0.06 atom fraction excess for Angelo, Sedgwick, and Hopland respectively, with upper ranges of 0.29, 0.36, and 0.21. The most commonly enriched taxa at each site include Acidobacteria, Actinobacteria, and Proteobacteria, as was observed in our amplicon sequences.

This research is based upon work supported by the LLNL 'Microbes Persist' Soil Microbiome SFA, funded by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under Award Number SCW1632 to the Lawrence Livermore National Laboratory, and subcontracts to the University of California, Berkeley and Northern Arizona University.