Functional genomics of replicating microbes and viruses following rewetting of a Mediterranean 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 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.*

Abstract: Rewetting of soil stimulates a succession of microbial growth and mortality, a process that could potentially become more frequent as climate change in semi-arid zones is predicted to lead to less rain events, potentially allowing for soil dry-down between events. Hypothetically, certain microbial traits, such as degradation of carbohydrates and acquisition of nitrogen, underlie this succession and confer advantages for growth as both the soil microbial community and available resources change over time. We hypothesized that some of the mortality during this succession is due to viral predation of growing organisms (i.e., Lotka–Volterra "kill the winner" dynamics). We also hypothesized that the summer dry down of soil would drive phages to integrate into host chromosomes and that wet-up of dry soil serves an environmental inducer of temperate phages.

To determine the mechanisms driving microbial growth and mortality during wet-up, we performed a wet-up experiment using soils that had been previously $^{13}CO_2$ labeled and maintained under one of two precipitation regimes: the historical average precipitation (100%) and a 50% water reduction. Following the annual summer dry period, soils were collected and incubated with multiple isotopic treatments. 'Heavy water' (^{18}O-H_2O) additions were used to specifically target the active portion of the microbiome and virome. Samples were harvested at six times following rewetting (0, 3, 24, 48, 72, 168 hr) for DNA-quantitative stable isotope probing (qSIP), metagenomics, viromics, and CO_2 production.

While total soil respiration did not vary between soils exposed to 100% versus 50% precipitation, respiration of new (labeled) rhizodeposits was higher in the 100% soils, implying functional differences between precipitation groups. This result was supported by differential abundance of traits found in growing (^{18}O labeled) microorganisms, which revealed differences between precipitation treatments, as well as successional patterns with time. Differential abundance of traits also revealed an extreme legacy effect of historic precipitation, which was 1-3 orders of magnitude higher than any temporal changes. The effect peaked at 0 h but waned quickly and disappeared by 168 h, implying that the community "restarts itself" annually. Abundance of pathways for carbohydrate degradation in growing organisms varied over time, e.g., an increase in abundance of cellulose degradation at 48 h and 72 h, implying changes in complex C availability. N acquisition, while varying little over time, appeared to depend mainly on ammonium transport and assimilation pathways, as well as extracellular proteases, but not other complex N degradation pathways or dissimilatory inorganic N processes.

In comparison to temporal abundance patterns in microorganisms, viruses displayed spatially heterogeneity in addition to temporal community changes. Actively replicating viruses were mostly phages and were detected in dry soil (0 h) as virus-like particles. The vast majority of viruses sampled (83%) did not encode an integrase gene, implying a non-lysogenic life cycle. The low prevalence of putatively lysogenic phage in this wet-up dataset is also underscored by the fact that non-integrase-containing viruses increased by 24 h after rewetting, while integrase-containing viruses did not increase in abundance with time, suggesting that wetting of dry soil does not induce integrated phages. We conclude that the role of lysogeny in soil viral infections is lesser than generally hypothesized.

In summary, we observed temporal changes in active microbial and viral communities following wet-up which were underpinned by organic carbon and organic nitrogen degradation capabilities, as well as by lytic infection by viruses.

This research is based upon work of the LLNL 'Microbes Persist' Soil Microbiome SFA, supported 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, with a subcontract to the University of California, Berkeley. Work at Lawrence Livermore National Laboratory was performed under U.S. Department of Energy Contract DE-AC52-07NA27344.