Cross-Kingdom Interactions: the Foundation for Nutrient Cycling in Grassland Soils

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Project Goals: Our project asks how cross-kingdom and within-kingdom interactions (involving viruses, bacteria, archaea, fungi, protists, microfauna, and plant roots) provide a functional framework for nitrogen (N) cycling in grassland soils. We are using stable isotope probing, NanoSIMS, metagenomic and metatranscriptomic sequencing, exometabolomics, network analysis, and ecosystem modeling to unravel how biotic interactions shape N availability and loss pathways and how these interactions and pathways differ among soil compartments (rhizosphere, detritusphere, hyphosphere, and bulk soil). Our primary goals are to: 1) determine how biotic interactions control key N-cycle transformations, such as depolymerization of macromolecular organic N compounds, N mineralization and immobilization, nitrification, and denitrification, and 2) assess how spatial compartmentalization and transfer between soil compartments (*e.g.*, by fungal hyphae and fauna) determine the occurrences and rates of N-cycling processes.

Decades of research have revealed key microbial mediators of terrestrial nutrient cycling, their edaphic sensitivities, and the functional genes and enzymes involved. While some aspects of bacterial, fungal, and microfaunal mediation of nutrient cycling are reasonably well understood, these and other organisms interact in a complex biotic milieu, and we know little about how such interactions shape nutrient cycling in soil. Considering interactions among viruses, bacteria, archaea, arbuscular mycorrhizal fungi (AMF), saprotrophic fungi, microfauna, and plant roots, we will explore the effects of predation, competition, and cooperative/antagonistic interactions on terrestrial N- and C-cycling. As an introduction to our new project, here we highlight relevant results from our previous work and describe several of our planned experiments.

Building on our large-scale field sampling and manipulative field experiments at the University of California's Hopland Research and Extension Center (HREC) over the last ~20 years, we will continue our work on HREC's Mediterranean grassland soils, which are dominated by the annual grass, *Avena barbata*. In our most recent precipitation manipulation experiment at HREC, *A. barbata* was labeled with $^{13}CO_2$ to trace C flow from the plant into active soil biota and to determine the impacts of reduced precipitation on soil food web dynamics and the C cycle. In Spring 2020 we will leverage the existing infrastructure and conduct a $^{13}CO_2$ field labeling and ^{15}N pool dilution experiment to establish cross-kingdom interactions and connect them to their gross N-cycle effects in the field. To identify how different N-cycling microbial groups, fauna, and viruses respond to N inputs, we will add a urea treatment. We will follow changes in microbial community composition and activity through SIP-enabled metagenomics, amplicon sequencing, and quantification of marker genes for N-cycling processes. Faunal communities

will be tracked via 18S rRNA gene sequencing, and viral dynamics will be measured via viral metagenomics (viromics). We will use liquid chromatography mass spectrometry (LC-MS) to identify changes in metabolites in response to urea addition and to link the dynamics of these molecules to changes in the relative abundances of microbial, viral, and faunal populations.

In a recent greenhouse experiment, we studied both rhizosphere and bulk soils associated with multiple growth stages of the annual grass, *Avena fatua*, labeled with $^{13}CO_2$. Analyses of metagenomes, ITS regions, and 16S rRNA genes revealed a strong influence of root development on the composition and associations of microorganisms and highlighted AMF as extensions of plant roots in mediating soil microbial interactions and associated nutrient cycling. Our upcoming greenhouse experiment will build on these results to measure microbial community dynamics and activity across trophic scales in four different soil compartments (rhizosphere, detritusphere, hyphosphere, and bulk soil), along with differences in N-cycling processes between compartments and how microbiota interact and/or travel between compartments to impact C- and N-cycling.

We expect that viral lysis will impact N-cycling processes, both indirectly through mortality of host populations responsible for specific N metabolisms and directly through the release of organic N-rich cellular contents. Some viruses of bacteria and archaea are capable of switching replication strategies between lysogeny (integration in microbial host genomes as prophages) and lysis, depending on host and environmental cues. We will perform laboratory experiments to link viral populations to their replication strategies, and then we will follow these populations in our field and greenhouse omic data to identify the conditions under which prophage integration or lysis is favored for a particular viral population and the conditions under which specific hosts can lyse. We will focus particularly on changes in N-cycling processes that could drive switches in viral replication strategies or that could result from host lysis.

We have previously shown a strong influence of root development on the composition and associations of microorganisms, including specific bacterial-fungal-protozoan co-occurrence patterns revealed through network analysis. Based on these previously identified patterns, we will prepare simple rhizosphere communities and iteratively add trophic complexity to elucidate changes in C- and N-cycling processes across trophic scales. Specifically, plants inoculated with AMF in EcoFab systems will be iteratively exposed to different bacterial, microfaunal, and viral populations, all isolated from HREC field soil. We will follow changes in community composition, dynamics of genes involved in N-cycling processes, and CO_2 and N_2O emissions.

Combining multiple novel stable isotope techniques with current molecular methods and modeling will allow us to explore, map, and quantify the complex web of biotic interactions that mediate and control N-cycling in soil.

This research is based upon work supported by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under Award Numbers DE-SC0016247 and DE-SC0020163 to UC Berkeley, UC Davis, University of Hawaii, and University of Oklahoma. Work at Lawrence Berkeley National Laboratory was performed under the auspices of the U.S. Department of Energy Contract No. DE-AC02-05CH11231, and work at Lawrence Livermore National Laboratory under U.S. Department of Energy Contract DE-AC52-07NA27344.