

Directing traffic in the rhizosphere: how phage and fauna shape the flow and fate of root carbon through microbial pathways

Katerina Estera^{1*} (rinaest@gmail.com), Anne Kakouridis¹, **Javier Ceja Navarro**², Henrik Krehenwinkel¹, Nhu Nguyen³, Evan Starr¹, Steve Blazewicz⁷, **Eoin Brodie**², **Trent Northen**², **Neo Martinez**⁴, **Zhili He**⁵, **Jizhong Zhou**⁵, **Mary Lipton**⁶, **Rosemary Gillespie**¹, **Jennifer Pett-Ridge**⁷, and **Mary Firestone**¹

¹University of California, Berkeley; ²Lawrence Berkeley National Laboratory, ³University of Hawai'i, Manoa; ⁴University of Arizona, Tucson; ⁵University of Oklahoma, Norman; ⁶Pacific Northwest National Laboratory; ⁷Lawrence Livermore National Laboratory

Project Goals: Our project is designed to explore the complex interactions controlling carbon (C) flow in the rhizosphere -- addressing two overarching topics: 1) How multi-trophic interactions control soil C dynamics, and 2) How changing precipitation regimes alter these interactions, and thus impact flow and fate of soil C. We will study interactions among key groups of soil organisms: 1) arbuscular mycorrhizal fungi (AMF), bacteria, archaea, and roots; 2) phage and their microbial hosts; and 3) rhizosphere fauna (proto- and metazoan) and their prey. Primary goals of this work are to expand the knowledge of food web dynamics in the rhizosphere, how these multi-trophic interactions play a role in terrestrial C cycling, and to investigate how drought alters these interactions and the fate of soil C. The resulting data and information will substantially expand our knowledge of microbial ecology, food web interactions, and terrestrial C cycling.

Rhizosphere soil immediately surrounding plant roots is a zone of abundant biological activity and an area of intense C cycling. Organisms that reside in the rhizosphere, including bacteria, fungi, viruses, and fauna, actively interact with each other as they utilize, transform, and transfer C from root exudates and root debris. These food web interactions enable C that has been fixed via photosynthesis to return to the atmosphere or remain in soil for varying periods of time. While food web interactions are generally well recognized, little is known about how rhizosphere multi-trophic interactions impact C transformation and persistence. Additionally, it is likely that future alterations in precipitation regimes will differentially impact the participants and interactions in multitrophic, root-C based food webs. Our research investigates two primary questions: 1) How do the complex interactions among bacteria, fungi, phage, and fauna mediate and control C flow and fate in the rhizosphere? 2) How do changing precipitation patterns alter these interactions?

We hypothesize that the major phage and faunal grazers of bacteria and fungi redirect a substantial portion of root-derived C towards mineralization. This means that a high abundance of faunal grazers could lead to more rapid mineralization. We expect drought to reduce the function and abundance of protozoa, nematodes, and phage, but it may have less impact on the abundance and function of arthropod fauna. To test these hypotheses, we have constructed 16 “trenched”, rainout plots in a Mediterranean grassland located in the Hopland Research and Extension Center (HREC) in Hopland, California. These plots contain monoculture stands of *Avena barbata*, a naturalized slender oat found throughout California. Precipitation will be manipulated such that half of the plots receive a 50% reduction of the 50-year rainfall average.

The other half of the plots will receive the full average rainfall amount. We will use ^{13}C labeled carbon dioxide (CO_2) to trace the pathway of CO_2 as it is fixed by the plant, and delivered belowground by the roots in the form of exudates and fresh root, and consumed by the various residents of the rhizosphere. Soil will be sampled at multiple time points to track the location and persistence of the recently fixed C in the soil. DNA and RNA will be extracted from rhizosphere soil for stable isotope probing (SIP) enabled -omic techniques. Results will help elucidate how C travels through the rhizosphere food web.

Until recently, our analyses of the faunal component of food webs has been limited by labor-intensive methods and a lack of molecular tools. Next generation sequencing approaches are just now becoming available to study the community assembly of higher eukaryote taxa. Based on ever-growing reference sequence collections of mitochondrial and nuclear DNA sequences, accurate predictions of species composition and richness are now feasible from mixed environmental samples. To identify components of the soil fauna, we are developing a metabarcoding approach based on mitochondrial *cytochrome oxidase I* (COI) and the nuclear ribosomal DNA markers 18S and 28S. We use a two-step PCR approach to simultaneously sequence all markers on by MiSeq. Our initial work on arthropod communities suggests an average recovery of 97% of the species in a mixed sample. We are currently optimizing sequence datasets with multiple gene fragments (e.g. COI) that allows for prediction of species composition and relative abundance in environmental samples. Combining these gene-based approaches with stable isotopes will allow us to follow C from roots to faunal food sources to faunal predators, and ultimately to CO_2 or heavy fractions of soil.

AMF are ubiquitous soil organisms with critical roles in ecosystems, notably as plant root symbionts. The development of molecular methods has made it possible to identify AMF taxa in plant roots and soil instead of relying on morphological characteristics of spores. Illumina sequencing offers new opportunities to investigate the molecular diversity and community ecology of AMF. We use two primer pairs spanning regions of increasing variability in the AMF nuclear ribosomal DNA (rDNA): WANDA/AML2, which targets a DNA sequence in the small subunit (SSU) and gITS7/ITS4, which targets a DNA sequence in the internal transcribed spacers (5.8S, ITS2). As different primer pairs may be biased towards certain groups of AMF, this use of primer pairs with complementary strengths leads to better resolution and inclusion during molecular analyses, as well as more robust information for understanding AMF community ecology.

We hypothesize that a myriad of cross-domain interactions provides pathways and controls for root C flow into the soil. The interactions of these organisms form complex ecological networks. We will be using random matrix theory (RMT) approaches to define the structures and connections of the root-C based food webs.

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