Characterizing the Multitrophic Interactions that Mediate Carbon Flow in Soil and their Responses to Drought

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Project Goals: Our project addresses both a fundamental understanding of carbon (C) cycling as mediated by multi-trophic interactions in the rhizosphere and the potential impacts of altered precipitation regimes on these interactions. Specifically, our work employs the use of stable isotopes to identify and quantify pathways of C-flow through multi-trophic interactions in the rhizosphere. Primary goals are to broaden knowledge of the roles played by multi-trophic interactions in terrestrial C cycling and to discover if drought alters the interactions and/or C cycling. This research will substantially expand our knowledge of soil microbial ecology, belowground food web, and terrestrial C cycling under a changing climate.

The objective of the research presented here is to illuminate the contribution of bacterial, archaeal, fungal, and other eukaryotic communities to carbon and nutrient cycling in soil by applying multi-omics approaches, stable isotope tracing, and field manipulations to the development of mechanistic network theory of material flows in the soil. In all terrestrial ecosystems, numerous populations of organisms such as protists, nematodes and arthropods interact with the soil's free-living and plant-associated microbes, defining biogeochemical and nutrient cycling processes. To predict the responses of C and nutrient cycling to environmental change, it is important to recognize that these environmental processes are the result of the interactions of multiple groups of organisms that in concert shape an ecosystem and its processes. We have optimized multiple approaches to characterize the belowground populations from all trophic levels from a Mediterranean annual grassland, while testing approaches to measure their contributions to C cycling.

Soil samples were collected from a field experiment where replicated plots were manipulated by the addition of different levels of water (50 and 100% local recorded precipitation), aiming to induce normal vs drought conditions. Arthropods were separated from soil samples using berlese funnels, quantified, and morphologically characterized. Nematode and protozoan populations were extracted from fresh soil samples using gradient centrifugation. Specimens were concentrated by filtration and their DNA extracted for amplicon sequence libraries. Sequences were analyzed and groups identified against our locally developed and manually curated dataset. Bacterial and fungal populations were characterized by amplicon sequencing from DNA extracted directly from soil samples. Both diversity and community structure of the analyzed populations showed changes in response to the different water treatments. After five months of differential water regimes, the diversity of nematodes and fungi remained unchanged between treatments, while their community structure changed with at least 10-15% of data variation explained by the type of treatment. Protozoa and bacterial communities, on the other hand, showed higher diversity in the soils under normal conditions without changes in community structure. Predicted functional roles of the

identified nematode and protozoan groups suggest direct interactions with the roots of plants in addition to bacterial and fungal predation. Co-occurrence analyses are being conducted to suggest possible interactions among groups detected from all trophic levels. Results from the co-occurrence analyses will inform laboratory experiments in which populations of organisms isolated from the field will be combined to further define their interactions and fine tune our food-web models.

Besides field water manipulations, our field experiment also included the incubation of *Avena fatua* plants with $^{13}CO_2$. Rhizosphere samples were collected and attached soil was separated, allowing us to physically separate nematode and protozoan populations. Isolated specimens were fixed and deposited onto a filter, imaged using electron microscopy, and ^{13}C levels of different organisms were screened using NanoSIMS isotopic imaging.

The approaches developed here will provide the foundation for molecular approaches to quantitative study soil trophic networks, and also have potential applications as diagnostic tools to identify and intervene for the early control of plant pathogenic organisms in bioenergy cropping systems.

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