Multitrophic and Metabolite Responses to Drought in Grassland Soils

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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 webs, and terrestrial C cycling under a changing climate.

In all terrestrial ecosystems, populations of organisms such as protozoa and nematodes interact with the soil's free-living and plant-associated bacterial and fungal populations. Together these multi-trophic interactions are key regulators of soil carbon and nutrient cycling processes. To predict the responses of these processes to environmental change, we have optimized multiple approaches to characterize the belowground populations from each trophic level, while also incorporating metabolomics analyses to associate changes in community composition with biological activity. Our primary objective 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.

At a Mediterranean annual grassland undergoing simulated drought (-50% average precipitation), soil was collected, and nematode and protozoan populations extracted using gradient centrifugation. Specimens were concentrated by filtration and their DNA extracted. Bacterial and fungal populations were characterized by amplicon sequencing from whole soil DNA extracts, and both diversity and community composition of the analyzed populations showed changes in response to drought. After five months of drought, while the diversity of nematodes remained unchanged, their community composition changed significantly, with 10-15% of variance explained by the water treatment. Protozoan and bacterial communities showed higher diversity in soils under ambient conditions, and protozoan community structure was significantly altered. Fungi, on the other hand increased diversity in soils under drought along with significant changes in community composition. Differential abundance analyses were identifid specific trophic groups that responded to drought. Bacterial groups from the genus *Arthrobacter*, *Gemmatimonas*, *Clostridium*, *Hydrogenispora*, and *Anaeromyxobacter* together with protists such as *Lagenidium*, *Eustigmatos*, *Phytophtora*, and *Theratromyxa* declined under drought conditions. Fungal populations including *Podospora*, *Rozellomycota*, and *Olpidium* increased under drought

Co-occurrence analyses using Random Matrix Theory was used to define network structure for all analyzed trophic groups. Network complexity was found to be higher in soils under ambient water conditions than under drought. Predicted modules in the interaction network will be experimentally tested using populations of organisms isolated from the field.

We used liquid chromatography mass spectrometry-based metabolomics (LC-MS) to identify differences in metabolites profiles from our field drought experiment. Our results show that the drought treatment significantly decreased the relative abundance of primary metabolites such as salicylic acid, threonine, guanine and disaccharides, and increased the abundance of metabolites known as organic osmolytes (e.g. aspartic acid, asparagine, sorbitol). Metabolite exchange impacts the abundance and physiology of organisms at multiple trophic levels and could help define multi-trophic interactions within rhizosphere communities. Further analyses in metabolite profiles will aim to identify molecules associated with signaling for microbial predation, exploratory microbial growth, and biofilm production.

In field $^{13}CO_2$ labeling of the *Avena fatua* plants was carried out and rhizosphere samples with attached soil were collected for DNA extraction and SIP-fractionation. Currently, labeled DNA fractions are being used to identify protozoan groups that had access to labeled C through predation of microbial cells.

The approaches developed here provide the foundation for quantitative molecular understanding of how soil trophic networks and cross-kingdom interactions influence C and N cycling and ecosystem resilience to environmental change.

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