Cross-Kingdom Characterization of Community Dynamics and C flow 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 determine the occurrences and rates of N-cycling processes.

Decades of research have identified key microbial mediators of terrestrial nutrient cycling, their edaphic sensitivities, and the functional genes and enzymes involved. While many individual aspects of bacterial, fungal, and microfaunal mediation of nutrient cycling are reasonably well understood, these organisms mediate biogeochemical processes in a complex biotic milieu. While soil biotic interactions are recognized, their impacts on nutrient cycling are poorly understood. We posit that cross-kingdom interactions shape carbon (C) and nitrogen (N) cycling and resulting plant/microbe nutrient availability and loss pathways.

In a Californian Mediterranean annual grassland undergoing simulated drought (-50% average precipitation), we collected soil at multiple timepoints corresponding to different plant phenological stages. Total DNA was extracted, and we analyzed communities of bacteria, fungi, and microfauna using amplicon sequencing. Our results show that the diversity of bacterial and fungal communities declined during the growing season and reached its lowest levels during the plants' exponential growth, only to recover to levels similar to the early vegetative stage during the plants' later phenological stages. Community composition analyses showed that sampling time had a significant effect on community structure, while the simulated drought influenced only the composition of bacterial groups. To infer community assembly mechanisms, we used a phylogenetic bin-based null model (iCAMP)[1], which indicated that homogenous selection, dispersal limitation, and drift were the key processes controlling bacterial community assembly, while dispersal limitation and drift were more influential in shaping the assembly of fungal communities. The analysis of microfauna dynamics is ongoing.

Microbial communities can be influenced by many factors, but rhizosphere metabolites are thought to play a particularly significant role in the assembly of root-associated microbiomes. We used liquid chromatography mass spectrometry-based exometabolomics (LC-MS) to analyze rhizosphere metabolite profiles of our mixed annual grassland communities collected in the field. We identified specific metabolites that were more abundant at the early stages of plant development, including organic acids and nucleotides/nucleosides (e.g., salicylate, lactate, 5-methylcytosine). Fifty-six metabolites, including sugars and amino acids, were more prevalent during later plant development (e.g., sucrose, maleic, L-proline). We found that amino acids, organic acids, and nucleotides/nucleosides (e.g., p-coumaric acid, L-threonine, guanine) decreased in abundance in water-limited treatments.

In the same drought simulated plots, we pulse labeled the annual grassland plants with $^{13}CO_2$ and then collected rhizosphere soil for DNA extraction and density-gradient stable isotope probing (SIP). Labeled DNA fractions are being used to identify bacterial, fungal, protozoan and metazoan groups that had access to labeled C through either direct uptake of plant-derived C or predation of microbial cells. Our results from the first five-day labeling event suggest that different groups of organisms consumed ^{13}C plant-derived substrates in the simulated drought versus normal moisture treatment soils. Highly enriched groups in the rhizosphere of soils under drought treatment included several members of the family Burkholderiaceae and the genus *Skermanella*, *Pseudonorcardia*, *Modestobacter*, and *Ramlibacter*. A fungal ASV (Chytridiomycetes) and the bacterivore protist *Cryptodyfflugia* were significantly ^{13}C-enriched in the drought treatment. In the rhizosphere of the normal moisture plants, we observed a larger number of significantly ^{13}C-enriched taxa, from multiple trophic levels. These bacterial and fungal groups included *Parviterribacter*, *Conexibacter*, *Geodermatophilus*, *Microvirga*, *Methylobacterium*, *Agaromycetes*, *Morteriella*, and *Olpidium*. ^{13}C-enriched microfauna in the normal moisture treatment included both protists and nematodes. The protists included the bacterivores *Pseudocyrtolophosis*, *Paracercomonas*, the omnivore *Cercomonas*, the eukaryvore *Bressalua*, green algae *Stichococcus*, and a plant pathogen from the Peronosporales. ^{13}C-enriched nematodes included bacterivores from the class Chromadorea and the plant parasite *Filenchus.*

Our results suggest an important connection between plant phenology and cross-kingdom soil microbial community interactions and demonstrate that multiple trophic groups participate in the movement of C from the rhizosphere into the soil ecosystem. We are measuring gross soil N fluxes and multiple C pools that will be used to further define the contributions of cross-kingdom interactions to biogeochemical cycles through ecological modeling.

1. Ning, D. *et al.* A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nat. Commun.* **11**, 4717 (2020).

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