Characterizing the Multitrophic Interactions that Mediate Carbon Flow in Soil

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Project Goals: This project aims to understand the effects of changing precipitation regimes on the composition, function and interactions among biological compartments (microbial and eukaryotic) of the soil food web and the consequences for C flow in the rhizosphere. Approaches for the separation, quantification, and identification of soil fauna are being optimized and combined with high-throughput sequencing and stable isotope probing techniques, to characterize fauna populations associated with the rhizosphere of *Avena fatua* and their contribution to the dynamics of C transformation and stabilization. A primary goal is to develop robust mathematical models of soil trophic networks that extend beyond bacteria and fungi, and thus require information on faunal community composition, body size, and feeding preferences.

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. The objective of the research proposed here is to illuminate the contribution of bacterial, archaeal and eukaryotic communities, as components of a multitrophic network, to carbon and nutrient cycling in soil, with their mechanistic basis illuminated through the application of multi-omics approaches, stable isotope tracing, and field manipulations.

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. The first step for the construction of mathematical models to define environmental multitrophic interactions is to characterize the different trophic nodes that are part of an ecosystem. For this, we have tested and optimized different approaches to characterize populations of arthropods, nematodes, and protists from two different soil ecosystems: a switchgrass plantation in a marginal soil in southern OK, and a mediterranean annual grassland.

Characterization of soil arthropod community composition. We separate total arthropod communities from soil samples using Berlese funnels. Arthropods are surface- cleaned, individually imaged and measured, and their DNA extracted for identification by sequencing and phylogenetic analyses. These approaches have allowed us to characterize arthropod dynamics during switchgrass development. Populations of fungal-feeding collembola and mites first emerge during plant growth, followed by increased densities of root-feeding beetle larvae and plant

pathogenic thrips. Next, spider populations' bloom while collembolan populations decline, possibly impacting soil fungal population dynamics.

Old meets new: physical isolation and sequence based characterization of soil nematode and protist populations. Our initial attempts to use direct DNA extraction and "universal" molecular marker amplification to quantify and characterize nematode and protist populations yielded eukaryotic populations that were mainly dominated by plant and fungal sequences with minimal contribution of micro- and meio-fauna. Based on these results, we re-directed our efforts to develop techniques for the physical separation of nematodes and protists from the soil matrix. We developed an optimized isolation protocol based on gradient centrifugation and size selection by differential filtration. Subsamples of the isolated pools of nematodes and protist are then used for DNA extraction, fixing of specimens, and imaging. Sequenced libraries yielded 50-70% fauna sequences; remaining sequences were mainly fungal. This technique has allowed us to characterize nematode and protist densities in bulk and rhizosphere, confirming that the rhizosphere is a hotspot for nematode and protist populations. Predicted functional roles of these species suggests direct root feeding in addition to bacterial and fungal predation. These functional predictions are being evaluated using isotopic methods. We are designing new primers (targeting specific fauna groups) to enable quantification of these groups by qPCR.

Physical isolation of arthropods, nematodes, and protists has allowed us to define the body size distribution of individual fauna populations; sizes range from 20 um to 1 cm – a property directly related to metabolic rates. This information, together with predicted functional roles, will later be used to parameterize and optimize food-web models.

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 arthropods and nematodes in bioenergy cropping systems.

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