Faunal Population Dynamics Throughout Switchgrass Developmental Stages

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Project Goals: The project, *Establishment to senescence: plant-microbe and microbe-microbe interactions mediate switchgrass sustainability*, aims to understand the bases of switchgrass productivity and the biotic and abiotic factors that mediate its establishment in marginal soils. With this research, we seek to define the multitrophic interactions that occur in the rhizosphere of superior switchgrass genotypes adapted to resource limitations and to elucidate how these interactions facilitate plant establishment.

Switchgrass (SG; *Panicum virgatum* L.), is one of the most promising bioenergy crops in the U.S., with the potential to provide high yield biomass while also improving the physical and hydraulic properties of marginal soils unsuitable for traditional crops. A persistent concern for bioenergy cultivation of SG, with low-input management, is improving seedling establishment and resistance to biotic and abiotic stress, a goal that may be reached by enabling beneficial interactions between the plant and the trophic compartments of biological activity in soil. This project is applying a holistic approach to define and understand the trophic interactions that occur during plant establishment and plant developmental stages, by considering that populations of nematodes, protists, and arthropods are critical elements of biological activity that together with free-living and plant-associated microbes define soil and plant productivity.

Our goal is to build 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 preference amongst other parameters. As a first step for the study of the multitrophic interactions that mediate SG establishment and productivity, we are analyzing faunal dynamics during SG developmental stages using two primary approaches **1. Funnelomics:** where faunal populations are screened using traditional methods. With this approach populations of arthropods and nematodes are physically isolated from soil samples using Berlese funnels, and wet sieving with sucrose gradient centrifugation respectively. Arthropods are individually imaged, measured, and their DNA extracted for identification. Nematodes are subsampled and pooled into populations for DNA extraction, sequencing, and identification. **2. Molecular Fauna:** where bulk molecular approaches are used to obtain a general inventory of faunal populations to begin to establish hypothetical nodes in these trophic networks. Here, ten grams of soil are used for DNA extraction using in-house protocols that target arthropods, nematodes, and protists, and the extracted DNA is analyzed using metabarcoding (universal metazoan primers), and for the

quantification of different faunal populations using quantitative PCR (qPCR) with primers designed to be group-specific based on the results of "Funnelomics".

Soil samples were collected from SG planted and fallow plots over 1, 2, 6 and 7 months post-seedling planting. During each sampling point four plants (SG treatment) and four fallow locations, were randomly selected and soil was collected to a depth of 20 cm within a 10-squared cm area. Fifty grams of soil were stored at -80°C for subsequent DNA extraction, 300 g of soil used for arthropod extraction, and 50 g for nematode isolation. A total of 250 individual arthropods have been individually imaged/measured and a collection of 36 pools of nematodes obtained. Protocols for the extraction of nucleic acids from large soil samples have been optimized and the DNA extracted.

A critical step for the identification of metazoan nodes in a trophic network is to identify appropriate PCR primers that will target the largest number of metazoan groups. We have tested different combinations of markers and primer combinations for cytochrome oxidase I, and 18S rRNA which are the markers most commonly used for metazoan identification. These primers were tested against different arthropod groups to identify primers that provide the best coverage and that will be used as 'universal primers' for subsequent analyses.

Screening of the isolated arthropod specimens has identified nine species of mites belonging to the order Mesostigmata (free-living predatory mites); eight species of mites from the order Oribatida (plant and fungal feeders); five different species of springtails from the families Onychiuridae and Isotomidae (fungal and root-feeders). Different specimens identified as members of family Agrypninae (including leaf-feeding beetles that feed on roots during their larval stages), together with arthropods from the infraclass Paraneoptera (Halothrips sp., Caliothrips sp., and Liposcelis sp.), some of which are known plant pathogens that feed and breed in the flowers of grasses and agricultural crops. Analyses of nematode populations and molecular fauna experiments are in progress.

Results to date have identified important arthropods groups whose presence relates to different developmental stages of SG. The emergence of these faunal groups may have consequences for plant productivity, rhizosphere microbial communities and the dynamics of other faunal nodes in the soil trophic network. The approaches developed here will provide the foundation for molecular approaches to quantitative study of soil trophic networks, also with potential applications as diagnostic tools to identify and intervene for the early control of plant pathogenic arthropods and nematodes in agricultural systems.

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