

An Overview of the Switchgrass Associated Microbes Belowground with Mutualistic Fungi and Soil Mesofauna as Potential Models for Sustainable Cultivation

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Project Goals: Our project, *Establishment to senescence: plant-microbe and microbe-microbe interactions mediate switchgrass sustainability*, aims to understand the bases of switchgrass productivity and potential biotic and abiotic effects in marginal soils by dissecting the key molecular mechanisms that differentiate soil organisms associated with superior switchgrass genotypes adapted to a range of resource limitations. Through our research we seek to provide a mechanistic understanding of the network of interactions that exists within the switchgrass root system, with the overall goal of understanding and synthesizing concepts that underlie soil food webs and their effects on ecosystem sustainability.

Successful cultivation of the biofuel crop switchgrass (*Panicum virgatum*) to produce high-yield biomass in marginal soils had been identified as an important goal to meet the US Department of Energy's goal to replace 30% of petroleum-based transportation fuels with biofuel by 2030. An important goal in this area is to identify ways to alleviate biotic and abiotic stresses while enhancing survival and growth during seedling establishment in marginal soils. We suggest that promoting a beneficial holobiome (consisting of bacteria, archaea, protists, fungi, nematodes and arthropods) is a logical and potentially sustainable way to support successful switchgrass establishment and sustainable cultivation. The network of interactions within this holobiome may provide key ecosystem services ranging from C sequestration and increased soil fertility. Yet, our understanding of the occurrence and regulation of mutualistic switchgrass-microbe interactions, carbon (C) and nutrient exchange between roots, soil food webs, rhizosphere C, nitrogen, phosphorus, and water cycles remains rudimentary. Over the next five years of this project, we aim to identify the holobiont of the switchgrass root system, and use and combination of 'omics and isotope techniques to understand the network interactions and nutrient dynamics.

We present an initial study of the switchgrass root system through characterization of the community of associated bacteria, archaea, and fungi from rhizosphere, rhizoplane, and bulk soil from 2 established field sites in Oklahoma. The soils from these sites represent nutrient limited areas in which future field experiments will be conducted. The results of the microbial community structure, including major microbial components, and soil characteristics from these sites will be discussed. The results of this study will inform our targets for designing a set of primers that will be used to amplify the holobiome of the switchgrass root-system, which will then be compared with deep sequencing of soil metagenomes.

We also present an overview of a set of experiments aimed to dissect plant-microbe and animal-microbe interactions in the context of nutrient solubilization and allocation. To study the interactions between switchgrass and microbes, we will collect rhizospheric soil as well as root

tissues in which to isolate bacterial and fungal endophytes. We have established high-throughput screens for many potential useful microbial traits, including N-fixation, ACC deaminase activity, and solubilization of inorganic phosphorus (CaPO₄). Endophytic bacterial or fungal strains found to contain one or more will be candidates for stable isotope probing (SIP) tracer studies and simplified community modeling and analysis.

We will also test the nutritional exchange between two types of mycorrhizae that are found on the roots of native switchgrass plants, the sebacinoid (SM) and arbuscular (AM) mycorrhizal fungi. SIP will be used to quantify the movement of phosphorus and nitrogen from the bulk soil through these two beneficial fungi, and into establishing switchgrass plants. While AM are known to extract a steep carbon cost to the host plant, we predict that sebacinoid fungi, themselves competent saprobes and scavengers, will require significantly less photosynthetically derived carbon. Thus, we will in parallel track the movement of labeled carbon from the plant into the symbiotic fungi.

To study the interactions between animals and microbes in the context of the switchgrass root system, we have established the protocols for the study of the soil mesofauna and their associated gut microbial communities. These approaches include the separation of metazoan from soil samples using Burslem and Baermann funnels, followed by grouping into morphospecies. Individual specimens are used for high throughput DNA extraction and for the amplification and sequencing of eukaryotic and prokaryotic biomarkers (16S, 18S rRNA, and Cytochrome Oxidase I genes). These approaches are being combined with large-volume soil DNA extractions to further assess the composition and distribution of soil mesofauna under different experimental conditions.

We will combine analytical chemistry and spectroscopic (NMR, μ XRF) methods to characterize and quantify the key phosphorus species from gut microbes. Similar to the endophytic microbe study described above, gut microbial isolates will be assessed for P solubilization activity by organic acid production, enzyme secretion and siderophores production on microbial growth media based on switchgrass root exudates and insoluble P (iron-, aluminum-phosphate, phytic acid) that we have developed. Effective P solubilizers will be sequenced and identified.

We will develop a novel framework for constructing directional networks for discerning the network interactions among different groups of microbes. As a proof of principle, we applied this new framework to *Saccharomyces cerevisiae* gene expression data, taking advantage of the availability of the information on the directions of gene interactions. Our results showed that among directed links predicted by this approach, 88.2% of the links are in the same direction to known genes in SGD (Saccharomyces Genome Database). These results demonstrate that the new method is capable of revealing biologically meaningful causal interactions in yeast. We predict that this method will be useful in discerning the causal network interactions in microbial communities when applied to metagenomes.

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