

## **Dissecting the Chemistry of Switchgrass-Microbe Interactions Using Cultivation, Exometabolomics and Mass Spectrometry Imaging**

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**Project Goals: Our project works towards a fundamental understanding of the molecular mechanisms driving beneficial plant-microbial interactions in superior switchgrass genotypes adapted to a range of resource limitations. Plant-microbe interactions are examined during establishment to gain insight into how symbiotic and associative microbes improve plant performance and carbon stabilization in marginal soils. We will combine focused (single plant-microbe pairing) and 'community' systems biology approaches to examine the complex interplay among plants, microbes, and their physio-chemical environment.**

Plant-soil-microbial interactions regulate the availability of nutrients and carbon stabilization in soil. Plants exude a diverse range of compounds into the soil surrounding their roots; these exudates are thought to attract and support microorganisms that may improve plant nutrient acquisition, drought tolerance, and resistance to pathogens. Here we used mass spectrometry-based metabolomics to identify key chemical mechanisms that drive bidirectional plant-microbe interactions in soil. These include nutrient uptake, metabolite exchange, and their patterns of regulation that influence plant productivity, adaptability to environmental change and the stability of carbon in soil.

Taking a multi-scale approach including field, greenhouse and highly controlled lab experiments our goal is to identify the chemical mechanisms that underlie plant-soil-microbial relationships. We are comparing switchgrass growth and exudation properties across these scales; creating a library of rhizosphere microbial isolates and combining these with plants in controlled systems to dissect their interactions and to link biology of specific microorganisms to the physiology and biochemistry of switchgrass.

**Field, switchgrass exudation and soil metabolites.** To identify how exudation of switchgrass changes during plant development in soil, we have collected samples from the rhizosphere of switchgrass and bulk soil during the first six months of switchgrass establishment (June-November) in two different field locations in Oklahoma. Currently these metabolites are being analyzed using mass spectrometry based metabolomics. Metabolite-microbe-mineral interactions are essential for processes involved in carbon stabilization in soil. To gain insights into these processes, we are examining soil metabolites across various soil depths and will use these samples to assess how the profile changes after several years of switchgrass cultivation.

Acquired data will be used to inform our lab experiments in highly controlled systems and to link back the results from the lab to the environmental conditions in the field.

**Switchgrass rhizosphere isolates.** We have now isolated more than 1000 heterotrophic bacteria from the rhizosphere of switchgrass and bulk soil using five media with different nutrient content to cover a variety of nutritional niches that may exist in the root zone. These isolates have been isolated in pure culture and preserved in glycerol stocks. Currently we are identifying representatives from this collection, which will be genome sequenced and used for further experiments.

**Switchgrass exudation, hydroponics.** Root exudation plays a major role in how plants alter the soil environment and define its rhizosphere microbiome. To complement our field analyses, we are analyzing switchgrass exudates during early plant development using a hydroponic approach.

**Switchgrass mesocosm studies.** We are using laboratory ecosystems to visualize bacteria and root growth and also enable collection and measurement of metabolites. Currently we are testing switchgrass growth conditions, exudation and response to the microbial inoculation using this platform. In future we plan to use the same laboratory systems to examine specific microorganisms isolated from the rhizosphere of switchgrass.

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