

## **Exometabolomics of Switchgrass-Microbe Interactions in natural and fabricated ecosystems**

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**Project Goals: Our project studies molecular mechanisms driving beneficial plant-microbial interactions in superior switchgrass genotypes growing under a range of resource limitations. The genomics and chemistry of plant-microbe interactions have been examined during switchgrass establishment to gain insight into how symbiotic and associative microbes improve plant performance and soil carbon persistence in marginal soils. We are integrating focused (single plant-microbe pairing) and 'community' systems biology approaches to examine the complex interplay among plants, microbes, and their physio-chemical environment.**

Plants exude a large amount of photosynthesis-derived carbon (C) into rhizosphere soil. These exuded, small, organic molecules (metabolites) shape rhizosphere community composition and potentially attract beneficial microbes that improve plant nutrition, reproduction, drought tolerance, and other stress responses. Additionally, these interactions partially define the future fate of root C, specifically whether it is respired to the atmosphere or stabilized in soil. In this study, we combined mass spectrometry-based metabolomics with genomic analyses of rhizosphere isolates to uncover key mechanisms of switchgrass-microbe interactions in soil. Using this integrated approach, we are studying the release of exudates by switchgrass under different nutrient limitations and metabolite exchange between switchgrass and rhizosphere microorganisms. These data will provide a detailed understanding of the substrate preferences of the rhizosphere microbiome as it is linked to dynamic root exudation and ultimately how these interactions impact C persistence in soil.

**Metabolite profiles of the switchgrass rhizosphere in field soil.** Switchgrass roots deposit large quantities of metabolites into soil and these metabolites likely shape the rhizosphere community. We have developed protocols for metabolite extraction and we have identified soil metabolite profiles in two field experiments in Oklahoma in which switchgrass was cultivated in marginal soils. Metabolite profiles from the two soils differed in composition. Switchgrass rhizosphere soils from the Red River field had proportionally higher amounts of nitrogen containing compounds, such as amino acids and nucleotides; while soils from the Third Street field had a higher content of carboxylic organic acids. Currently we are analyzing metabolite profiles at the main stages of switchgrass establishment and at the different root depths. To identify how metabolites released by roots shape the rhizosphere communities, we will link metabolite profiles to the taxa identified in the soils from the field.

**Synthetic rhizosphere community of switchgrass.** To dissect how different microbial taxa interact with switchgrass and potentially improve plant biomass yield, we isolated and identified 300 heterotrophic bacteria from the switchgrass rhizosphere. We determined how abundance of each isolate changes during plant development by mapping 16S rRNA of isolates to OTU-representative sequences in the field where switchgrass was cultivated. We selected 40 representative isolates for further genome sequencing and for designing synthetic rhizosphere communities to be used to test potential mechanisms defining switchgrass-microbe interactions.

**Switchgrass exudation during development and under nutrient stress.** We hypothesize that switchgrass exudes specific molecules to select microbial taxa to mitigate stress and improve its nutrition. We identified signature molecules released by switchgrass under nitrogen and phosphorus limitation. We are currently identifying microbial taxa that metabolize these molecules.

**Fabricated laboratory switchgrass ecosystems.** To dissect plant-soil-microbe interactions in the lab we are using 3D printing technologies to create ‘controlled laboratory ecosystems’ (EcoFABs) developed in the Northern Lab. Under simplified, controlled, sterile conditions we combine switchgrass with isolated bacteria to collect and identify metabolite profiles under different environmental stresses and to visualize bacteria and root architecture.

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