Connecting switchgrass-microbe-soil interfaces for sustainable bioenergy crop production on marginal soils: stable-isotope labeling, genomics and exometabolomics

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Project Goals: Our project studies molecular mechanisms driving beneficial plant–microbial interactions in switchgrass cv. Alamo (*Panicum virgatum*) growing in marginal soils under a range of resource limitations. Genomics and chemistry of plant-microbe interactions are 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.

Switchgrass (SG) is a perennial grass and one of the most promising bioenergy crops in the United States. SG is well-adapted to resource-limited environments, such as low-nutrient or droughty soils. We hypothesize that SG releases exudates to recruit beneficial rhizosphere microbial communities. These microbial communities are associated with improved plant performance during environmental stresses and increased C stocks in marginal soils. However, the molecular mechanisms that underlie SG-microbe relationships in the rhizosphere remain poorly understood. In this study, we analyzed complex exudate chemistry of SG grown under nutrient stresses, and link SG exudates to the abundance, activity and substrate preferences of bacteria found in the SG rhizosphere.

We used field, greenhouse and controlled reductionist approaches to dissect SG-microbe interactions. We performed a greenhouse study to investigate plant-associated microbes connected to the improved plant performance and C and N transformations in marginal soils, and used $^{13}CO_2$ stable-isotope labeling, genomics, and exometabolomics to identify the nature and dynamics of C in the SG rhizosphere. For this study, we reconstituted three nutrient-deplete soil horizons with different physio-chemical properties in one-meter deep soil columns in greenhouse mesocosms. We grew SG clonal plants in these reconstructed soil horizons and subjected these mesocosms to N and/or P amendments and two watering regimes. We collected rhizosphere and bulk soil from $^{12}CO_2$- and $^{13}CO_2$-labeled SG and extracted DNA and exometabolites.

Analysis of switchgrass exudate chemistry. We used liquid chromatography mass spectrometry-based exometabolomics (LC-MS) to analyze small organic molecules (metabolites) released by SG grown in soil mesocosms and hydroponic systems. We identified specific molecules exuded by SG during phosphorus (P), nitrogen (N) stresses and water limited conditions. We found that aromatics such as shikimic acids, salicylic acids, were more abundant in the exudates of N-stressed SG plants. In contrast, N-containing compounds such as amino acids and nucleosides were depleted in this treatment. During P stress, SG increased production

of carboxylic organic acids (e.g. succinic, malic acids), whereas osmolytes were more abundant in water-limited treatments.

Linking plant chemistry to microbial community composition. To connect rhizosphere metabolites and SG rhizosphere communities, we also identified dynamics of different microbial groups under N, P, N/P and reduced water treatments in the mesocosms. We found that N addition decreased the relative abundances of Actinobacteria and Bacteroidetes. Phosphorus treatment had lower relative abundances of Alphaproteobacteria and Verrucomicrobia. Planctomycetes, Chloroflexi and TM6 significantly decreased when any type of nutrients were added. A 50% reduction in water resulted in decreased relative abundances of Enterobacteriales and Planctomycetales.

Using a reductionist approach, we sought to disentangle the complexity of interactions between exudates and SG rhizosphere microbial communities, and to identify specific linkages between exudates and microbial substrate preferences. First, we isolated and characterized 300 bacteria from the SG rhizosphere. We then analyzed the abundances of these isolates during SG development in soil and identified bacteria associated with SG development. Finally, we used exometabolomics to connect stress molecules released by SG under N and P stresses to the metabolite uptake preferences of the rhizosphere isolates and related plant growth phenotypes. Our results indicate that during N and P nutrient stress SG mediates the release of stress-specific exudates to regulate the abundance of specific microbial taxa in the rhizosphere.

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