Plant-microbe and microbe-microbe interactions mediate switchgrass sustainability: following rhizosphere microbial communities during switchgrass establishment

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Project Goals: Switchgrass (SG; *Panicum virgatum* L.) is a perennial C_4 grass native to the tallgrass prairies and a promising feedstock in the U.S. for bioenergy production. Capable of abundant biomass yield with minimal fertilizer or water, SG can survive on marginal soils, and even thrive once established. Thus, SG is a model for low-input agriculture as well. A major barrier to large-scale cultivation of SG under low-input management is seedling establishment, somewhat of an Achilles heel for all or most perennial plants. Further, little is known regarding how SG responds to abiotic and biotic stressors, nor what beneficial relationships the plant may forge to deal with these types of stresses. We hypothesize that successful establishment and sustainable cultivation of SG in marginal soils is in part enabled by beneficial plant-microbial interactions. We are investigating the succession of rhizosphere microbial communities associated with high- and low-performing SG plants grown in nutrient-limited soils at two Oklahoma fields. The outcome of this research will provide a better genomic basis for SG cultivation in marginal soils, expand our knowledge of the interactions between soil microbiomes, plants and ecosystems, and ultimately guide efforts for translation into agronomic row crops.

In the soils surrounding roots (rhizosphere), biotic, chemical and physical drivers enrich for specific bacterial and fungal communities. These organisms can play multiple roles, and some may benefit plant productivity through assisting in nutrient acquisition, water uptake and/or pathogen suppression. We are investigating the composition, succession and function of rhizosphere microbial communities during SG cultivation in an effort to better understand the plant-microbiome interactions that enable plant survival and adaptation under stressed conditions, such as drought and nutrient deficiency.

To study plant-soil microbiome characteristics of SG growing in 'marginal' nutrient or water-limited soils, we selected two research farms, both remnants of the Dust Bowl Era in Oklahoma. Red River Farm lies near the border of Oklahoma and Texas, and has sandy loam soil that is low in NO_3-N and organic matter. The other one, Third Street Farm, has a silt loam soil and relatively low phosphorus availability. Five hundred genetically distinct Alamo seedlings were planted into each field in May-June of 2016. Other than hand weeding during the summer, no management, water, or nutrients were supplied to the fields. Thirty plants were randomly selected at the onset for monitoring of rhizosphere community succession, plant performance and soil physio-chemical characteristics including: gravimetric moisture, pH, and NO_3/NH_4. Rhizosphere and bulk soil of these plants were sampled over the first growing season (Year 1) at 5 time points (T1-T5): early and late vegetative growth, reproductive growth, maximal growth, and senescence. Although plant biomass exhibited large variation within plots due to plant genetic

diversity, we believe the selected thirty plants are representative of the whole plots in terms of biomass production. We are investigating microbial community profiles by amplicon sequencing of marker genes specific to bacteria, fungi and other soil eukaryotes. To date, we have finished 16S amplicon sequencing for T2, T3 and T4 samples and here present some of the preliminary data. Proteobacteria, Actinobacteria, Acidobacteria and Firmicutes are dominant in all the soil samples, accounting for ~75-80% of the microbial communities. Third Street (clay soil) has more Verrucomicrobia and Firmicutes present when compared to Red River (sandy soil). Proteobacteria is significantly more abundant in the rhizosphere soil (directly around the root surface) relative to bulk soil (no close proximity to SG plants) in both sites. Proteobacteria abundance appeared to decrease when plants transitioned from active growth to senescence. Overall, microbial communities of Red River soil exhibits higher alpha-diversity than those found at Third Street. As expected, rhizosphere microbial communities are less diversified than the corresponding bulk soil at both sites, indicating a selective effect of root exudation. Significant differences in soil microbial communities were observed between both sites as well as between different habitats (rhizosphere vs. bulk soil). However, site effect appears to be greater than the rhizosphere effect. Intriguingly, Third Street soil differences between rhizosphere and bulk soil are less pronounced than those found at Red River. Community shifts in rhizosphere soils were observed between most time points at both sites, suggesting there is indeed a succession pattern of rhizosphere microbial communities over growth season.

In order to dissect the changes of rhizosphere communities *between* growth seasons, we performed our rhizosphere community survey of these thirty plants in Year 2 (year of 2017). To enable this additional research, an "in-growth core strategy" was adopted, and used to collect roots and the rhizosphere soil produced in Year 2. To maintain consistency with Year 1, soil samples were collected at three time points: vegetative growth, reproductive growth, and senescence. In addition, 40 plant genotypes from the plots were selected based on plant performance, to establish replicated field experiments. This collection includes 12 high- and eight low-performing genotypes originating from each of Red River and Third Street plots. The new plots of clonal plants have been planted and established at both sites adjacent to the Year 1 plots in May 2017. These clonal plots will allow us to investigate the relationship between host plant genetic diversity and their associated rhizosphere communities. Rhizosphere and bulk soil of plant clones of 18 genotypes (12 high-performing and 6 low-performing genotypes) were sampled in Sep. 2017. DNA extraction and marker gene sequencing of these soil samples is ongoing.