

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

Yuan Wang¹, Javier Ceja Navarro², Erin Nuccio^{3*}, Renmao Tian⁴, Travis Simmons¹, Katerina Estera-Molina⁵, Christina Fossum⁵, Jialiang Kuang⁴, Colin Bates⁴, Lauren Hale⁴, Na Ding¹, Josh Barbour¹, Nameer Baker⁵, Abelardo Arellano², Kateryna Zhalnina⁶, Eoin Brodie², Trent Northen⁶, Wolf Scheible¹, Michael Udvardi¹, Jennifer Pett-Ridge³, Malay Saha¹, Liyou Wu⁴, Jizhong Zhou⁴, **Mary Firestone**⁵ and Kelly Craven¹ (kdcraven@noble.org)

¹Noble Research Institute, Ardmore, Oklahoma; ²Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, California; ³Lawrence Livermore National Laboratory, Livermore, California; ⁴University of Oklahoma, Norman, Oklahoma; ⁵University of California, Berkeley, California; ⁶Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California.

Project Goals: Switchgrass (SG; *Panicum virgatum* L.) is a perennial C₄ grass native to the tallgrass prairies and a most 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. 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, and ecosystem-scale effects of high- and low-performing SG plants grown in nutrient-limited soils in southern Oklahoma. 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 enhanced 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 in marginal soils.

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 a silt loam soil low in NO₃-N and organic matter. Third Street Farm, has a clay 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 for monitoring of rhizosphere community succession, plant performance and soil physio-chemical characteristics including: gravimetric moisture, pH, and NO₃/NH₄. For each plant, rhizosphere and bulk soil 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. Due to plant genetic diversity, plant biomass was highly variable within the plots, and the selected thirty plants are representative of this diversity. This provided the basis for assessing correlations between plant biomass production and plant associated microbial communities—assessed by amplicon sequencing of marker genes specific to bacteria (16S), fungi (ITS), and soil protozoa (18S).

Overall, microbial and protozoan communities of the silt loam soils (Red River) exhibited higher alpha-diversity (species richness) relative to clay loam soils (Third Street). In contrast, clay loam soils (Third Street) had more variation in microbial community composition within plots, consistent with greater variability observed in soil physical characteristics. In both sites, beta-proteobacteria were significantly enriched in the rhizosphere soil relative to bulk soil. Both microbial and protozoan communities were less diverse in the rhizosphere soil than in corresponding bulk soil, indicating a selective plant effect at multiple trophic levels. Site, habitat type (rhizosphere vs. bulk) and plant developmental stage had significant effects on soil microbial and protozoan community composition. The site effect was greater than the rhizosphere effect, followed by effects of plant developmental stage. Soil nutrient availability (P, K, pH) was the most significant environmental driver for microbial community assembly, and its influence increased in magnitude over the growing season particularly in rhizosphere communities. The rhizosphere communities from different trophic levels dynamically changed over the growing season, where the early successional phase (first 3 months after planting) was most distinct from bulk soil. A few microbial OTUs were found to be significantly correlated with plant biomass production at the early phase. Although plant biomass was correlated with both rhizosphere and bulk communities, plant height was more significantly correlated with rhizosphere communities. In conclusion, switchgrass rhizosphere communities are highly correlated with plant biomass production, and early establishment phase (1-3 months) may be an important time frame for the microbial stimulation of plant biomass production.

This research is based upon work supported by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under Award Number DE-SC0014079 to UC Berkeley, Noble Research Institute, the University of Oklahoma, Lawrence Livermore National Laboratory and Lawrence Berkeley National Laboratory. Part of this work was performed at Lawrence Berkeley National Lab under contract DE-AC02-05CH11231 and at Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344.