Experimental Approaches to Understand Rhizosphere Processes for Improved Bioenergy Crop Productivity

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Project Goals: This project couples novel lab and field studies to develop the first predictive model of grass-microbiomes based on new mechanistic insights into dynamic plant-microbe interactions in the grasses *Sorghum bicolor* and *Brachypodium distachyon* that improve plant nitrogen use efficiency (NUE). The results will be used to predict plant mutants and microbial amendments which improve low-input biomass production for validation in lab and field studies. To achieve this goal, we will determine the mechanistic basis of dynamic exudate exchange in the grass rhizosphere, with a specific focus on the identification of plant transporters and proteins that regulate root exudate composition and how specific exudates select for beneficial microbes that increase plant biomass and NUE. Further, we will develop a predictive plant-microbe model for advancing sustainable bioenergy crops and will predictively shift plant-microbe interactions to enhance plant biomass production and N acquisition of N from varied forms.

Up to 25% of all photosynthetically fixed carbon is released into the rhizosphere through root exudates. Exudates, consisting of >100 distinct metabolites, provide a critical source of growth substrates for the root microbiota, consisting of diverse microorganisms and fauna actively recruited by plants from bulk soil. Yet, we lack the foundational understanding of plant exudate biology including if and how exudation is controlled by active transporters and regulatory mechanisms. Unraveling the nature of plant and microbial drivers behind these dynamic interactions is crucial for understanding the dynamics of N cycling and uptake in the rhizosphere, and will guide the development of predictive models to inform the design of sustainable bioenergy systems.

Plants use multiple forms of N from soil including nitrate (NO_3^-), ammonium (NH_4^+), and various forms of organic N. To investigate how inorganic N affects plant root exudation, we grew *B. distachyon* in sterile fabricated ecosystem devices (EcoFABs) supplied with NO_3^-, NH_4^+, or N-depleted media and then characterized the resulting plant phenotypes, Additionally, we applied LC-MS/MS-based metabolomics to analyze root exudates. Our untargeted metabolomics results demonstrate strong modulation of root exudation by N supply. N source significantly changed root and shoot biomass and N uptake kinetics. To examine the circadian rhythms of root exudation, we quantified the diurnal root exudation patterns of *B. distachyon* cultivated in EcoFABs supplied with inorganic N. This analysis revealed more sugars and amino acids, such as hexose, isoleucine, and glutamic acids, are exudated during the daytime. Finally, to elucidate how plant transporters regulate the root exudation, we analyzed metabolites secreted by *B. distachyon* ABC and N transporter mutants grown in EcoFABs. Together these studies are providing new foundational insights into the regulation of plant-microbe interaction by evaluating the dynamics and transporter of plant exudation.

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0021234.