Unravelling Rhizosphere-Microbial Interactions in the Rhizosphere of Alamo Switchgrass (*Panicum virgatum*) under Abiotic Stresses

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Project Goals: Switchgrass (*Panicum virgatum* L.) is a perennial C_4 grass native to the tallgrass prairies and a promising feedstock for U.S. bioenergy production. Capable of abundant biomass yield with minimal fertilizer or water, switchgrass can survive on marginal soils, and even thrive once established. We hypothesize that successful establishment and sustainable cultivation of switchgrass 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 switchgrass plants grown in nutrient-limited soils in southern Oklahoma. The outcome of this research will provide a better genomic basis for switchgrass 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 rhizosphere, root exudation is a key process for C transfer into the soil, influencing the role of soil microbial communities in organic matter decomposition and in nutrient cycling. Root exudates increase the number and activity of soil microbes and fauna found in the rhizosphere through a myriad of complex interactions. Soil microorganisms depend upon plant C and, in turn, provide plants with nitrogen (N), phosphorus (P) and other mineral nutrients in part through decomposition of soil organic matter. We grew Alamo switchgrass (SG) in two types of greenhouse experiments to investigate how SG growth development and exudates shape rhizosphere microbial community composition, nutrient fractions (i.e. P species) and soil characteristics, as well as how these interactions are affected by abiotic stresses. In the first experiment, we tested the hypothesis that SG rhizosphere microbes enhance its access to organic P (Po) pools by stimulating root growth and/or liberating and solubilizing P from Po. In a second experiment, we assessed the response of soil extracellular polymeric substances (EPS) stocks to SG cultivation under varying N, P, and water stress, testing the hypothesis that soil microbes produce EPS (particularly polysaccharides) in response to nutrient and moisture limitation.

We collected marginal soils for the first experiment from three locations in Oklahoma (Ardmore 3rd Street, Red River, Anadarko). We amended soils with 50 mg/kg inorganic-P (KH_2PO_4, phosphate) or organic-P (phytate), and used non-amended soils as a control. Phosphate significantly enhanced plant biomass, total root length and surface area in all three soils after 8-weeks growth. Interestingly, phytate was an equally good source of P for plant growth promotion in 3rd Street soil. At all sites, phosphate increased resin-P in bulk soils, but substantially decreased resin-P in rhizosphere soils from 3rd Street and Anadarko soils, consistent with substantial uptake by plants. Phytate addition increased resin-P in the rhizosphere relative to bulk soil and decreased NaOH-P_o species in the rhizosphere relative to bulk soil in 3rd Street soil, consistent with mobilization by the microbiome and plant uptake of P from phytate in this soil.

SG accumulated more Pi at Red River in both shoots and roots than soils at other sites; phytate addition decreased Pi and increased Na^+ in shoots and roots at 3rd street. We also analyzed the bacterial and fungal microbiome from the bulk, rhizosphere and root associated compartment (endosphere + rhizoplane), respectively. Rhizosphere and bulk soils had a similar number of OTUs (α-diversity), and significantly more than were observed in the root associated compartment for both bacteria and fungi. Bacterial and fungal communities differed at the genus level across compartment and soil types. Soil type (rhizosphere or bulk), compartment and P treatment strongly influenced bacterial and fungal community composition. HCl-P and total P strongly influenced bacterial community composition at Red River. Organic P pools ($NaHCO_3$-Po and NaOH-Po) markedly impacted bacterial community composition in soils with added phytate. Interestingly, Tichomeriaceae were positively correlated with NaOH-Po in both rhizosphere and root-associated soils.

For a second greenhouse experiment, we collected marginal soils from Anadarko, OK. We grew ramets of a single switchgrass genotype for 18 weeks in three reconstituted field soil horizons, subjecting them to five treatments: added N, P, N plus P, 50% water, and controls. We then labeled plants either with $^{12}CO_2$ or $^{13}CO_2$ for 12 days before destructively harvesting plants and soil horizons. To determine how abiotic stresses altered the size and nature of EPS stocks and soil characteristics, we assessed root biomass, soil chemistry, EPS content, the monosaccharide composition of EPS, and the amount of water-stable aggregates.

Soils with both added N and P had the highest EPS content, root biomass, and percentage of water-stable soil aggregates. Multiple linear regression analysis showed root biomass was the most important determinant for soil EPS production, potentially by controlling carbon supply and diurnal changes in soil water stress. Root biomass and soil water potential were also correlated with water-stable aggregates, indicating that EPS concentration and soil aggregation have similar drivers in this soil. High mannose content confirmed the microbial origin of EPS. $^{13}CO_2$ labeling indicated that 0.18% of newly fixed plant carbon was incorporated into EPS. Analysis of field soils obtained via deep coring indicates that EPS concentrations are significantly enhanced under long-term switchgrass cultivation, suggesting a mechanism by which deep-rooted perennial grass cultivation may positively affect soil aggregation in soils with low organic material.

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