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

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Project Goals: Our project works towards a fundamental understanding of the key molecular mechanisms driving beneficial plant-microbial interactions in superior switchgrass genotypes adapted to a range of resource limitations. Plant-microbe interactions are examined during establishment to gain insight into how symbiotic and associative microbes improve plant performance and carbon stabilization in marginal soils. We will combine focused (single plant-microbe pairing) and 'community' systems biology approaches to examine the complex interplay among plants, microbes, and their physio-chemical environment.

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 have been shown to 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, potentially 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 transcriptomes and exudates shape rhizosphere microbial community metagenomes and soil characteristics, as well as how these interactions are affected by abiotic stresses. The first set of experiments explore the gene-to-metabolite networks responsible for coping with N or P starvation by investigating the Alamo SG transcriptome, metabolome and physiology under a range of N and P supply conditions. The second experimental approach applied $^{13}CO_2$ stable-isotope labeling to trace Alamo SG photosynthate through root exudates and debris into the metagenomes of the microbial communities that consume them in 1-m soil profiles recreated in the greenhouse.

Sand-based: Alamo seedlings SG were grown in sand culture over a 4-week period under various N or P stresses with nutrient media containing KNO_3 (0.2-6.0 mM) and KH_2PO_4 (20-600 µM). Treatment with 6mM·N and 600 µM·P supply was served as control. Compared to control plants, moderately- (200 µM P_i) and severely-reduced (20 µM P_i) P-supply resulted in a decrease of biomass by 35% and 96%, respectively. Plants grown under severe P-limitation had comparable primary root length, but more and longer root hairs than P-replete plants. P-limitation induced the expression of genes involved in regulation of transcription, phospholipid degradation and phosphate transport in shoots and roots, phosphorylation in shoots, tryptophan and glycolipid biosynthetic processes in roots. Conversely, genes involved in phospholipid synthesis and secondary metabolism in shoots and roots, and photosynthesis, chlorophyll biosynthesis, starch and cell wall degradation in shoots were repressed. Switchgrass invested more organic acids (oxalate) and sugars (sucrose and trehalose) in roots relative to shoots in response to P-limitation. Shoots and roots showed distinct P-stress acclimation patterns at the transcriptional and metabolic levels, which reflect, in part, changing priorities for C-allocation to root growth and P-acquisition at the expense of C- and P-investment in shoot growth.

As expected, N levels strongly impacts plant biomass, root to shoot ratio and length of primary seminal roots. N starvation reduced total N and protein compared to control with replete N supply. Providing 2.0 mM N to switchgrass satisfied with the N requirement of plant growth, but reduced total N, total protein and modified metabolites profiles in relative to control. Metabolites composition was altered depending on plant organs and N availability. Some organic acids were elevated in N-stressed roots compared to control, such as malic acid, phosphoric acid, glycolic acid, hydracrylic acid, octanoic acid as well as quininic acid. Sugar metabolism was also influenced by N availability, with the increase of sucrose accumulation and slight decrease in glucose upon N starvation. The number of induced or repressed genes had negative relationship with the degree of nutrient limitation. Some high and low affinity transporters were highly induced during N starvation. In addition, genes encoding nitrate reductase (NR) and nitrite reductase (NIR) were also induced during N deficiency.

Soil-based: Alamo SG were grown in marginal soil with N and/or P amendments and under two watering regimes. We observed strong effects of root biomass in our soils, particularly in the +N/+P treatment where enhanced root biomass may have strongly affected soil water potential. Nitrogen fertilization also affected soil chemistry, as was evident in observed pH and dissolved organic carbon (DOC). Reduced pH observed in soils that received N amendments probably resulted from microbial ammonia oxidation of the coated urea fertilizer, a process that releases protons. However, enhanced [DOC] observed under nitrogen additions may be due to increased release of exudates by switchgrass roots.

We also observed significant differences between control and nutrient-amended soils in extracellular polysaccharide (EPS) production. EPS protects and binds soil microbial communities together, maintains favorable soil-water relations, and acts as an adhesive agent to increase soil aggregate stability. The highest soil EPS content and concentration of ^{13}C-enriched EPS was found in our +N/+P treatment, and was significantly correlated with observed root biomass. In addition, we did not observe significant differences between treatments in bulk soil microbial biomass as measured by phospholipid fatty acid analysis. However, the high mannose content of recovered EPS indicates that these polysaccharides are microbial in origin rather than being a direct root product. Notably, these high-DOC, high-EPS soils also contained more water-stable aggregates, indicating that enhanced root biomass and the resulting exudates and mucilage were potentially mediated through the microbial community to form a polysaccharide matrix that has enhanced a critical aspect of soil health in a marginal soil.

We observed a significant shift in density between the microbial DNA recovered from our $^{13}CO_2$-labeled mesocosms and that recovered from $^{12}CO_2$-labeled controls. This enables us to isolate microbial genomes within our recovered metagenomes that became significantly enriched in ^{13}C as a result of consuming ^{13}C-enriched photosynthate over the course of our labeling experiment. We aim to investigate whether these microbes contain traits that would facilitate the conversion of plant photosynthate into microbial EPS, as well as other traits that could be of potential mutualistic benefit to the plant providing C to the rhizosphere microbial community.

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