

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 the decomposition of organic matter 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 communities, metagenomes, and metatranscriptomes and how these interactions are affected by abiotic stresses. The first group of experiments focused on the Alamo SG transcriptome, metabolome and physiology under a range of P_i supply conditions to explore the gene-to-metabolite networks responsible for coping with P starvation. The second experimental approach used Alamo SG clones growing 1-m soil profiles recreated in the greenhouse and applying ¹³CO₂ stable-isotope labeling to trace SG photosynthate into fresh root exudates and the metagenomes and metatranscriptomes of the microbial communities that consume root exudates and debris.

Sand-based: Alamo SG seedlings were grown in sand culture over a 4-week period with nutrient media containing KH₂PO₄ concentrations ranging from 20 μM to 600 μM (control). Plants growing at 200 μM KH₂PO₄, accumulated only 64.7% of the biomass present in plants grown at 600 μM, whereas plants grown in the presence of 20 μM only had 3.8% of the control biomass. Severe P limitation (20 μM) did not inhibit primary root growth, as has been frequently reported (e.g. *Arabidopsis thaliana*) and is considered a typical root system architectural change resulting from inhibition of primary root elongation by P limitation. At the transcriptional level, SG showed expected (previously known) and novel responses to P limitation. The number of gene transcripts and the strength of their response increased with the severity of P limitation both in shoot and root tissues. RNA-Seq data were analyzed with MapMan software to identify coordinated, system-wide changes in metabolism. During P limitation, a large number of gene transcripts related to lipid degradation, glycolipid biosynthesis as well as tryptophan synthesis

were induced. For example, NPC4, encoding nonspecific phospholipase C, was markedly induced upon P limitation and is known to play an important role in the supply of phosphate from membrane phospholipids during P limitation. SG under P stress also showed large changes in the expression of genes involved in secondary metabolism and photosynthesis. It is surprising that a suit of genes related to light reaction were sensitively down-regulated in shoots, but also in roots experiencing P limitation. Analyses of metabolites confirmed that P limitation led to a shift towards the accumulation of sugars and organic acids in roots relative to shoots. Shoots and roots show distinct adaptation patterns at the molecular and metabolic levels towards P limitation, suggesting that distinct P starvation response strategies are used for different plant organs in response to a shortage of P. Lipid remodeling is known to be a dramatic metabolic response to P starvation. The abundance of phospholipid species (PC, PE, PS) was significantly decreased in shoot under 20 μ M treatment; however, this response was not observed during more moderate P limitation (60 μ M and 200 μ M). Membrane glycolipids, such as MGDG and DGDG, accumulated in roots during P limitation as compared to control.

Soil-based: Alamo SG clones were grown in mesocosms containing three horizons of Oklahoma pasture soil packed into 1m columns, to which one of five treatments (+N, +P, +N/+P, -H₂O, and control) was applied. These treatments had a significant ($p < 0.05$) effect on plant height, shoot biomass, and relative root biomass, with the greatest height and biomass observed in the +N/+P treatment and the least in the -H₂O treatment, as expected. The +N/+P treatment also had a significant ($p < 0.05$) positive effect on exopolysaccharide (EPS) abundance in top horizon bulk soil, though further analysis is required to determine if this EPS is plant or microbial in origin. After nine weeks, a subset of SG plants of each treatment were labeled with 99 atom-percent ¹³CO₂ for 6 days, and after 14 and 18 weeks, another two subsets were labeled for 12 days each. Labeled mesocosms were cored or destructively harvested to recover roots, rhizosphere soil, and bulk soil at different depths within the rooting profile. Bulk soil chemistry, density fractions, and microbial metagenomes are being used to determine how growing SG alters bulk soil characteristics with depth. Rhizosphere microbe metagenomes and metatranscriptomes, root exometabolites, and root transcriptomes extracted from these samples will be used to determine how SG-microbe interactions in the rhizosphere are delineated and how these networks respond to abiotic stress.

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