

## How Plant-Microbial Interactions Shape Resource Allocation in Perennial Bioenergy Grasses

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**Project Goals: The LLNL Bioenergy SFA seeks to support sustainable and predictable bioenergy crop production through a community systems biology understanding of microbial consortia that are closely associated with bioenergy-relevant crops. We focus on host-microbial interactions in algal ponds and perennial grasses, with the goal of understanding and predicting the system-scale consequences of these interactions for biomass productivity and robustness, the balance of resources, and the functionality of surrounding microbial communities. Our approach integrates ‘omics measurements with quantitative isotope tracing, characterization of metabolites and biophysical factors, genome-enabled metabolic modeling, and trait-based representations of complex multi-trophic biological communities, to characterize the microscale impacts of single cells on system scale processes.**

Microbe-host interactions have long shaped the biosphere and geosphere and can fluctuate between mutualistic, commensal and parasitic. In bioenergy systems, microbial symbionts may have direct effects on crop productivity, resilience, or stress tolerance of the feedstock crop. We hypothesize that foliar endophytes regulate a diverse set of genes putatively related to C use, stress, and secondary metabolites. In the first stage of our newly-funded SFA Plant Bioenergy focus area, we will determine how foliar fungal endophytes influence belowground carbon cycling and feedstock robustness during water and nutrient stress by altering aboveground foliar processes.

To understand the effects of foliar endophytes on stress tolerance in bioenergy plants, we have identified fungal endophytes in the leaves of *P. virgatum* and *P. hallii* exposed to drought (5% vs. 15% soil moisture). These isolates represent a broad spectrum of symbionts, ranging from mutualists to antagonists. The transpiration efficiency of *P. hallii* infected by these endophytes varied from 0.1x to 25x, and wilt resistance from 4 to 22 days, which can scale up to enormous effects at the ecosystem level both directly and via constraints on C available for transfer belowground. Plant physiological outcomes were largely predicted by independent fungal traits related to osmotic stress tolerance, and results were generally consistent between *P. virgatum* and *P. hallii*. Based on *P. hallii* transcriptomics for plants grown under drought with one of two mutualists or three antagonists, we found no genes stimulated by all five fungi, and antagonists

shared more genes than mutualists under drought. These preliminary results suggest there are likely multiple genetic pathways by which endophytes benefit plants and fewer pathways by which they cause harm. Identifying the genetic and ecological mechanisms that drive fungal endophyte effects on host plants will provide both a basic biological understanding of these symbioses, and could ultimately guide use of such biotic mutualisms to improve bioenergy plant production.

In our future work, we will determine if there are functional categories of belowground C allocation that are generalizable to different types of symbioses (antagonists to mutualists), and the robustness of these strategies to drought. While endophytic fungi are ubiquitous plant symbionts and play important roles in plant physiology and responses to environmental stress, we have little understanding of their belowground impacts. We hypothesize that both foliar fungal endophytes will affect belowground C allocation during drought, with expected trade-offs for plant stress tolerance vs. biomass or stress and nutrient transfer. The degree and type of impact belowground will depend on the specific fungus and the mechanism by which they affect drought stress tolerance/resilience in the plant. We will initially test our hypothesis in a simplified sterile system with our model *Panicum* grass, *P. hallii*, grown individually with each of our well-characterized 20 foliar fungal endophytes in C-free sand soils with low inorganic nutrient addition. Our goal is to predict these interspecies exchanges under deficient conditions to support sustainable and predictable bioenergy crop production.

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