

Plant-microbe interfaces: Interrogating poplar fungal microbiome interactions using meta-transcriptomics and constructed communities

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Project Goals: The goal of the PMI SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. *Populus* and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic *Populus*-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.

Poplar trees (genus *Populus*) are host to diverse root fungal microbiomes that include ectomycorrhizal, arbuscular mycorrhizal, and endophytic fungi. These fungi perform services for the plant host including growth promotion, nutrient acquisition, protection from pathogens, and conferral of abiotic stress tolerance. Meta-transcriptomics can provide large amounts of data on the function and taxonomic composition of the poplar root fungal microbiome. We developed an RNA-seq method using a synthetic spike-in standard curve that allows for the calculation of absolute abundances of fungal transcripts on poplar roots. We implemented a bioinformatics workflow that provides taxonomic and functional annotations of assembled fungal contigs from meta-transcriptomic data. These methods were applied to an ecosystem-scale, time-series field experiment to document taxonomic and functional shifts of the poplar fungal microbiome in response to a historic drought in the semi-arid American West during the summer of 2021. We identified transcripts from a previously isolated dark septate endophyte in the genus *Hyaloscypha* as a highly active root colonizer across our field sites. Dark septate endophytes are a functionally diverse group of root associates that have been described as either mutualists, commensalists, or latent pathogens. We conducted further work to understand the characteristics of the *Populus*-*Hyaloscypha* association. *In vitro* inoculations with this fungus demonstrated compatibility with both *Pinus* and *Populus*, suggesting that it engages in antagonistic interactions with arbuscular mycorrhizal fungi during plant host colonization. We were also able to establish simplified constructed communities with this fungus and three common ectomycorrhizal fungi, ranging in diversity from one to four species. These constructed communities will allow us to identify interactions between fungi during root colonization and evaluate the effects of fungal diversity on plant performance and nutrient uptake. Future work will also 1) dissect the molecular mechanisms of the antagonistic interaction with arbuscular mycorrhizal fungi, 2) evaluate the ability of this fungus to confer drought tolerance to *Populus*, and 3) identify common and unique symbiosis-induced genes when colonizing different plant hosts.

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