

209. Plant-Microbe Interfaces: Understanding the factors shaping microbiome structure and function within *Populus* species.

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Project Goals: The goal of the PMI SFA is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. *Populus* and its associated microbial community serves as the experimental system for understanding how these molecular events manifest themselves within the spatially, structurally, and temporally complex scales of natural systems. To achieve this goal, we focus on 1) characterizing host and environmental drivers for diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

Populus spp. (Poplar and Cottonwood) are broadly distributed in the temperate environments of North America. They are typical of important riparian habitats, making them ideal ecological model species. Additionally, their rapid growth rates, ability to form both ecto- and endomycorrhizae, clonal propagation, and growth on land otherwise not suitable for food production, make them good candidates for bioenergy production scenarios. Because of these reasons, *Populus* are also now emerging as a model system for understanding the role of the plant microbiome. We have been investigating microbial communities of *Populus deltoides*, *P. trichocarpa* and their TxD hybrids in natural riparian habitats as well as clonal plantation populations. Community level microbiome structure is examined with Roche-454 and Illumina- MiSeq analyses of rRNA amplicons, and we are currently developing techniques for metagenomic analysis. Microbiome data for both bacteria and fungi are analyzed against the corresponding bulk soil properties and chemistry, tree phenotypic measures, tree metabolomics data, and tree genotype data in order to understand how such properties influence microbiome structure. Most work to date has focused on root endosphere and rhizosphere communities, but we have also recently examined a variety of different plant tissue/sample type across the ecosystem that is a *Populus* tree.

In our studies of *P. deltoides* we have shown that the environments of rhizo- and endosphere compartments feature highly developed, diverse and to a large degree often exclusive communities of bacteria and fungi. Endophytic bacterial diversity is found to be highly variable, but typically contains tenfold lower diversity than the rhizosphere, suggesting root tissues provide a distinct environment supporting relatively few species more heavily dominated by Actinobacteria and γ -Proteobacteria when compared with the rhizosphere. *Populus* spp. appear to be highly enriched for *Pseudomonas fluorescens*-like species/OTUs in the endosphere when compared to rhizosphere habitats, and as compared to the endophytic habitats of surrounding (non-*Populus*) tree species. Fungal endophytic communities are more diverse than corresponding bacterial diversity, but less diverse than corresponding fungal rhizosphere

communities. Both fungal and bacterial rhizosphere samples showed distinct phylogenetic composition patterns compared to the more variable endophyte samples. Contrary to initial expectations, *Populus deltoides* has low natural levels of colonization by ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi, but high levels of presumed fungal endophytic taxa such as, *Mortierella*, *Ilyonectria* and members of the *Atractiellales*. However at the overall community level endosphere, rhizosphere, and soil communities more closely resemble others of the same type, regardless of the plant species from under which they were collected.

Overall *P. trichocarpa* communities studied in the Western US in two separate Oregon common gardens have shown that rhizosphere and soil microbiomes significantly group by the common garden site at which they were sampled: Clatskanie and Corvallis. There is also a significant separation of endophyte microbiome communities by common garden although not as strong as soil and rhizosphere. While at the overall community level there appeared to be no pattern of *Populus* genotype specific selection among clones, at the OTU level certain bacteria appear to be enriched or depleted in the endosphere of several clones across all replicates. *Pseudomonad* OTUs are highly enriched in both the rhizosphere and endophyte samples while being nearly undetectable within bulk soil. Additionally, in contrast to our prior work in *P. deltoides*, *Acinetobacter* were also prominent in many *P. trichocarpa* endosphere samples. Finally, the effects of *P. trichocarpa* genotype on the composition of its root microbiome appear to be limited compared to the effects of local soil environment at the community level, but OTUs/species may respond to genotypic/phenotypic specific cues. We are currently reanalyzing these results in the context of newly available metabolomic profiles to clarify mechanisms for such responses.

We have also been developing methods for enriching microbiomes from plant tissues for metagenomic sequencing that avoid host DNA background contamination. Ongoing research will take advantage of these metagenomic techniques as well as 1) move beyond the rooting zone to total microbiome studies of *Populus* inclusive of stem and leaf tissue, and 2) functional studies of defined phenotypic and genotypic variants of *Populus* to clarify the mechanisms and effects of microbiome interactions in natural and agroforestry environments.

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