

6. Plant-Microbe Interfaces: Understanding the factors shaping microbial community structure within root and rhizosphere microbiomes of *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 a genetically diverse genus of tree species that are broadly adapted to the temperate environments of North America and typical of important riparian habitats, making them ideal as ecological model species. Additionally, their fast growth rates and the ease with which *Populus* clones and hybrids can be grown on land otherwise not suitable for food production, make them good candidates for production bioenergy research. For these reasons, it is important to understand the role of the root microbiome as it relates to the health and productivity of *Populus*. We have been examining the root, rhizosphere and soil communities of *Populus deltoides* in natural riparian habitats in the Eastern US, as well as controlled common garden populations of *P. trichocarpa* in the Western US, using a Roche-454 and Illumina-MiSeq analysis of rRNA amplicons respectively. Microbiome data for both bacteria and fungi are analyzed against the corresponding bulk soil properties, tree phenotype, and tree genotype data in order to understand how such properties influence microbiome structure.

In our studies of *P. deltoides* we have shown that the rhizo- and endosphere environments 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 on average tenfold lower 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. especially appear to be highly enriched for *Pseudomonas fluorescens*-like species/OTUs when compared to other *Populus* habitats as well as endophytic habitats of surrounding (non-*Populus*) tree species. Fungal endophytic species are more numerous than bacteria, but also less than rhizosphere spp. Both fungal and bacterial rhizosphere samples showed distinct phylogenetic composition patterns compared to the more variable endophyte samples. Contrary to initial expectations, both *Populus* spp. have low natural levels of colonization by ectomycorrhizal (ECM) and arbuscular

mycorrhizal fungi, but high levels of presumed fungal endophytic taxa such as *Nectria*, *Mortierrella*, and members of the *Atractiellales*.

Overall, the *P. trichocarpa* rhizosphere communities studied in the Western US separate based on the two Oregon common gardens in which they were sampled: Clatskanie and Corvallis. Conversely, the endophyte communities between the common gardens were similar. Pseudomonad OTUs dominated both the rhizosphere and endophyte samples while being nearly undetectable within bulk soil. 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. These results are similar to what we have observed for natural populations in the Eastern US for *P. deltoides*.

Future research plans will 1) expand both the number and geographic range of species of *Populus* examined, 2) move beyond the rooting zone to total microbiome studies of *Populus*, as well as 3) move towards additional functional examinations enabled by developments in metagenomic analyses.

The Plant Microbe Interfaces Scientific Focus Area is sponsored by the Genomic Science Program, U.S. Department of Energy, Office of Science, Biological and Environmental Research