Plant-Microbe Interfaces: *Populus deltoides* supports distinct fungal and bacterial root associates from other ectomycorrhizal tree hosts

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Project Goal: 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 an initial 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 will focus on 1) characterizing the natural variation in *Populus* microbial communities within complex environments, 2) elucidating *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships, and 3) performing metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

Populus deltoides is a common riparian tree species in areas of southeastern North America and is largely dependent on flooding for seedling recruitment. This species is unique from co-occuring tree species in that it forms root associations with both arbuscular and ectomycorrhizal fungal species. *Populus deltoides* also harbors both bacterial and fungal endophyte communities within its roots. To address the influence of edaphic or genotypic factors on the structuring of rhizospheric assemblages, we carried out a series of trap-plant experiments in growth chamber environment using rooted cuttings of *P. deltoides* and two other tree species (*Quercus alba* and *Pinus taeda*) grown in soils from our field sites. We used 454 multiplex amplicon pyrosequencing to characterize rhizospheric fungal and bacterial diversity for each plant in the experimental treatments. Specific objectives of this research were to:

1) Determine the effect of host species (*P. deltoides*, *Q. alba*, *P. taeda*) on the structuring of rhizosphere bacterial and fungal communities
2) Determine the influence of *P. deltoides* genotype on the structuring of rhizosphere bacterial and fungal communities
3) Determine the effect of different field soil inoculum on bacterial and fungal rhizosphere communities of individual host species and *P. deltoides* genotypes

Field soils from our ORNL *P. deltoides* research sites in NC and TN were used as the source of microbial inoculum in these trap-plant studies. Cuttings from different *P. deltoides* genotypes were planted into a 50% mixture of sterile sand and field soil. For the host treatment we grew *P. deltoides*, a *P.deltoides x P.trichocarpa* hybrid, oak (*Quercus alba*) and pine (*Pinus taeda*) in the same soil. For the soil treatment a single *P. deltoides* genotype was grown in multiple soil types. Plants were harvested after five-months of growth. Soils were washed off the root systems, roots visually assessed for ectomycorrhizas and fungal infection, and samples of bulk roots used for DNA extraction. The fungal community from each plant was sequenced at the ITS and LSU

rDNA regions using the fungal specific primers ITS1f/ITS4 and LROR/LR3. Arbuscular mycorrhizae were preferentially amplified selectively with the primer set AML1 & a modified AML2 primer. Bacterial 16S rDNA primers that amplify across the V4 region and discriminate against plastid DNA were used to compare rhizosphere bacterial communities in selected samples. Pine and oak seedlings showed high ectomycorrhizal colonization (>80%), while most of the *P. deltoides* genotypes had low ectomycorrhizal colonization (<30%). This observation was verified through sequence-based assessments, with more than 75% of the sequences from *Populus* belonging to endophytic fungi and over half of those from oak and pine belonging to ectomycorrhizal fungi. The most abundant ectomycorrhizal fungi on *P. deltoides* belonged to the genera *Peziza, Inocybe* and *Hebeloma*. The *Peziza* and *Inocybe* taxa also co-occurred on oaks and pines. Ectomycorrhizal species of *Tuber* and *Laccaria* were recovered from both *Populus* and oak roots. Although *Populus* hosted fewer ectomycorrhizal taxa than either oak or pine, a more species-rich assemblage of endophytic fungi was detected in *Populus* roots. The total richness of root associated fungi (238) and bacteria (283) taxa was significantly greater for *Populus* than oak (175-f;184-b) or pine (157-f;185-b). For the bacteria, *Populus* was characterized by a higher relative abundance of *Actinobacteriales* and *Sphingobacteriales* than were oak or pine, and a lower abundance of *Rhizobiales* and *Berkholderiales.* Nonetheless, a core set of fungal (43) and bacteria (103) taxa were shared between the three hosts. There were some minor responses of bacterial and fungal communities to *Populus* genotype. In particular, the *P. deltoides x P. trichocarpa* hybrid had a wider spread across species space (based on PCA ordination) and was represented by a greater frequency and relative abundance of ectomycorrhizal taxa (e.g. *Inocybe, Tomentella, Hebeloma*). Arbuscular mycorrhizal fungi belonging to the Glomerales (*Glomus* sp.) and Paraglomales (*Paraglomus* sp.) were present in all genotypes, but sequences belonging to the Diversisporales were only detected in the hybrid genotype. Soils differed significantly in their effect on microbial communities. For example, a novel species of *Atractiellales* was the most abundant species in some assayed soils, but was absent in other soils. Similar patterns were observed for other microbial taxa. While we did detect fungi and bacteria in our negative controls, these clustered tightly in ordination space and showed little overlap with taxa from the experimental treatments. In conclusion, the microbiota associated with roots of *P. deltoides* is diverse and unique from oak and *pinus*, and appears to be structured both by the microbial inoculum available in soils and (to a lesser extent) plant genotype.

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