Plant-Microbe Interfaces: The *Populus* Microbiome Atlas Project - Dissecting the microbiome landscape of trees from soil to canopy.

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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, Cottonwood or Aspen) are broadly distributed in temperate North American habitats, making them ideal ecological model species. Additionally, their highly developed genetic toolsets, rapid growth rates, association with ecto- and endomycorrhizal fungi, and ability to grow on land not suitable for food production make them good candidates for bioenergy production scenarios. *Populus* spp. are also now emerging as model systems for understanding the role of the plant microbiome. Most of our and other's work to date on tree microbiomes has focused on comparisons of individual habitats within and across tree species or a few habitat types such as root endosphere, rhizosphere, mycorrhizae, or leaf endosphere versus phyllosphere communities. Surprisingly, a comprehensive comparison of the overall phytobiome of such woody tree species across tissue and habitat types from the soil to the canopy has been lacking. Additionally, high host DNA background levels have limited the ability of microbial ecologists to apply shotgun metagenomic techniques to the sequencing of host endophytic habitat types.

In our ongoing work, we are examining 30 different plant tissue/habitat types across five *Populus deltoides* and five *P. trichocarpa x deltoides (*TxD*)* hybrids (replicated identical genetic clones) collected from destructive whole tree harvests in East Tennessee in August of 2014. Microbiomes of these tissue-level habitats are being analyzed by 16S rRNA bacterial/archaeal amplicons and fungal ITS2-rRNA amplicons for community comparisons across 300 total samples. These samples encompass multiple belowground tissue types (e.g., fine and coarse roots, rhizosphere, soil - from deep and shallow soil locations), aboveground tissue types (e.g.,

wood, live xylem, bark/phloem/cambium tissues – from large structural roots and each of the 3 main stem age segments), as well as leaf endosphere, petiole and phyllosphere samples from developing and mature leaves. For select belowground tissues and habitats, we are also applying new differential centrifugation methods to enrich the microbial cells from root tissues prior to DNA extraction and Nextera-based metagenomic sequencing in order to avoid host DNA background contamination. These metagenomes have enabled us to contrast the fine root endosphere and rhizosphere communities as well as bulk soils in the shallow rooting zone of each *P. deltoides* and TxD hybrid tree (30 metagenomes).

To date we have completed the bacterial amplicon sequencing for all samples and the majority of metagenome samples (28/30). Across each tree type, bacterial community structure from rRNA gene amplicons varied significantly across leaf, stem, roots and soil/rhizosphere tissue/habitat types ($p < 0.01$). Leaf and stem habitat types had significantly lower OTU richness compared to root and stem habitats and featured decreasing abundance of Proteobacteria from leaf, to stem, to root and finally to soil. The oldest woody stem tissue (3-year-old heartwood) was also distinguishable from the younger 1st and 2nd year tissues, and featured enrichment of Firmicutes, consistent with potentially anaerobic/fermentative taxa in this habitat. Within the leaf, stem, root, and soil habitats, bacterial community structure in *P. deltoides* samples could also be differentiated from TxD hybrid samples ($p < 0.01$). Belowground rhizosphere and bulk soil habitats could be differentiated by soil depth ($p < 0.01$). Fungal ITS2 amplicon sequencing of these same samples has been optimized to incorporate improved primer designs for phylogenetic representatives among fungi. In addition, we have incorporated a newly designed ITS2 PNA blocker that reduces host nuclear contamination from >90% in many tissue samples to <1%. Using these optimized protocols, we were able to reduce host contamination to <10% of metagenome reads in 9/10 root endosphere metagenomes samples. Comparative metagenomics-based analyses between tissue/habitat types (e.g., soil, rhizosphere, root endosphere) and with amplicon-based datasets are ongoing.

When complete, these studies will allow us to comprehensively dissect the plant microbiome both phylogenetically and functionally. The results from this work should greatly enhance our understanding of plant microbiomes in general, and the important model species *Populus* in particular.

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