

Plant-Microbe Interfaces: Dissecting the microbiome of *Populus* tree species from the soil to the canopy using amplicon sequencing and shotgun metagenomic analyses

Melissa A. Cregger^{1*} (creggerma@ornl.gov), Allison M. Veach,¹ Miranda Crouch,¹ Ian Hodges,^{1,6} Zamin K. Yang,¹ Debbie Weighill,^{1,2} Piet Jones,^{1,2} Carissa Bleker,^{1,2} Armin Geiger,^{1,2} Mircea Podar,^{1,2} Rytas Vilgalys,³ Timothy Rials,⁴ Susannah G. Tringe,⁵ Dale A. Pelletier,^{1,2} Daniel A. Jacobson,^{1,2} and **Christopher W. Schadt**^{1,2}

¹Oak Ridge National Laboratory, Oak Ridge, TN; ²Univ. of Tennessee, Knoxville, TN; ³Duke Univ., Durham, NC; ⁴Univ. of Tennessee Institute of Agriculture, Knoxville TN; ⁵DOE Joint Genome Institute, Walnut Creek, CA, Stanford University, Palo Alto, CA

Populus trees are broadly distributed in nature, widely used in pulp and paper production, and a potentially important bioenergy feedstock. In recent efforts, we examined 30 plant tissue/habitat types extracted from plantation grown *P. deltoides* and hybrid *P. trichocarpa x deltoides* (TxD) trees. The microbiome composition was assessed by 16S-rRNA gene (bacteria) and ITS2-rRNA gene (fungal) measurements of 300 samples across multiple tissue types and tissue ages. For select habitats (root endosphere, rhizosphere, and soil), we also applied a differential and density gradient centrifugation method to enrich the microbial cells from plant tissues prior to DNA extraction and shotgun metagenomic sequencing. For each tree type, bacterial and fungal community structure from rRNA gene amplicons varied significantly across leaf, stem, roots and soil/rhizosphere tissue/habitat types. Leaf 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, to soil. The oldest woody stem tissues were also distinguishable from the younger 1st and 2nd year tissues, and enriched in *Firmicutes*, consistent with potentially anaerobic environments. Within the leaf, stem, root, and soil habitats, bacterial community structure in *P. deltoides* samples could be differentiated from TxD hybrid samples. Interestingly, the leaf environment from the TxD hybrid samples had high levels of fungal pathogens relative to the *P. deltoides* leaf samples (primarily *Septoria musciva* and a *Marsonina* sp.). These data suggest the differential abundance of these pathogens between the species, may be driving changes in the overall microbiome structure, and follow on investigations are underway to address this. Comparative metagenomic analyses and amplicon-based datasets were completed for root endophyte, rhizosphere and soil samples and show a high degree of congruence and confirm our methods reduced host DNA contamination to < 2%. Metagenome functional gene profiles based on ordination and network analyses of PFAM enrichment patterns, show distinct clustering based on sample type (endosphere, rhizosphere, and soil) and host genotype (*P. deltoides* vs. TxD). Results from this and future work should greatly enhance our community-based and functional understanding of plant microbiomes in this important model species.

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