Plant-Microbe Interfaces: Temporal dynamics of the *Populus* microbiome across scales

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Project Goals: The goal of the PMI SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. *Populus* and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic *Populus*-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.

Our understanding of the plant microbiome is clouded by the fact that the majority of studies on the plant microbiome represent "snapshots", as they present data from a single time point. It is well known, however, that microbiomes are temporally-dynamic resulting from external forcing factors and intra-community interactions. Hence, a predictive understanding of the relationship between the plant and its microbiome, and the ways these events manifest themselves across the various temporal scales relevant to natural systems, is a challenge that requires long-term fundamental research. Using amplicon and metagenomic sequencing of the plant microbiome in combination with metabolomic measurements, we are characterizing how the *Populus* microbiome changes over time in three projects. Studying temporal changes in the microbiome of a long-lived plant species, such as *Populus*, can give us unique insights into the ecological processes shaping microbiomes when compared to annual plant species (e.g., *Arabidopsis*, *Maize*, etc.) that often serve as models plant-microbe interaction studies.

The first project leverages a multi-year common garden experiment planted with 10 genotypes of *P. deltoides* and *P. trichocarpa* with varying degrees of disease resistance. In 2018 and 2019, we collected soils, roots, and leaves from these genotype four times each year representing the major seasons to determine intra- and inter-annual variations in in the *Populus* microbiome. We found that in the rhizosphere, the microbial community changed both seasonally and annually, suggesting a non-cyclical pattern of rhizosphere microbiome composition. However, while the taxonomic composition of ectomycorrhizal (EM) fungi (important plant symbionts) changed, the relative abundance of fungi followed a cyclical pattern and was highest in the spring. Functionally, there was a greater genetic potential for nitrate and nitrite assimilation over time regardless of the season in the rhizosphere, suggesting that these microbial populations are rapidly growing. Analysis for of leaf and root microbiomes is currently underway. We hypothesize that these endospheric microbiomes will vary with time, but to a lesser extent than the rhizosphere due to relatively stronger plant genetic effects.

The second project further characterizes how the broader *Populus* holobiome is moderated following infection by *Sphaerulina musiva*, a well-characterized fungal pathogen, which infects *P. trichocarpa* and *P. trichocarpa x deltoides* hybrids. For this project, we investigate the microbiome and metabolome of *Populus* leaves and roots and the microbiome of their associated surfaces (to understand the impact *S. musiva* abundance (quantified by qPCR) and infection systematically in the same common garden field setting as above. We found that *S. musiva* is present in all trees and tissues, but *S. musiva* abundance as measured by qPCR was unrelated to stem canker onset and development. We also find that the leaf and root metabolomes significantly differ between the two *Populus* species and that certain leaf metabolites, particularly the phenolic glycosides salirepin and salireposide, are diminished in canker-infected *P. trichocarpa* trees compared to their uninfected counterparts. Furthermore, we found significant associations between the metabolome, *S. musiva* abundance, and microbiome composition, particularly in *P. trichocarpa* leaves. This suggests that the effects of *S. musiva* on *P. trichocarpa* trees are widespread and not confined to the site of canker infection.

The third project extends the temporal scale of our previous work to the lifespan of a tree. To accomplish this, we are taking advantage of the availability of large (10s to 100s of acres) clonal stands of *P. tremuloides* in the intermountain west, including the grove nicknamed "Pando", which is one of the largest organisms on earth. By using defined chronosequences within these clonal stands, we are able to isolate the effects of tree (ramet) age, from the soil and genotypic factors that we know from previous work also exert large controls on microbiome composition. During the summer of 2021, we collected leaf, stem and root tissues as well as associated soils from 140 trees/ramets representing four aspen clones, including Pando, ranging in age from 1 to 140 years old. Microbiome and metabolomic analysis is currently underway. We hypothesize that differences in the microbiome with tree age will attenuate in older trees in concert with changes in the metabolome. Furthermore, we hypothesize that microbiome and metabolomic differences will be greater in older tissues (e.g., heartwood) compared to younger tissues that regenerate each year (e.g., leaves).

By integrating these three projects we are gaining an understanding of the temporal dynamics in the *Populus* microbiome from seasons to centuries (Figure 1).

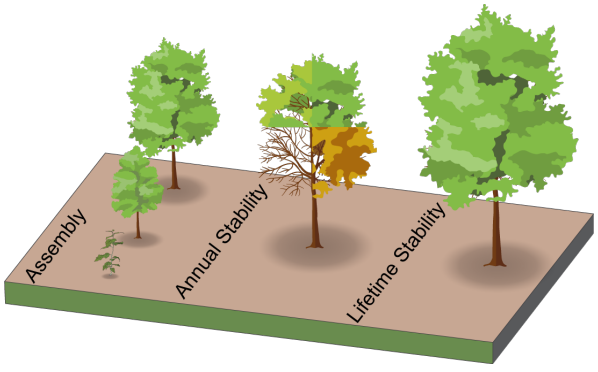

Figure 1: Temporal scales of the *Populus* microbiome.

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