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 on the length and time scales of natural systems, is a challenge that requires long-term fundamental research. Using amplicon and metagenomic sequencing of the plant microbiome in combination with ecological assembly and source tracking models, we work to characterize alterations in the *Populus* microbiome 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.

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. Over the course of a year after planting in the spring of 2017, we collected soils, roots, and leaves from these genotypes to assess microbial community assembly patterns and processes. We found that the initial assembly of the *Populus* microbiome is time-, genotype-, and habitat-dependent, and is moderated by both selective and stochastic factors. We hypothesize that the initial assembly of the plant microbiome may establish the trajectory for forthcoming microbiome states (via priority effects) and could

determine the overall future health of the plant. We have continued to collect and analyze samples from this site, now in its 4th year, to address this hypothesis and determine intra- and inter-annual changes in the *Populus* microbiome and how they vary with the health of the plant.

The second project further characterizes the initial *Populus* microbiome assembly as moderated by an ecologically significant disturbance—wildfire. For this project we took advantage of a high-intensity prescribed fire in Central Utah and collected soils, roots, and leaves of < 1 y old regenerating *P. tremuloide*s saplings in control, moderate, and severely burned areas. We found that fire severity influences the relative dominance of microbial inoculum and the vertical inheritance of the sapling microbiome from the parent tree resulting in changes to the leaf microbiome, notably a three-fold increase in fungal pathogens with increasing severity. Overall, this work demonstrates, for the first time to our knowledge, that fire impacts the plant microbiome, outside of the bulk soil and rhizosphere and highlights potential for further research towards increasing plant fitness and ecosystem recovery after fire events.

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 ha) 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 these clonal stands, we will be 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. In the initial year of study, we characterized the age, genotype, and soils of >200 trees in multiple clonal stands, and our first microbiome sampling campaign is planned for summer 2021.

Integrating these three projects we work to understand the temporal changes in the *Populus* microbiome from seasons to centuries (Figure 1).

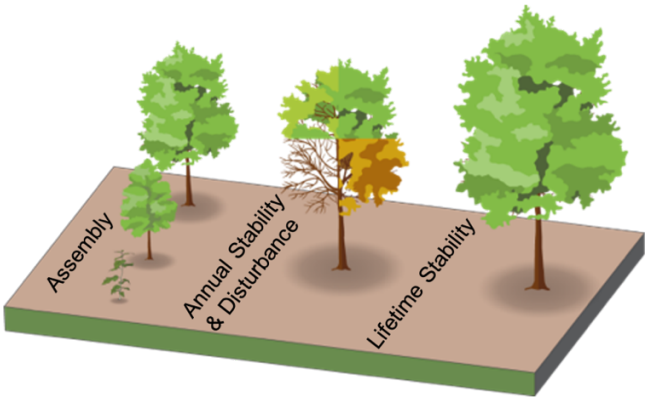

Figure 1: Temporal scales of the *Populus* microbiome.

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