## Environmental Drivers of the North American Populus Microbiome

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Project Goals: Plant-microbe symbioses play a critical role in the health and nutrition of all plants, and especially so for *Populus* tree species targeted for bioenergy feedstock programs. The primary goal of this project is to test quantitative predictions about the climatic, edaphic, and historical variables that best control *Populus*-microbe symbioses and their impacts on ecosystem function. Identifying the factors that influence the distribution of different *Populus* species and their symbiotic microbial communities (e.g., rhizosphere bacteria, mycorrhizal fungi) has practical implications for human management of forest systems and sustainable production of biofeedstocks that depend on these associations. Results from this project will identify areas where *Populus*-symbiont relationships may be especially critical for maintaining natural forest productivity in marginal habitats, indicating a potential probiotic source for tree plantations under future environmental conditions.

Microbial communities form symbiotic relationships with plants above- and belowground, but we lack a general understanding of the biogeographic drivers of such relationships. This is important because the distributions of host-associated symbionts (i.e., fungal mutualists) are likely to vary across the host species' range and affect their ability to tolerate certain abiotic pressures. This creates a long-term disadvantage to sustainably manage biofeedstock programs and accurately forecast ecosystem productivity as plant-microbiome interactions will likely be disrupted or altered by environmental change. However, examining how microbial communities vary throughout a host species' range can reveal the environmental factors most important for shaping plant-microbiome symbioses, thus refining our ability to predict the dynamics of such relationships under future conditions. We sequenced bacterial and fungal communities in leaf, root, and soil material from five ecologically and economically important *Populus* species across the United States (*P. angustifolia*, *P. deltoides*, *P. fremontii*, *P. tremuloides*, and *P. trichocarpa*). Here, we asked two main questions: (1) how do microbial communities associated with *Populus* leaves, roots, and soils differ? (2) what are the major environmental gradients that structure microbial community composition within and among *Populus* species?

We find differences in foliar, root, and soil microbial community composition among the five *Populus* species, with the greatest divergence between species from disparate habitats. Moreover, the environmental gradients identified as the most important for driving microbial community turnover differed by sample type (leaves, roots, and soil) and among *Populus* species. This indicates that the climatic and edaphic factors important for shaping *Populus* microbial communities (and potentially their impact on plant performance and functioning) varies

depending on how they interact with the host (i.e., within or surrounding plant tissues) and respond to abiotic stress. Species differed in the level of belowground fungal symbioses, with *P*. *fremontii* having the least (mean =  $5.4\% \pm 3$ ) and *P. tremuloides* having the greatest (mean =  $28.7\% \pm 3.5$ ) root colonization by ectomycorrhizal fungi. We also found higher total numbers of ectomycorrhizal taxa from *Populus* species with larger range sizes, suggesting a positive link between the diversity of fungal mutualists and plant host distributions on the landscape. The dominant environmental gradients corresponding with microbial community turnover differed among *Populus* species, indicating that the role of abiotic effects on plant-microbiome symbioses may be species-specific. Finally, analysis of the most common taxa found across all *Populus* species shows variation in the connectivity and modularity of co-occurrence networks, suggesting there are differences in how community members interact or respond to the same environmental pressures. Collectively, these results demonstrate the key environmental drivers of the *Populus* microbiome, which can be used to inform management decisions and potential planning of *Populus* biofeedstock programs based on the diversity and biogeography of their natural microbial symbionts.

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