

## How Does Precipitation Impact the Taxonomic and Functional Diversity of the *Populus trichocarpa* Soil Microbiome?

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**Project Goals: Plant-microbe relationships are vital to plant health and nutrition, with strong geographic patterns in their form and function. Our primary goal is to test quantitative predictions about the climatic, environmental, and historical variables that best control *Populus*-microbe relationships (including symbioses between *Populus* and mycorrhizal fungi). We recently collected soil and root samples beneath *P. trichocarpa* across a strong rainfall gradient in the Pacific Northwest, and preliminary data show that ectomycorrhizal fungal colonization declines with precipitation. From this, we are actively pursuing three main research questions with amplicon and metagenomic sequencing.**

One of the first global maps of mycorrhizal distributions hypothesized that ectomycorrhizal fungi (EMF) dominate in northern biomes—where plant carbon (C) to nitrogen (N) ratios are high and N mineralization is low—based on their ability to acquire N directly from organic matter (Read 1991). In contrast, arbuscular mycorrhizal fungi (AMF) were thought to have limited ability to decompose organic matter. For three decades, this hand-drawn map largely shaped ecologists' thinking on the causes and consequences of EM- versus AM-dominated forests. However, new data have challenged some of the central assumptions in this early hypothesis, raising questions about the accuracy of these categorical maps and the mechanisms that determine the dominance of the various symbiosis forms on the landscape.

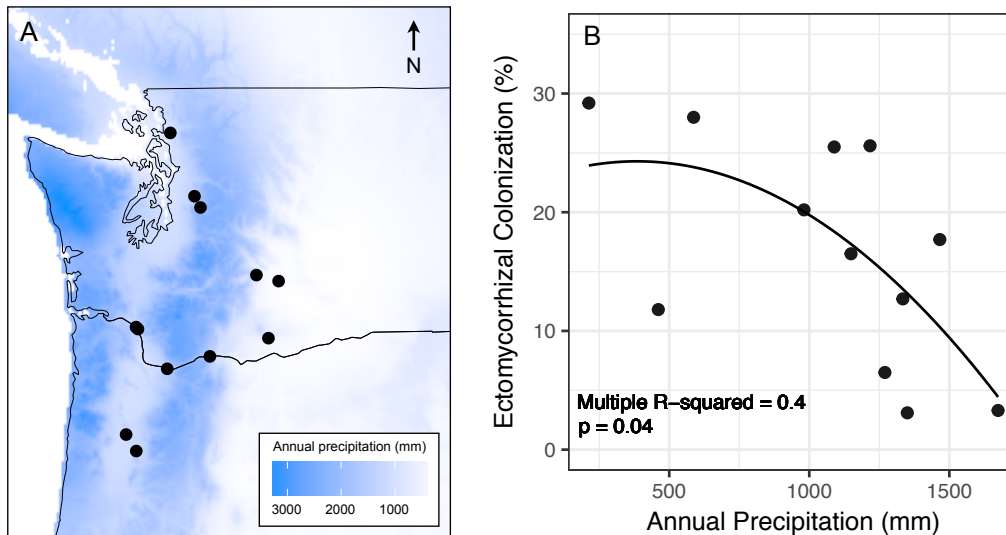
Our goal is to test quantitative predictions about the climatic, environmental, and historical variables that best control *Populus*-microbe symbioses and their impacts on ecosystem function. *Populus* is an ideal candidate for this research because: (a) it is ecologically relevant as an important component of natural forests in the northern hemisphere; (b) *Populus* species vary naturally in their degree of EM symbiosis; and (c) there are tremendous *Populus* genetic resources available due to its commercial importance and potential bioenergy feedstock. We recently conducted a field survey of soils and root samples from sites dominated by *P. trichocarpa* that differ markedly in annual precipitation (range = 213-1674 mm yr<sup>-1</sup>; **Fig. 1a**). Preliminary data from these sites show that EM colonization declines with precipitation (**Fig. 1b**). As these samples undergo amplicon (16S & ITS2) and metagenomic sequencing, we will focus on three main research questions:

1. How does the taxonomic and functional of soil and root microbial communities vary with precipitation? Because we surveyed across a continuous climate gradient, does diversity respond non-linearly such that we might identify thresholds or tipping points in microbial responses to precipitation?
2. Are there other climate or edaphic factors that structure metagenomic diversity by amplifying or dampening the effect of precipitation (i.e., potential counter-gradients)?

3. Is precipitation associated with changes in the structure/topology of co-occurrence networks, and are there connections between the soil and root microbiomes? Is there a “core” belowground microbial network that is associated with *P. trichocarpa*?

First, we expect to see significant turnover associated with precipitation in the community composition and function of soil and EM-enriched metagenomes (*Question 1*). Second, we will use a combination of PCoA and multiple regression approaches to test the strength and direction of additional variables (bioclim variables and soil C, phosphorus (P), organic N, NH<sub>4</sub>, pH, cation exchange capacity, and moisture) that might further influence microbial diversity (*Question 2*). These results will provide an important baseline from which we can: (a) predict how soil microbiome diversity and function will differ for other *Populus* species based on assumptions about the strength and importance of environmental gradients underlying their distributions, and (b) compare plant-microbial symbioses among *Populus* species that occur across gradients of different factors and sizes. Third, we will test how microbial network structure varies with precipitation (*Question 3*) using random matrix theory co-occurrence models derived from four site replicates of three discrete precipitation categories: High (>1300 mm yr<sup>-1</sup>), Medium (<1300 and >1000 mm yr<sup>-1</sup>), and Low (<1000 mm yr<sup>-1</sup>). Importantly, this will reveal co-occurrence patterns between specific prokaryotic and fungal taxa that may be indicative of facilitative or antagonistic relationships between these groups. Finally, we will identify any relationships among common microbial taxa that are consistent across all sites, creating a candidate “core” belowground microbial network for *P. trichocarpa*.

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**Figure 1. *Populus trichocarpa* sampling locations and ectomycorrhizal colonization across the precipitation gradient.** (A) We recently sampled soils and roots from *P. trichocarpa* (taking care to trace roots back to focal trees) across 12 sites that vary in the amount of annual precipitation. (B) Preliminary data from these sites shows that ectomycorrhizal colonization is negatively related to precipitation.