Title: Elucidation of the Roles of Diazotrophic Endophyte Communities in Promoting Productivity and Resilience of *Populus* through Systems Biology Approaches

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Project Goals: The overall project goal is to move toward an understanding of the holobiont, how plants and the microbial community within them interact in ways that promote the productivity of the whole. Integration of plant physiology data with the molecular plant-microbe interactions (multi-omics) data from greenhouse and field experiments will allow us to develop a systems-level understanding of the genetic and molecular basis for diazotrophic endophytic mutualism in *Populus*. This deeper level of understanding of the plant responses will guide construction of microbial communities in order to optimize the impacts of bioinoculants for environmental sustainability of bioenergy crops.

Abstract text: Poplar trees are important feedstocks for bioenergy and ecosystem services, but more efficient and resilient growth is essential for sustainability. Some of the micro-organisms which make up the wild poplar microbiome can help poplar grow by providing the required nutrients of nitrogen and bioavailable phosphorus. In addition to increasing nutrient acquisition, the micro-organisms may also promote plant tolerance to other environmental stresses including drought. Previously we demonstrated that adding microorganisms from wild poplar to a variety of other plants including grasses, conifers, and cultivated poplar, increased the health and growth of these plants under nutrient limitation and drought. Since the start of this grant in autumn 2020, a suitable field site in a nutrient- and water-controlled site near Prosser, Washington was prepared for planting in spring 2021. However, after a record-breaking heat wave and physiological impacts on the plants, we had to abort the 2021 planting, re-start the experiment in August, and conduct greenhouse testing of the bio-inoculants instead in preparation for a spring 2022 field planting. Growth, physiological parameters and nitrogen levels of the inoculated and control greenhouse poplar trees are being assessed. Random barcoded TnSeq experiments to identify bacterial endophyte genes required for phosphate solubilization and nitrogen fixation were conducted. An additional set of diazotrophic strains was isolated from wild poplar roots that significantly increased growth of *in vitro* grown poplar plants in nitrogen-free media. Genomic analysis of the new endophyte strains are in progress. A new consortium of strains optimized for nitrogen fixation and poplar growth promotion will then be added to the greenhouse poplar prior to field planting. In parallel, methodology for proteomics and metabolomics of a ^{15}N-labeled diazotrophic endophyte strain is underway. Preliminary analysis of peptides conservatively identified nearly 200 peptides with substantial ^{15}N enrichment. Optimization of the analytical pipeline for ^{15}N proteomics is on-going in preparation for experiments with the finalized consortium of strains. This will enable elucidation of the molecular microbe-microbe interactions for optimal nitrogen fixation. This first year of the grant

project is setting the stage for in-depth investigations of the mechanisms by which bacterial endophytes promote the growth and health of trees for sustainable bioenergy crop production.

Funding statement: This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0021137. Pacific Northwest National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-76RL01830. This program is supported by the U. S. Department of Energy, Office of Science, through the Genomic Science Program, Office of Biological and Environmental Research, under FWP 76183.