Plant-Microbe Interfaces: Towards a rhizosphere on a chip for understanding physical and chemical transitions in multi-kingdom systems

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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.

The mechanisms by which plants, bacteria, fungi and other microorganisms negotiate spatial networks defined by the soil to ultimately shape and define interactions in the rhizosphere are multifaceted and complex. It is clear that a combination of physical and chemical interactions affect movement through such networks, but their relative importance and the molecular signals that dominate such behaviors are yet to be elucidated. Microfluidics, as engineered habitats, provide a path for experimentally teasing apart the answers to such questions, and provide a means by which scientists can carefully explore the impact of environmental structure and composition on multi-kingdom interactions, niche establishment, and complex community dynamics. In effect, tractable systems that combine known quantities of community members in well-defined initial conditions, can be observed and quantified over extended periods of time to track the position and activity of different biological species, while simultaneously mapping the chemical composition of the environment in which they are interacting. In this study, we have begun to map and image the chemical environment of *Populus* cuttings grown over extended periods of time within engineered habitats that mimic soil structure in two dimensions. These rhizosphere-on-a-chip platforms provide optical access to capture fine morphological changes and growth in living plant and microbial systems. This enables local and global assessments of structure and chemical composition. In complementary efforts, we have refined methodology and completed the baseline assessment of fungal growth and hyphal elongation rates in microfluidic networks with varied levels of confinement and network complexity. Distinct differences in growth rates, branching,

penetration potential, and exploration motifs were evident across the species that were examined and quantified.

Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR22725. The Plant-Microbe Interfaces Scientific Focus Area is sponsored by the Genomic Science Program, U.S Department of Energy, Office of Science, Biological and Environmental Research under FWP ERKP730.