

# **Window to the Rhizosphere: Creating Engineered Habitats to Understand Plant and Microbial Community Development Underground**

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The development of terrestrial ecosystems depends on complex physical and chemical exchanges that occur over space and time. Underground, the chemical exchanges between plant roots and microbes are mediated by local changes in physical confinement and connectivity within soil pores and can undergo significant fluctuations with changes in microbial community member abundance, plant and microbial growth and senescence, and hydration. Capturing the ephemeral nature of the dynamically changing host and community organization and correlating those with chemical drivers in the local environment is extremely challenging in Nature. However, engineered habitats that combine microfluidics with complementary imaging, chemical, and genetic sampling strategies can be used to quantify and correlate changes in plant and microbial phenotypes with changes in the local environment. In this work, we describe the development of a suite of tools for these tasks and describe the challenges and opportunities for looking at these complex community interactions.

In previous work, a combination of conventional microfluidic networks and microfluidic soil analogues have enabled the characterization of root colonization patterns by plant-growth promoting bacteria isolated from *Populus*, and allowed visualization of biofilms within microstructures that mimic the physical organization of soil. Here, we utilize these engineered habitats to examine the effects of confinement on microbial communities and root development using a combination of time-lapse optical and chemical imaging. In complementary work, methods for systematically varying the complexity of soil-like microfluidic networks have been refined to explore the impact of spatial confinement and network complexity on the growth, migration, and development of fungal species in soil-like environments. Taken together, these efforts are enabling observations of synergistic interactions among plants, fungi, and bacteria in environments that mimic the pore networks within natural soil.

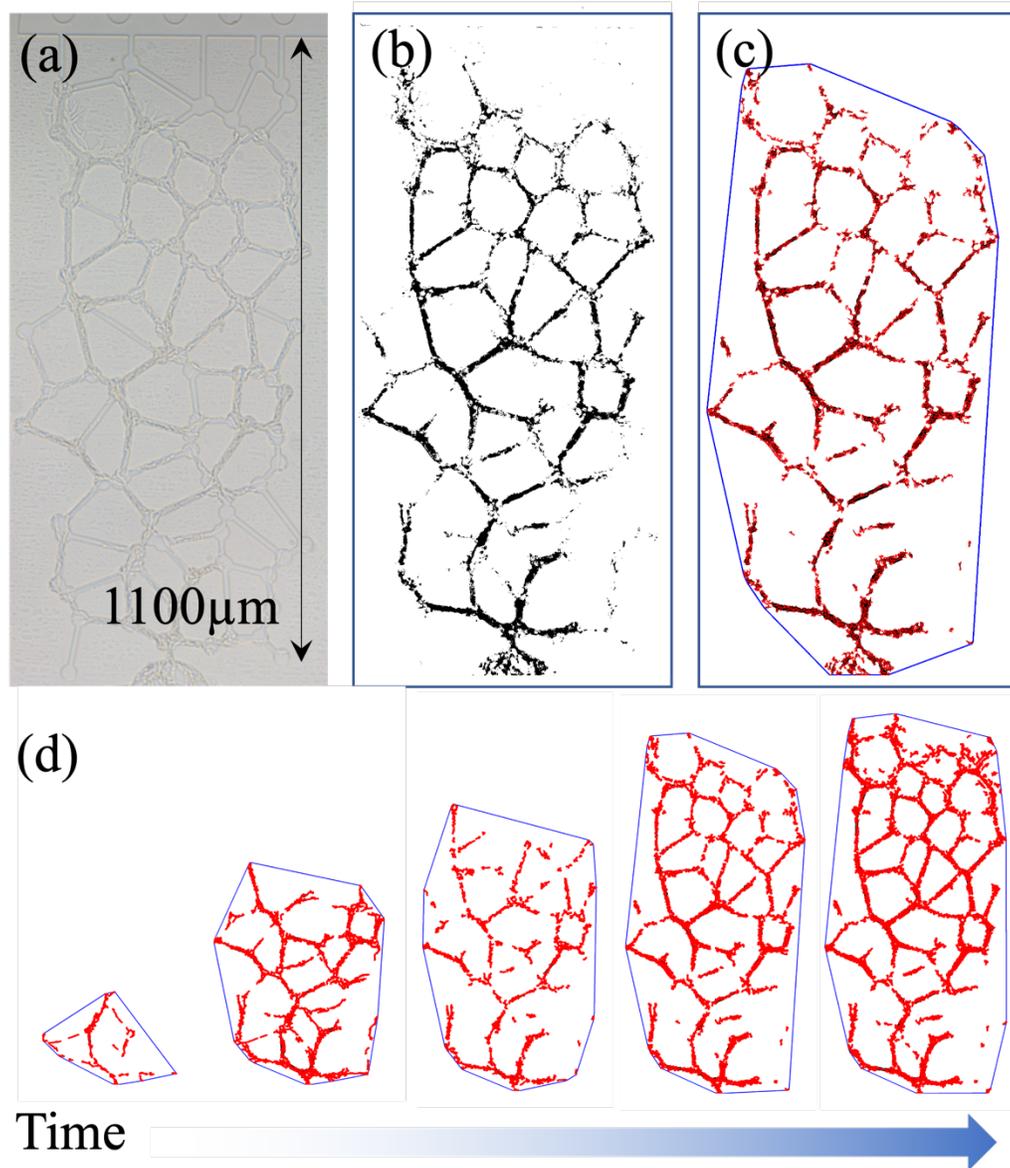


Figure 1: Hyphal growth of fungi through engineered habitats with soil-mimicking microfluidic networks is captured via optical time-lapse microscopy, segmented, and quantitatively analyzed. (a) optical micrograph of *Banniella erionia* in a Polydimethylsiloxane microfluidic 'soil' network. (b) Segmentation of the image in ilastik using a Random Forest Network. (c) Subsequent analysis of the segmented image is performed in Rhizovision Explorer. (d) A representative time-lapse series of hyphal growth.