Fabricated Ecosystems (EcoFABs) design for controlled and reproducible habitats to investigate plant-microbe-soil interactions

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Project Goals: Microbial Community Analysis and Functional Evaluation in Soils (mCAFEs) will use fabricated ecosystems (EcoFABs) in combination with CRISPR-Cas and phage-based approaches for interrogating gene and microbial functions *in situ,* gaining critical new insights into the rhizosphere and advancing a mechanistic understanding of microbial ecology. We will use 'bottom-up' defined microbial assemblies that enable detailed characterization of both constituent isolates and synthetic communities. We will complement this with 'top-down' investigation of native soil-derived microbial community enrichments, expanding our approaches to more diverse communities that include uncultivated microbes. Predictive models will be developed and iteratively refined through integrated simulations and experimentation.

Most of what we know about microorganisms, including their gene functions and growth dynamics have been derived either from single-organism studies in benchtop experiments or from surveys from complex natural ecosystems. To bridge the gap between fully-constrained single organism laboratory experiments and high-dimensional ecosystem analyses, we have developed EcoFABs (Ecosystem Fabrication; www.eco-fab.org; https://doi.org/10.3791/57170) that are small plant growth chambers constructed using common microfluidic procedures that can be used to study and engineer microbiomes living on and around the root surface. The devices are designed to be amenable to a number of analyses including microscopy of the root zones and soils, metabolomics analyses, and spatial sampling of the microbial communities. Over a dozen different plant varieties have been grown in EcoFABs, and a number of growth conditions are supported including hydroponics, synthetic soil and field soils. A multi-laboratory EcoFAB study growing the model plant *Brachypodium distachyon* demonstrated reproducible plant phenotypes and metabolomics including significant differences between treatments that were retained across laboratories (https://doi.org/10.1111/nph.15662). This highly controlled, reproducible plant growth platform will allow for more detailed studies on the complex interactions that take place in the plant root-microbe interface.

Over eight different iterations on the original EcoFAB design have allowed for specialized investigations, including mycorrhizal plant interactions (MycoFAB), chemical and oxygen gradients (μEcoFAB), and *in situ* imaging of fluorescently labeled bacteria on plant roots (imaging EcoFAB). We have demonstrated reproducible rhizosphere assembly and similar microbial community structure between *B. distachyon* and switchgrass in EcoFABs, and have shown that EcoFABs can support *B. distachyon* through flowering and senescence for longitudinal studies. Additionally, we have performed both chemiluminescent imaging and multi-color fluorescent rhizosphere imaging with single bacterial cell resolution within this highly reproducible plant growth system.

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