

## Phenotypic Characterization of *Brachypodium distachyon* and a Synthetic Community for Dissecting Plant-Microbial Community Interactions in Fabricated Ecosystems (EcoFAB)

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<http://mCAFEs.lbl.gov>

**Project Goals: Understanding the interactions, localization, and dynamics of grass rhizosphere communities at the molecular level (genes, proteins, metabolites) to predict responses to perturbations and understand the persistence and fate of engineered genes and microbes for secure biosystems design.** To do this, advanced fabricated ecosystems are used in combination with gene editing technologies such as CRISPR-Cas and bacterial virus (phage)-based approaches for interrogating gene and microbial functions *in situ*—addressing key challenges highlighted in recent DOE reports. This work is integrated with the development of predictive computational models that are iteratively refined through simulations and experimentation to gain critical insights into the functions of engineered genes and interactions of microbes within soil microbiomes as well as the biology and ecology of uncultivated microbes. Together, these efforts lay a critical foundation for developing secure biosystems design strategies, harnessing beneficial microbiomes to support sustainable bioenergy, and improving our understanding of nutrient cycling in the rhizosphere.

Plants grow in environments rich with microbes, where negative (pathogenic), neutral, or beneficial plant-microbial interactions may develop. These microbes are found as a complex community, and so interactions must be understood in the context of a community, rather than as an interaction between a single microbe and the plant. However, mechanistic understandings of plant-microbe interactions often derive from these reductionist single microbe systems. This oversimplification creates several gaps in translating lab-produced knowledge to the field. In addition, the involvement of plant genetics in shaping these interactions remains largely unknown. The m-CAFEs project intends to overcome these gaps by adapting fabricated ecosystems (EcoFAB; [www.eco-fab.org](http://www.eco-fab.org)) to study plant-microbial community-interactions. The EcoFAB is a robust and reproducible system that enables plant microbial research with ecologically relevant experimental parameters<sup>1,2</sup>. To understand the interplay of plant-microbial community interactions, we use *Brachypodium distachyon* and a bacterial community composed of a defined synthetic community (SynCom) as our model system. The SynCom is composed of specific taxa enriched in grass soil, isolated from the switchgrass rhizosphere. For each SynCom bacterium, we sequenced its genome and developed metabolic models within KBase (Kbase.us). Next, we measured physiological traits for these microbes and constructed whole-genome, randomly barcoded transposon mutant libraries, when feasible. We also characterized several plant growth promoting traits of the individual strains, including siderophore and phytohormone production and mineral solubilization assays. Finally, we have characterized *B. distachyon*

growth and cell wall composition when grown in the EcoFAB, providing a robust baseline dataset for use in future experiments, including inoculation with the SynCom. Together this rich set of physiological data combined with predictive models will identify bacterial genes that mediate interactions within this *B. distachyon*-SynCom system, which we will validate using community editing technologies.

## References

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