

## **Title: Generating a Stable, Reproducible Rhizosphere Microbial Community**

**Authors:** Joanna Coker<sup>1\*</sup> (jkcoker@health.ucsd.edu), Kateryna Zhalnina<sup>2</sup>, Clarisse Marotz<sup>1</sup>, Karsten Zengler<sup>1</sup>, Trent Northen<sup>2</sup>

**Institutions:** <sup>1</sup>University of California, San Diego, <sup>2</sup>Lawrence Berkeley National Laboratory

**Website:** <https://ecofab-teams.lbl.gov/>

**Project Goals: Short statement of goals.** The vital role soil and plant microbiomes play in ecosystems is increasingly apparent, yet the lack of standardized and reproducible experimental systems represents a major challenge for microbiome science. Fabricated ecosystems (EcoFABs) are devices that provide unique capabilities in the control and measurement of simplified microbial communities with tremendous potential to advance a mechanistic understanding of soil and plant microbiomes. The Trial Ecosystems for the Advancement of Microbiome Science (TEAMS) project is creating, validating, and disseminating EcoFAB technologies, complete with standardized model microbial communities, that are tailored for users of DOE's unique resources and user facilities, such as [EMSL](#), [JGI](#), [KBase](#), and [NMDC](#), as well as other DOE stakeholders. EcoFAB technology is being distributed as EcoFAB kits that have been validated through multi-laboratory reproducibility studies, through development of the EcoBOT, which automates EcoFAB experiments for the highest degree of standardization, as well as through online resources. Together, these standardized and reproducible experimental capabilities will help advance microbiome sciences.

**Abstract text:** Microbial soil communities form commensal relationships with plants to promote the growth of both parties. Optimization of plant-microbe interactions to advance sustainable agriculture is an important field in agricultural research. However, investigation in this field is hindered by a lack of model microbial community systems. Here, a model community of 17 soil organisms, isolated from the switchgrass rhizosphere, has been developed and optimized for use with EcoFAB devices. EcoFAB devices allow reproducible research in model plant systems, with precise control of environmental conditions and easy measurement of plant metrics. This model rhizosphere community grows reproducibly *in vitro* between replicates and experiments. Highest community alpha diversity is achieved with low-nutrient media and starting composition ratios adjusted for the growth rate of individual organisms. Controlled printing of cells at picoliter scale enables exact cell number ratios from the start. Community cryopreservation with glycerol allows robust regrowth of the community from frozen stocks, allowing for dissemination of communities. Our results demonstrate the generation of a stable rhizosphere microbial community that can be used with EcoFAB devices and shared between research groups for maximum reproducibility. This community will be used as part of an international ring-trial experiment examining the reproducibility of plant host selection of this model rhizosphere community within standardized EcoFAB devices.

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