m-CAFÉs -EcoFABs: Model Ecosystems to Advance Microbiome Science II

Trent Northen[1]* (TRNorthen@LBL.gov), Joelle Schlapfer Sasse[1], Peter F. Andeer[1], Peter Kim[2], Jens Heller[1], Trenton Owens[1], Andrew Klein[5], Borjana Arsova[6], Josefine Kant[6], Benjamin Cole[1], Michelle Watt[6], John Vogel[1], Eoin L. Brodie[1], Jeff Dangl[5], Adam M. Deutschbauer[1], Jennifer A. Doudna[3], Jill Banfield[3], Mary K. Firestone[3], Peter S. Nico[1], Anup K. Singh[2], Rodolphe Barrangou[4], and **N. Louise Glass[1]**

[1]Lawrence Berkeley National Laboratory, Berkeley; [2]Sandia National Laboratory, Livermore; [3]University of California, Berkeley; [4]North Carolina State University, Raleigh; [5]University of North Carolina at Chapel Hill, NC, USA, [6]Institut für Bio-& Geowissenschaften IBG-2, Forschungszentrum Jülich, Germany

http://eco-fab.org

Project Goals: To derive mechanistic understandings of plant-microbe-soil interactions using reproducible, simplified ecosystems

The m-CAFÉs program is a collaborative, coordinated and integrated, mission-driven program to interrogate the function of soil microbiomes with critical implications for carbon cycling and sequestration, nutrient availability and plant productivity in natural and managed ecosystems. One of the major challenges for generalized understanding of microbiomes in these complex ecosystems is extreme variation in microbiomes and environmental conditions. In many fields, simplified model systems have been developed and adopted by many researchers to accelerate the study various aspects of biology. In contrast, there is no agreed upon model system for studying soil microbiomes, and thus, nearly every researcher in the field is studying a different soil system. This heterogeneity of study systems and an inability to replicate experiments in different laboratories limits determination of causal mechanisms and the ability for scientists to build on each other's work.

A major effort within the m-CAFÉs program is developing precisely controlled ecosystem fabrications (EcoFABs) that reflect key functional attributes of plant-microbe interactions within soil, focusing on progressively increasing complexity that honor the physical, chemical and biological properties of soils. Each EcoFAB system is contained within a sterile plant-sized container with independent lighting. 3D printing is used to create root chambers (1.5-5 mL) attached to microscope slides, enabling the use of hydroponics, soil, or sand as substrate, as well as high-resolution imaging. We have shown that these systems are suitable for growth of diverse plants, including *Brachypodium distachyon, Arabidopsis thaliana*, and switchgrass for >1mo. The integrated fluidics system using in the EcoFABs facilitates selective sampling and introduction of microbes, metabolites, etc. Metabolomic analysis of EcoFAB culture is used to examine metabolite exchange within soil and rhizosphere communities and sequencing is used to link activities to specific community members. We have now performed a reproducibility study in which the model grass *Brachypodium distachyon* was grown in three environmental

conditions in four different laboratories. Plant growth, root morphology, phosphate content of tissues, and root exudation were analyzed and compared between the participating laboratories. To interrogate microbial functions in EcoFABs, we are pioneering CRISPR-Cas and environmental RNAi technologies to systematically determine functions of bacteria and fungi in the rhizosphere/soil. The results of these studies will be transformative for our understanding of soil metabolism and microbiome science, with applications to DOE missions in energy and environment.

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