Evaluating Plant-Microbe Interactions, Persistence and Movement of Microbial Communities Across Scales

Albina Khasanova[1*](akhasanova@lbl.gov), Daniel Naylor[1], Jonathan Diab[1], Shweta Priya[1], Jenny C. Mortimer[1,2], Aindrila Mukhopadhyay[1], John Vogel[1], Kateryna Zhalnina[1], Adam M. Deutschbauer[1], and **Trent R. Northen**[1*](TRNorthen@LBL.gov)

[1]Lawrence Berkeley National Laboratory, Berkeley; [2]University of Adelaide, Australia

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.

Plant root exudates recruit microbes and regulate interactions which vary over the plant life cycle. For example, root secreted metabolites can regulate plant-soil-microbiome interactions by affecting the colonization of symbiotic microbes in the root rhizosphere. These relationships in turn play an important role in plant health by altering nutrient availability and both biotic and abiotic stress tolerances over the course of plant development. Here we examine how these interactions influence plant productivity and morphology across scales using fabricated ecosystems (centimeter-scale EcoFABs and meter-scale EcoPODs). EcoFABs and EcoPODs facilitate the examination of complex interactions under highly controlled and replicated conditions to explore plant-microbial-soil interactions. Both systems are specifically designed to be compatible with biosafety workflows. EcoFABs allow for the extremely controlled manipulation of abiotic and biotic factors in small scale, high-throughput experiments with plant seedlings, while the EcoPOD system allows for precise control and monitoring of conditions across several growth parameters, including light intensity, temperature, humidity, water availability, and other important climatic parameters both above and below ground over the main developmental stages of the plant. The EcoPOD's large size and soil depth allows for the experiments where plants experience conditions similar to the field, yet provides for a high degree of control in the manipulation of engineered microbial communities. Together, these two systems allow for complete system development to study the persistence and fate of engineered genes, microbes and SynComs over the entirety of plant life cycles as well as the role of these interactions in tolerances to biotic and abiotic stressors. Currently we investigate plant-microbe interactions across scales (**i**) to uncover the role of aromatic acid in rhizosphere microbiome

assembly and stability; (**ii**) to engineer and test microbial enrichments under limited N conditions; (**iii**) to study the effect of drought stress on plant-microbial relationships.

Aromatic acids are one of the important classes of metabolites involved in rhizosphere-microbiome interactions and are widely exuded by plants. However, the role and mechanisms through which these molecules influence rhizosphere microbiome establishment are unknown. To dissect the role of aromatic acids as metabolic handoffs that strengthen plants-microbe interactions we identified *Brachypodium distachyon* accession lines with altered aromatic acid exudate profiles using EcoFABs. We pair these lines with SynComs utilizing these aromatic acids and evaluate dynamics of SynComs in response to different levels of exuded aromatic acids. In parallel, we utilized a RB-TnSeq mutant library of *Bulkholderia* and identified the microbial genes protocatechuate 3,4-dioxygenase alpha chain and 3-oxoadipate CoA-transferase subunit B, which are related to the microbial degradation of aromatic acids, as required for optimal growth on shikimic acid. We are currently constructing targeted mutants of these genes to demonstrate how the structure of SynCom changes when microbes are unable to utilize aromatic acids. Lastly, we used the EcoPOD to conduct an experiment that paired SynComs in tandem with *Brachypodium distachyon* accession lines that expressed altered production of aromatic acids at a field-relevant scale.

Limited N availability is a significant factor affecting plant growth. In this project, we aim to use a host-mediated microbiome engineering approach to develop a beneficial microbiome for low-N conditions. In this approach, *Brachypodium distachyon* plants are inoculated with a native soil microbiome, exposed to low-N inside EcoFABs over several rounds of selection, and the best-performing plants will have their microbiomes harvested and perpetuated onto the next round of selection, in this way 'evolving' a beneficial microbiome with a tight host association. We have successfully established an assay for inducing negative growth phenotypes in *Brachypodium* in response to decreasing N levels and performed pilot inoculation with soil enrichments of microbes. Future experiments will involve testing alternate soil sources to find one with more beneficial microbes. Once the effect of the enrichment is established in the EcoFAB, it will be tested at a more field-relevant scale using the EcoPOD.

Plant response to drought stress may be in part affected by root microbial relationships. We have used the EcoFAB system to develop a baseline dataset of *Brachypodium distachyon* response to drought in the presence of PEG6000, and identified an osmotic stress level that reduced growth without inducing mortality. We then used a soil bacterium (*Pseudomonas putida* KT2440) expressing mCherry as a proof of concept to test the effects of drought on root colonization. The EcoPOD system carefully controls water availability and enables mimicking field drought in the lab. We are currently testing the *Brachypodium-Pseudomonas*-drought system in the EcoPOD-mini, and comparing the findings with our small scale EcoFAB results.

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