Designing Synthetic communities for dissecting plant-microbe interactions in fabricated ecosystems (EcoFABs)

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https://mcafes.lbl.gov

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

Plants release a large fraction of photosynthetically-derived carbon into the rhizosphere. The soluble metabolites and root biopolymers released by plants serve as primary carbon sources for supporting microbial growth resulting in the well-known "rhizosphere effect" in the soil surrounding its root. Metabolite exchange is thought to be important in both the recruitment of microbial communities to plant roots as well as a driver in the formation and stability of microbial communities. Already, several plant exudates involved in the putative recruitment of rhizosphere colonizing bacteria have been identified in the grass *Avena barbata*. However, the specific molecular mechanisms of microbial community assembly in the rhizosphere remain elusive. **Here we hypothesize that specific root exudate components are selectively used by rhizosphere bacteria *in situ*, enabling plant modulation of community structure using exudate composition**. **In this work we assemble and perturb defined rhizosphere communities to investigate metabolic networks, interactions, localization, and activities.**

To dissect plant-microbe interactions, we use EcoFABs technologies that enable precise and reproducible control and characterization at a level that is not yet possible in complex soil systems (https://eco-fab.org). We design synthetic communities (SynComs) of rhizosphere microbes, analyze chemical signaling between these SynComs and model grasses, and connect dynamics and activities of the microbial taxa to the plant growth phenotypes observed in EcoFABs.

Design of synthetic communities for investigation in EcoFABs.

To identify rhizosphere colonization by soil bacteria and plant phenotypes in response to this inoculation, we inoculated seedlings with a defined 105-member synthetic community (SynCom) and analyzed dynamics of the different microbial taxa within this SynCom in response to plant growth. We found that after a week of incubation in EcoFABs with introduced seedlings the rhizosphere SynCom was significantly enriched with specific taxa (e.g. *Dyella*, *Leifsonia* and *Burkholderia*). Liquid chromatography mass spectrometry-based metabolomics was used to analyze the modulation of plant exudates by the added SynCom. We observed that inoculation of plants with the SynCom increased relative abundances of *p*-coumaric acid and dehydroshikimic acid, while some primary metabolites (e.g. arginine) decresed when compared to the non-inoculated plants. We used our 105-member SynCom to test the dynamics of SynCom members in response to the added mix of aromatic acids, as well as consumption of these acids by SynCom isolates as C source. Correlation analysis between aromatic acid consumption and rhizosphere community restructuring shows the enrichment of several isolates in the SynCom via 16S rDNA gene sequencing correlated with uptake of specific aromatic acids.

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