Spanning laboratory ecosystem scales: insights into environmental complexity with EcoFABs andEcoPODs.

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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.

Microbial rhizosphere communities are critical players in biogeochemical cycles, influencing plant nutrient availability, plant health, development, and stress tolerance. Plants fix CO_2 and release a large quantity of readily available carbon into the rhizosphere. This diverse pool of metabolites and root biopolymers affects microbial community assembly via a process known as the 'rhizosphere effect'. However, little is known about how these plant-microbe interactions are formed and regulated, and how they feedback to the environment. **Here we hypothesize that plants release specific metabolites that are selectively used by rhizosphere bacteria and this enables the plant to tailor its community assembly using exudate composition.** In this work, we use a suite of fabricated ecosystems, which span spatial and temporal scales to investigate complex interactions between plants, microbes, and the environment - from the small-scale **µEcoFAB**, the single-plant scale **EcoFAB,** and the larger **EcoPOD** (see below for descriptions)**.** These platforms enable us to explore complex interactions under fully controllable and highly replicated conditions and evaluate soil biogeochemical processes driven by plant-microbe interactions. These devices are compatible with conventional biosafety workflows to advance secure biosystems design by using contained and controlled environments of EcoFABs and EcoPODs to understand persistence and fate of engineered genes, microbes and synthetic communities through the environment.

µEcoFAB is a mm-scale fabricated ecosystem that mimics chemical gradients in the environment known to create micro-niches and contribute to the diversity of the microbial

communities in soil. Using a multi-layered design, the μEcoFAB generates 3 dimensional gradients (such as oxygen, synthetic exudates, and pH) in the microbial incubation chamber. This enables detailed examination of interactions and functions observed in the larger scale fabricated ecosystems We hypothesize that microbes known to colonize the rhizosphere will be enriched in regions with the highest synthetic exudate concentrations and low pH. **The EcoFAB** is a cm-scale chamber that captures key features of an ecosystem of interest (e.g., soil, plants). It includes main components of the ecosystem, including native, synthetic, or engineered microbial communities and different microbes (bacteria, archaea, fungi), plants, growth media (soil, hydroponics, sand), and is integrated with multiple measurement technologies, including high resolution imaging, and ports for collecting samples. The EcoFAB provides a sterile and highly controlled system that enables plant growth, phenotyping and plant-microbe imaging, sampling for metabolite, and microbial community composition analysis. We have been able to see unique establishment patterns in the rhizosphere by imaging multiple fluorescent strains of *Pseudomonas simiae* within EcoFABs. Since the presence of microorganisms in the rhizosphere does not necessarily indicate that they are metabolically active within the environment, we are identifying an active fraction of microbes in the rhizosphere using bio-orthogonal noncanonical amino acid tagging (BONCAT) combined with fluorescence-activated cell sorting (FACS). This was accomplished by using a modified EcoFAB that allowed cultivation of switchgrass in soil followed by BONCAT labeling and cell sorting, single-cell shotgun sequencing, and metabolomics. **The EcoPOD** is a meter-scale fabricated ecosystem that provides a contained and controlled environment equipped with sensors to regulate temperature, humidity, and other important climatic parameters both above and below ground thus complementing EcoFAB capabilities. Specifically, EcoPODs provide contained and controlled environments that reflect key aspects of field conditions to focus EcoFAB experiments on key microbes, metabolites, and genes. In turn, EcoFABs provide relatively low-cost and high-throughput capabilities for deconstructing molecular mechanisms to develop generalizable models, mechanisms, and principles for testing in EcoPODs. In the longer-term the EcoPODs will be linked to field studies to provide a complete translational path between laboratory and field experiments.

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