Title: The Development of Plant and Soil Fabricated Ecosystems (EcoFABs) for Standardized Microbiome Experiments

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Project Goals: Short statement of goals. The TEAMS project (Trial Ecosystems for the Advancement of Microbiome Science) is focused on the development of technologies and protocols to facilitate plant and soil microbiome experiments using fabricated ecosystems (EcoFABs). EcoFABs are intended to provide standardized methods for understanding microbial interactions in complex environments through the creation of environments that capture relevant aspects of their natural environment like spatial heterogeneity, but do so in a reproducible manner better suited for inter-laboratory investigations and designed to allow for multiple avenues of investigation (e.g., microscopy, metabolomics). Through the TEAMS project two types of EcoFABs have been developed, the EcoFAB 2.0, which can be mass produced and is compatible with robotics for automated plant-microbiome experiments, and the soil EcoFAB, which is designed to study microbial interactions within a defined spatial structure. Finally, the EcoBOT is being developed to facilitate automated EcoFAB experiments in a single system containing an integrated growth chamber, liquid handling robot, inverted microscope and hyperspectral camera. An automated arm allows the remote or programmed transfer of EcoFABs between stations to monitor plant development and collect metabolite and microbial samples throughout its growth to facilitate automated EcoFAB experimentation.

Abstract text:

The TEAMS project has organized inter-laboratory experiments to evaluate the reproducibility of microbial community analyses in plant microbiome experiments within the EcoFAB 2.0. These experiments follow up on a previously published inter-laboratory study that demonstrated that the impacts of growth media on the exudation, and root and shoot phenotypes of *Brachypodium distachyon* 21-3 were reproducible between laboratories when cultivated in EcoFABs[1]. This iteration is focused on the interactions between *B. distachyon* 21-3 and microbial communities and includes DNA sequencing to evaluate microbial colonization as well as metabolomics and phenotyping analyses. This study utilizes a standardized consortium of isolates made from a field grown switchgrass plant and selected to represent a phylogenetically diverse set of bacteria. All 17 of these isolates have been sequenced and evaluated for their reproducible growth *in vitro* under different nutrient conditions. Pilot EcoFAB experiments have demonstrated that inclusion of the entire community vs 16 members that exclude a

Paraburkholderia sp. have a significant impact on shoot fresh weight and root/shoot mass ratios. The details of this process will be investigated as part of a five institution intercomparison study. The results of this study will lay the foundation for an comparison study by scientists at all four of the DOE Bioenergy Research Centers. Together these studies will establish a common plant-microbiome experimental system to enable collaborative research studies. The results of these intercomparison studies will also be used to benchmark the automated EcoBOT system vs. conventional experimentation.

Researchers at PNNL have developed soil EcoFAB devices designed to complement the plant-microbe focused devices described above. These EcoFABs take advantage of custom polymers compatible with advanced mass spectrometry approaches to facilitate spatial mass spectroscopy imaging. They also provide solid structures of defined sizes and spacing to support microbial growth. Initial experiments were performed by inoculating each quadrant of the EcoFABs with 1 of 4 phylogenetically diverse bacteria expressing a fluorescent protein. After a week, confocal microscopy revealed that the bacteria each colonize the device in distinct, but reproducible manners. The reproducibility of the developed soil EcoFAB experiments will also be assessed using inter-laboratory evaluations involving laboratories at 4 separate institutions.

Together these capabilities provide a powerful new platform for scientists to build on each other's research and collaborate to advance microbiome science.

References/Publications

1. Sasse, J., Kant, J., Cole, B. J., Klein, A. P., Arsova, B., Schlaepfer, P., … Northen, T. R. (2019). Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass. New Phytologist, 222(2), 1149–1160. https://doi.org/10.1111/nph.15662

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