IMAGINE BioSecurity: Mesocosm-based Methods to Evaluate Biocontainment Strategies and Impact of Industrial Microbes Upon Native Ecosystems.

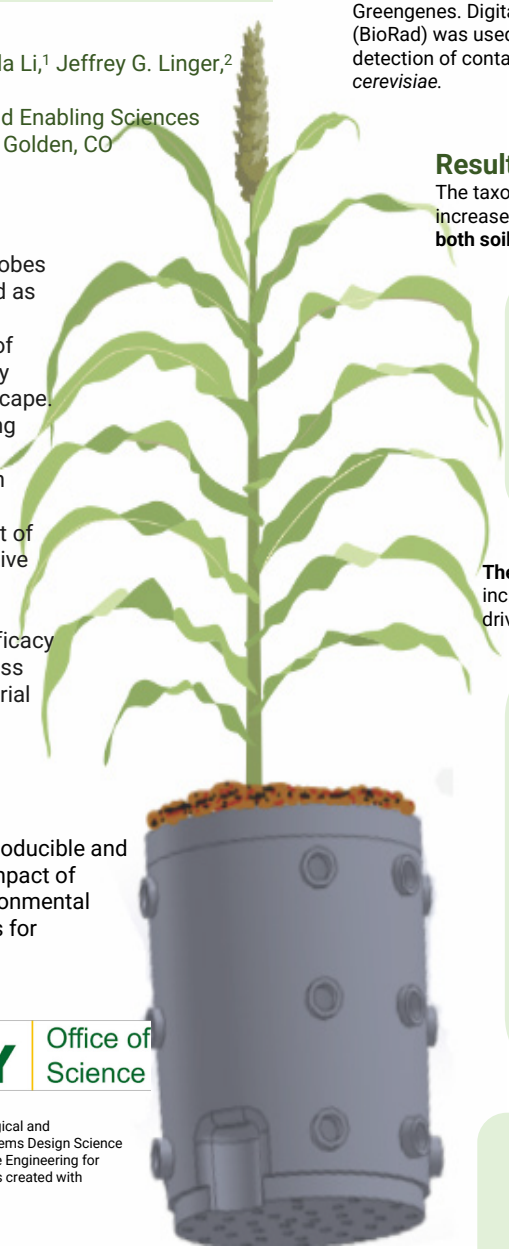
Kathleen L. Arnolds,[1]* Riley C. Higgins,[1] Gabriela Li,[1] Jeffrey G. Linger,[2] Michael T. Guarnieri[1] (katie.arnolds@nrel.gov)
[1]Biosciences Center; [2]Renewable Resources and Enabling Sciences Center, National Renewable Energy Laboratory, Golden, CO

Background

Genetically modified industrial production microbes and their associated bioproducts have emerged as an integral component of a sustainable bioeconomy. However, the rapid development of these innovative technologies raises biosecurity concerns, namely, the risk of environmental escape. Current laboratory-based biocontainment testing systems do not accurately reflect complexities found in natural environments, necessitating an environmentally relevant analysis pipeline that allows for detection of rare escapees, the effect of associated bio-products, and the impact on native ecologies. To this end, we have developed an approach that utilizes soil mesocosms and integrated systems analyses to evaluate the efficacy of novel biocontainment strategies and to assess the impact of production systems upon terrestrial microbiome dynamics.

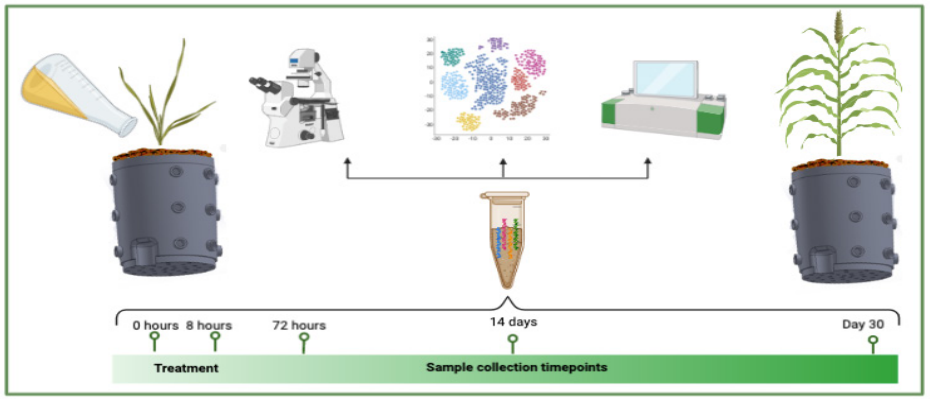
Goal

Develop an approach that will allow for the reproducible and streamlined assessment of the behavior and impact of engineered microbes in controlled versus environmental conditions to predictively devise new strategies for responding to biological escape.



Experimental design

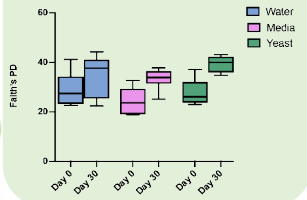
Mesocosms consist of 3D-printed pots with sampling ports, potting soil, and *Sorghum bicolor* seedlings. Contamination was modeled by inoculation with *Saccharomyces cerevisiae*. Soil was collected over 30 days, gDNA was extracted, and the ITS & 16S regions were sequenced and analyzed with Qiime2, UNITE, and Greengenes. Digital droplet PCR (BioRad) was used for the targeted detection of contaminating *S. cerevisiae*.



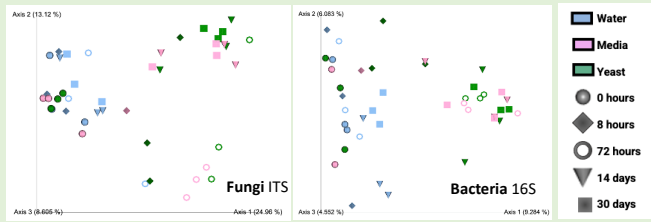
Results

The taxonomic diversity of microbes in a given sample, or the **alpha diversity, was increased** in all groups over time. The greatest increase was in mesocosms that received yeast. The overall composition of the soil microbial communities, **the beta-diversity of both soil bacterial and fungal communities was significantly shifted as a function of time and treatment**

Alpha diversity Faith's PD



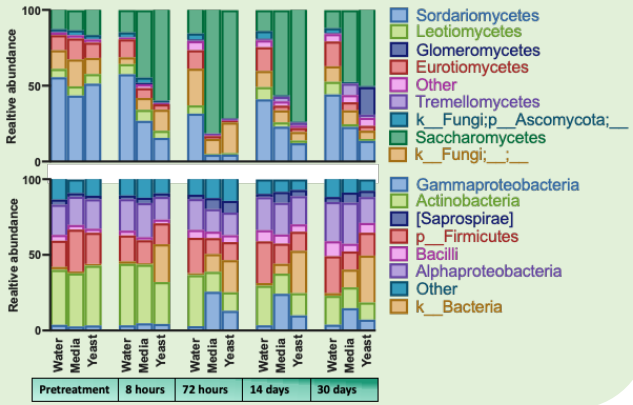
Beta diversity PCoA Bray-Curtis



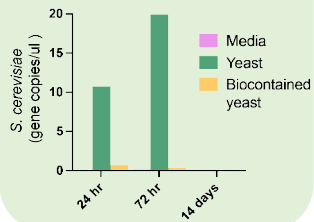
Each point on these plots represents the entire fungal (ITS) or bacterial (16S) community from one sample, the closer two points are the more similar their microbial communities

The dominant classes of fungi and bacteria were shifted over time and as a function of treatment with the *Sacchromycetes* increasing and *Sordariomycetes* decreasing, and expansion of *Glomeromycetes*. Shifts in the bacterial community were largely driven by an expansion of the class *Gammaproteobacteria*, coinciding with reductions in the *Firmicutes* and *Actinobacteria*.

Relative Abundance Class



Direct Detection (ddPCR)



Bio-contained *S. cerevisiae* does not propagate and is detected at lower levels than an uncontained strain as measured by ddPCR

Key Points

- Contamination with *S. cerevisiae* altered microbial community dynamics for duration of the experiment
- Bio-contained yeast did not propagate or thrive in the mesocosm
- Mesocosms model dynamic terrestrial ecosystem that can be used to evaluate biocontainment and assess the environmental impacts of industrial microbes

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), Genomic Science Program, Secure Biosystems Design Science Focus Area | IMAGINE BioSecurity: Integrative Modeling and Genome-scale Engineering for Biosystems Security, under contract number DE-AC36-08GO28308. Figures created with BioRender.com. Design credit to S. Kutos (Fordham U) and E. Barnes (JGI)

2024 GSP and ECR PI Meeting
Bethesda, Maryland
April 2-3, 2024
NREL/PO-2700-89066