

**Title:** Simplified Microbial Communities Provide Diverse Complementation Potential for Genome-reduced Microorganisms

**Authors:** Citlali Fonseca<sup>1\*</sup> ([fonseca-garcia@berkeley.edu](mailto:fonseca-garcia@berkeley.edu)), Joshua Elmore<sup>2</sup>, Ryan McClure<sup>2</sup>, Henri Baldino<sup>2</sup>, Ritu Shresthra<sup>2</sup>, Andrew Wilson<sup>2</sup>, Andrew Frank<sup>2</sup>, Pubudu Handakumbura<sup>3</sup>, Devin Coleman-Derr<sup>1</sup>, **Robert Egbert<sup>2</sup>**

**Institutions:** <sup>1</sup>Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, United States. <sup>2</sup>Biological Sciences Division, Pacific Northwest National Laboratory, Richland WA, 99352. <sup>3</sup>Environmental Molecular Sciences Laboratory, Richland WA, 99352.

**Website:** <https://genomicscience.energy.gov/research/sfas/pnnlbiosystemsdesign.shtml>

**Project Goals:** The Pacific Northwest National Laboratory Persistence Control Scientific Focus Area aims to gain a fundamental understanding of factors governing the persistence of engineered microbial functions in rhizosphere environments. From this understanding, we will establish design principles to control the environmental niche of native rhizosphere microbes. In our first funding period, we are examining the efficacy of genome reduction and metabolic addition to plant root exudates in environmental isolates as persistence control strategies using the bioenergy crop sorghum and defined microbial communities as a model ecosystem. Effective persistence control will lead to secure plant–microbe biosystems that promote stress-tolerant and highly productive biomass crops.

**Abstract Text:** The beneficial interactions between plants and rhizosphere microorganisms have been well documented over the past several years. More recently, researchers have explored ways of engineering microbial species to enhance these interactions and further promote plant growth. However, the introduction of engineered organisms to soil presents questions about how they can be restricted to their intended roles and not persist outside their functional or geographic niches. Genome reduction to remove key metabolic or stress-response pathways is one strategy to achieve this goal of niche restriction but its efficacy in the presence of genetic or metabolic complementation potential from the complex soil microbiome is not known.

To investigate complementation dynamics for engineered soil microbes, we have developed several Synthetic Communities (SynComs) that were derived from a native soil microbiome harvested from sorghum fields. We established these SynComs directly from complex soil samples (top-down) or from mixtures of soil isolates (bottom-up) and collectively they represent defined microbial communities that provide a palette for genetic or metabolic complementation of genome-reduced species. Using growth on agar plates supplemented with sorghum root-exuded metabolites we reduced native soil microbiome complexity and developed SynComs comprised of tens of species with relatively even representation and containing a number of key soil microbial taxa. We show that these SynComs are initially dynamic in their development before stabilizing and falling in complexity and richness. Of the methods tested for cold storage we show that glycerol, and in some cases lyophilization, enabled reconstitution of communities with the least dissimilarity from the original SynComs. Finally, by growing SynComs across a range of single carbon sources we highlight their complementation potential. Among several C sources tested arginine, adenosine, mannitol, and hydroxycinnamic acid led to SynComs that maintained the most

diversity, suggesting that in soils with these C sources these SynComs could serve as wide ranging complementation sources. These experiments reveal how synthetic microbial communities can be generated and stored as tools for microbiome engineering and begin to show the species and carbon utilization pathways that represent the highest potential for metabolic complementation of genome-reduced species. Future experiments will focus on incubation of genome-reduced species with SynComs to characterize their complementation effects, pathways and kinetics to improve our ability to control the persistence of engineered bacterial functions in native soil systems.

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