

Targeted DNA Editing Within Microbial Communities

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Project Goals: Microbial Community Analysis and Functional Evaluation in Soils (mCAFEs) will use 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 will use ‘bottom-up’ defined microbial assemblies that enable detailed characterization of both constituent isolates and synthetic communities. This will be complemented by ‘top-down’ investigation 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.

Our understanding of microbial life has largely been achieved by observing the effect of targeted genetic manipulations on isolated microbial species in laboratory settings. Unfortunately, this provides limited insights into the complex and societally relevant communities of microorganisms that exist in nature. We have created a generalizable toolset for targeted genetic manipulation within laboratory microbial communities that will allow microorganisms to be altered and studied in a community context. As a first step, we have developed environmental transformation sequencing (ET-Seq) to determine *in situ* what microbes can be edited within a community with what efficiency. The roadmap for tractability produced by ET-Seq is then used to guide CRISPR-Cas based targeted editing within the community. Editing events have currently been measured *in situ* in a 9 member synthetic soil community, and an ~60 member thiocyanate degrading community. The ability to make targeted DNA perturbations to microbial communities will open up the field of *in situ* microbial genetics which will greatly improve our basic science understanding of microbial genomics and microbial interactions.

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