**Title: Mycorrhizal Fungi Mediate Plant and Bacterial Response to Water Limitation in a Marginal Soil**

**Authors:** Rachel Hestrin[1*] (hestrin1@llnl.gov), Megan Kan[1], Rebecca Ju[1], Jeffrey Kimbrel[1], Christina Ramon[1], Prasun Ray[2], Rina Estera-Molina[3], Steven Blazewicz[1], **Kelly Craven[2], Mary Firestone[3], Peter Weber[1], Rhona Stuart[1], Erin Nuccio[1], Jennifer Pett-Ridge[1]**

**Institutions:** [1]Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; [2]Noble Research Institute, Ardmore, OK; [3]University of California, Berkeley, CA

**Website:** https://bio-sfa.llnl.gov/

**Project Goals: Algal and plant systems have the unrivaled advantage of converting solar energy and $CO_2$ into useful organic molecules. Their growth and efficiency are largely shaped by the microbial communities in and around them. The µBiospheres SFA seeks to understand phototroph-heterotroph interactions that shape productivity, robustness, the balance of resource fluxes, and the functionality of the surrounding microbiome. We hypothesize that different microbial associates not only have differential effects on host productivity but can change an entire system's resource economy. Our approach encompasses single cell analyses, quantitative isotope tracing of elemental exchanges, 'omics measurements, and multi-scale modeling to characterize microscale impacts on system-scale processes. We aim to uncover cross-cutting principles that regulate these interactions and their resource allocation consequences to develop a general predictive framework for system-level impacts of microbial partnerships.**

**Abstract text:**

Background: Multipartite mutualisms between plants, mycorrhizal fungi, and other soil biota can enhance plant productivity, resilience to stress, and carbon (C) allocation below ground. A better understanding of these relationships can inform more sustainable management of cellulosic bioenergy crops, such as switchgrass (*Panicum virgatum* L.), a C4 perennial grass championed for its high biomass yields and tolerance to a broad spectrum of climatic conditions and soils unsuitable for intensive agriculture. We are investigating context-dependent multipartite relationships between *Panicum hallii*—a model species closely related to switchgrass, two mycorrhizal lineages with different genomic repertoires—the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and the Sebacinales fungus *Serendipita bescii*, and microbial communities in a marginal soil. We hypothesize that both mycorrhizal lineages mediate microbial community composition and function, plant and microbial tolerance to resource limitation, and soil organic matter dynamics. However, we anticipate that due to their different genomic repertoires, each fungus will elicit a distinct biotic response.

Experimental design: We grew *P. hallii* with and without *R. irregularis* or *S. bescii* in microcosms containing 'live' soil harvested from a marginal Oklahoma pasture. Each microcosm contained a hyphal ingrowth core that excluded plant roots. This allowed us to investigate rhizosphere and hyphosphere processes separately. We maintained half of the microcosms under water-replete conditions and the other half under water-limited conditions. We also maintained half of the microcosms in a $^{13}CO_2$ atmosphere, which allowed us to track plant- and mycorrhizal-derived $^{13}C$ into other soil C pools (live and dead microorganisms, metabolites, mineral associated organic matter, particulate organic matter) and C fluxes ($CO_2$, dissolved C, volatile

compounds). We harvested the microcosms at 5, 8, and 12 weeks after the onset of $^{13}CO_2$ labeling. After 12 weeks, we also conducted quantitative stable isotope probing (qSIP) with $H_2{}^{18}O$ to assess microbial growth rates in hyphosphere soil. Although mycorrhizal fungi were present in the native soil microbial community, qPCR analyses show that the *R. irregularis* and *S. bescii* inoculant strains were more abundant in roots and soils harvested from respectively inoculated microcosms. Thus, differences observed in mycorrhizal-inoculated microcosms represent the additional effect of these inoculants despite the presence of a background microbial community.

Results: Soil moisture regime and mycorrhizal inocula altered plant and microbial activity, with significant effects on soil C cycling. In uninoculated microcosms, rhizosphere soil $^{13}C$ content was similar under both moisture regimes. In mycorrhizal-inoculated microcosms, rhizosphere soil $^{13}C$ was lower in water-replete compared to water-limited soil. This suggests that under water-replete conditions, plants inoculated with *R. irregularis* and *S. bescii* either allocate less C below ground (possibly due to a reduced need to invest in resource acquisition) or that the C that they allocate below ground is consumed and respired more quickly than under water-limited conditions. Following water limitation, bacterial growth rates, microbial growth efficiency, and the diversity of the active bacterial community were suppressed in uninoculated soils, but not in soils inoculated with either fungus. Several of the bacterial taxa that responded positively to mycorrhizal inocula in water-limited soil belong to lineages that are considered drought-susceptible. Although both fungi supported bacterial communities exposed to water limitation, *R. irregularis* elicited a stronger positive response than *S. bescii.* Together, our findings demonstrate that context-dependent mycorrhizal relationships influence biotic response to resource limitation.

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