

## Investigating Chloroplast Signal within the Hyphae of Diverse Fungi

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**Project Goals: The vast taxonomic diversity and the complexity of interactions within the soil microbiome presents a unique challenge. Many of the interactions between soil-dwelling bacteria and fungi are not yet well understood, and a more comprehensive understanding would lead to substantial agricultural, environmental, and energy-focused advancements. These potential developments align with the foci of the DOE, and would influence multiple scientific fields. The aim of this Science Focus Area (SFA) is to better understand the diverse and abundant interactions within the soil rhizosphere and decipher the mechanisms behind their communication. Herein, we explored an unexpected endo-hyphal signal that was revealed following an amplicon screen of fungi from diverse culture collections.**

The endo-hyphal microbiome contains many uncharacterized inhabitants and interactions. This fungal microbiome, coupled with an extensive network of extracellular interactions with bacteria and plants within the soil, contribute to the complex ecosystem services facilitated by fungi. We sought to characterize the members of the intracellular fungal microbiome as a way to better understand the roles of fungi and their associated endosymbionts. Based on a 16S rRNA amplicon screen of four distinct fungal collections from different geographic locations, we identified taxonomic signatures of many bacteria not previously known to be associated with fungi. Rather unexpectedly, one of the amplicon sequence signatures that was found across all culture collections, was a recurrent signal for various plant chloroplasts. Several techniques were utilized in validating the potential associations between fungi and the chloroplasts (which we anticipate are represented by these signatures), including FISH staining, phylogenetic analyses, qPCR and PCR amplifications, as well as broader bioinformatic screens. This discovery is leading to several new avenues of research to explore the acquisition, maintenance, evolution, as well as any physiological or ecological function of chloroplasts within fungi.

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