## Spatiotemporal Mapping of Lignocellulose Decomposition by a Naturally Evolved Fungal Garden Microbial Consortium

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This project will carry out a multi-omics approach to uncover the mechanisms that drive cooperative fungal-bacterial interactions that result in the degradation of lignocellulosic plant material in the leaf-cutter ant fungal garden ecosystem. This approach will provide the knowledge needed for a predictive systems-level understanding of the fungal-bacterial metabolic and signaling interactions that occur during cellulose deconstruction in an efficient, natural ecosystem.

Naturally evolved microbial systems that are capable of efficient deconstruction of plant cell wall biomass exist. Biomass deconstruction in these natural communities is often dependent on bacterial-fungal symbiosis, yet the molecular underpinnings of these interactions are poorly understood. An excellent example of such a system is the leaf-cutter ant fungal garden ecosystem, which employs inter-kingdom interactions to liberate energy rich carbohydrates from plant lignocellulose biomass. Unfortunately, the microbial community dynamics of the leafcutter ant fungal garden ecosystem are a challenge to assess because of the high heterogeneity of species composition and phenotype occurring across space and time during plant biomass deconstruction.

To understand how the fungal garden is able to degrade plant matter with such efficiency, it is necessary to study the metabolic interactions and biochemical pathways utilized by its microorganisms in each microscopic region of the fungal garden. This research will accomplish that with novel microscale metabolomics, lipidomics, and proteomics approaches that can analyze very small samples, providing detailed information on the location and function of fungal and bacterial molecules. In this initial study, we evaluated the lipidomic differences between the leaves feeding the gardens, microscopic hyphae called gongylidia produced by the fungus to feed the ants, and spatially-resolve regions of the fungus garden at initial to advanced stages of leaf degradation. The lipid species identified in the sample types and garden regions varied significantly. Lipids containing alpha-linolenic acid from the leaves were enriched in the top of the gardens, but not dominant in the middle or bottom regions. Furthermore, gongylidia were dominated by lipids containing linoleic acid. These microscale lipidomic measurements deciphered biomass lipid metabolism and bacterial-fungal lipid syntheses by spatially resolving those regions where specific deconstruction activities are enriched, thus functionally reducing the heterogeneity of the system.

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