A Novel Examination of the Diversity of Three Leafcutter Ant Colony Microbiomes

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Abstract

Leafcutter ants are a group of fungus-farming ants belonging to the tribe *Attini*, including any of the 47 species of ants belonging to the *Atta* or *Acromyrmex* genera. They collect leaves and plant matter from their habitat and use it to cultivate fungus, which the larvae of the colony feed on. The fungal gardens develop a microbiome, which to our knowledge, has not been thoroughly explored. Some members of the microbiome even play a role in the health of the fungus and their symbiote ants. For example, *Pseudonocardia* bacteria grow on the ants and play a role in suppressing the growth of other harmful microbes in the gardens. Given the possible benefits these organisms afford both the ants and fungi, it is possible that the garden microbiome may have played a role in shaping the evolution and success of leafcutter ants.In this project, we used Illumina sequencing techniques to identify microbial species profiles and data which was processed by the DNA Learning Center's DNA Subway Purple Line metabarcoding tool. Euthanized leafcutter ants and fungal samples were obtained from Long Island Aquarium, Audubon Zoo and myrmecological hobbyists from around the US. Members of the genus *Atta* and *Acromyrmex*, and associated fungus, were examined for evolutionary relationships between them and their associated microbiome profiles. While most species had cyanobacteria and proteobacteria present on their fungus, some had unique bacteria present that play vital roles in their given colony.

Introduction

Leafcutter ants comprise 47 species in the genus, for example *Atta*, *Acromyrmex,* and *Trachymyrmex* develop symbiotic relationships with fungi they grow. The bacteria differs based on ant species which has taxonomic benefits as it allows for better identification of ants and fungi. According to McGill University's Ada Mcvean, Leafcutter ants grow the fungi with the help of leaves as fertilizer for the fungi. In addition to the support of fungal growth, leaf cutter ants can protect the fungi from pests, molds, decay, and garbage (Mcvean, 2019). Fungi help the leafcutter ants as they act as a food source for the ant larvae; however, the adult ants do not feed on the fungi because they obtain nutrients through the sap from leaves. According to Douglas Woodham, On the ant, a certain bacteria, Pseudonocardia, grows (Woodham, 2013, 2013). The purpose of the Bacteria is to prevent disease from spreading to the ants and their fungal gardens, which allows them to develop through generations without the dangers of disease (Woodham, 2013, 2013). Additionally, as the bacteria multiplies and grows, the DNA is able to signal the ancestry of the host they originate from. In our project, we investigated the difference in species of bacteria present in the fungal colonies of leafcutter ants.

Relative Abundance of Bacteria Present in Three Leafcutter Ant Colonies

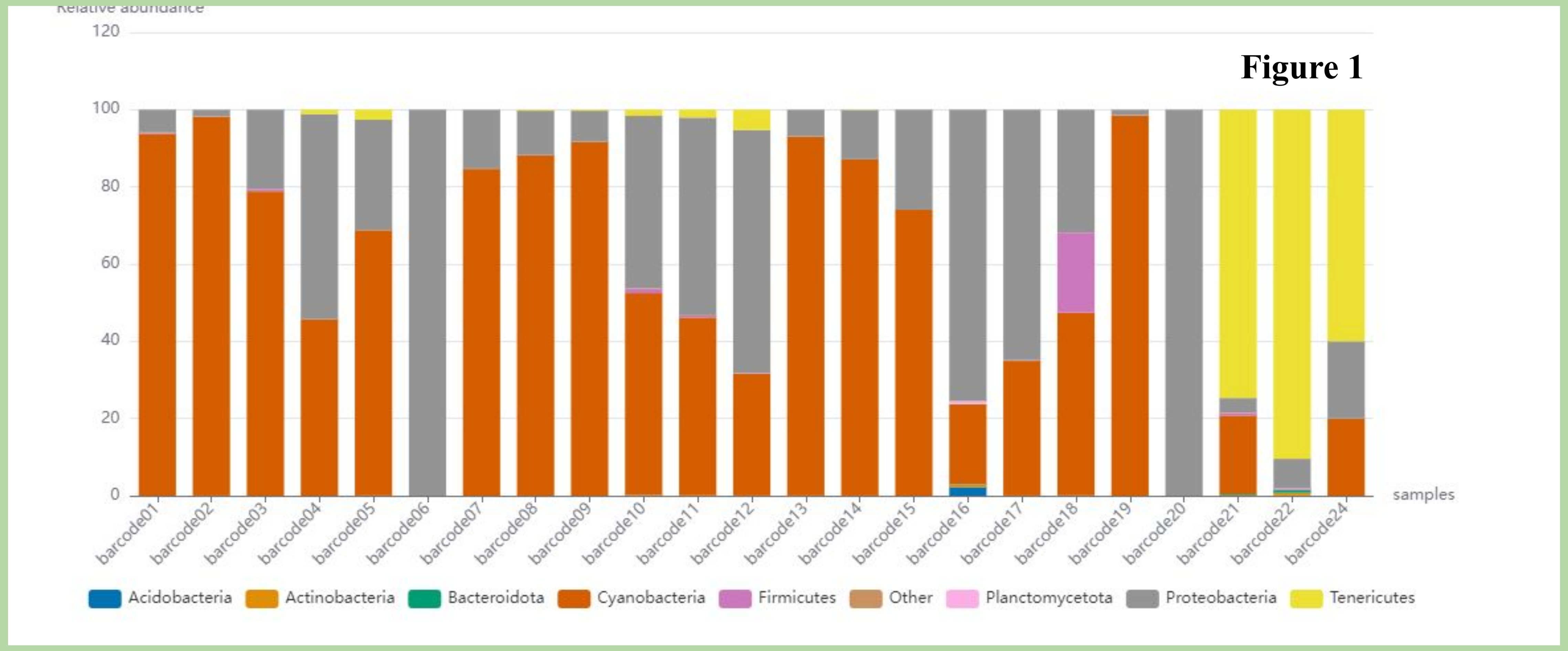

Figure 1

- The Trinidad Colony: Barcodes 1-15 These barcodes exhibited a consistent pattern with predominantly cyanobacteria, primarily from the orders *Chroococcales* and *Oscillatoriales*. These cyanobacteria are likely involved in nitrogen fixation, contributing to the nutrient availability within the fungal gardens. proteobacteria, specifically *Hyphomicrobiales*, were present in smaller proportions, suggesting a potential role in nutrient cycling or other metabolic processes.
- The Long Island Aquarium Colony: Barcodes 16-19 The prevalence of proteobacteria (*Hyphomicrobiales*) and cyanobacteria (*Chroococcales*, *Oscillatoriales*) may play critical roles in nutrient cycling, nitrogen fixation, and disease suppression within the fungal gardens. This bacterial community structure likely contributes to the colony's health and longevity by maintaining a balanced ecosystem.
- Audubon Colony: Barcodes 20-22 has a prevalence of cyanobacteria (*Chroococcales*) and cyanobacteria (*Chroococcales*, *Oscillatoriales*) which suggests adaptations to specific environmental niches and ecological functions within each colony. Overall this colony was dominated by proteobacteria from the order Entomoplasmatales, which belong to the class *Mollicutes* (*Tenericutes*). These bacteria are often associated with insects and can influence host physiology and immunity.

Metadata Discussion

The variations in metadata between the Audubon, Long Island Aquarium, and the Trinidad colony are essential to understanding microbial diversity. For instance, the different plant species provided to each colony can affect the nutrient composition of the fungal gardens, potentially influencing the microbial communities. Additionally, differences in temperature and humidity levels could create unique environments within each colony, leading to distinct microbial profiles. These microbial profiles refer to the composition and abundance of different bacterial and fungal species present in the colonies. Understanding these variations is crucial for understanding the differences within the leafcutter ant colonies and their associated microbiomes. The specific environmental conditions, such as the temperatures higher humidity of 80 degrees and 80 percent in the Long Island Aquarium colony, favors the growth of certain bacterial taxa like proteobacteria, which are known to thrive in such conditions, which is why we see a large abundance of proteobacteria. On the other hand, the cooler temperatures and lower humidity in the Trinidad colony creates conditions conducive to the proliferation of different bacterial species, such as actinobacteria, which are known to prefer cooler and drier environments. This is likely why we see this actinobacteria abundant in the colony. Understanding that environmental factors shape these bacterial communities is essential for comprehending the relationships between ants, fungi, and associated bacteria and their role in colony health.

References and Research Paper

Materials & Methods

- When collecting our ants and fungi we and our partners recorded metadata such as temperature, humidity, species of ants, and other metadata

Figure 2: Leaf Cutter Ant Colony & Fungal Garden

- To isolate the DNA of ants for verification of their species, we used the Chelex isolation
- To isolate the DNA of the microbiome, we used Qiagen's Powersoil Pro Kit tol target and extract the V4 region of the microbial 16S rRNA gene. We did PCR to amplify the DNA and gel electrophoresis to verify amplification. PCR cleanup was performed by the DNALC. Our samples were sequenced using Azenta's Amplicon-EZ

Figure 3: Metadata	Long Island Aquarium Colony	Audubon Colony	Trinidad Colony
Temperature	80℉	75℉	73℉
Humidity	80%	75%	80-90%
Light cycle	8 hours ambient light	12 hours	Varied from 12-24 hours
Plant Species Fed	*Rubus, Ligusturm, Euonymus,* Olive,and *Fortuni*	White Clover, Glossy, *Ligustrum*, and Chinese Elm	*Rubus armeniacus* and *Prunus laurocerasus*

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