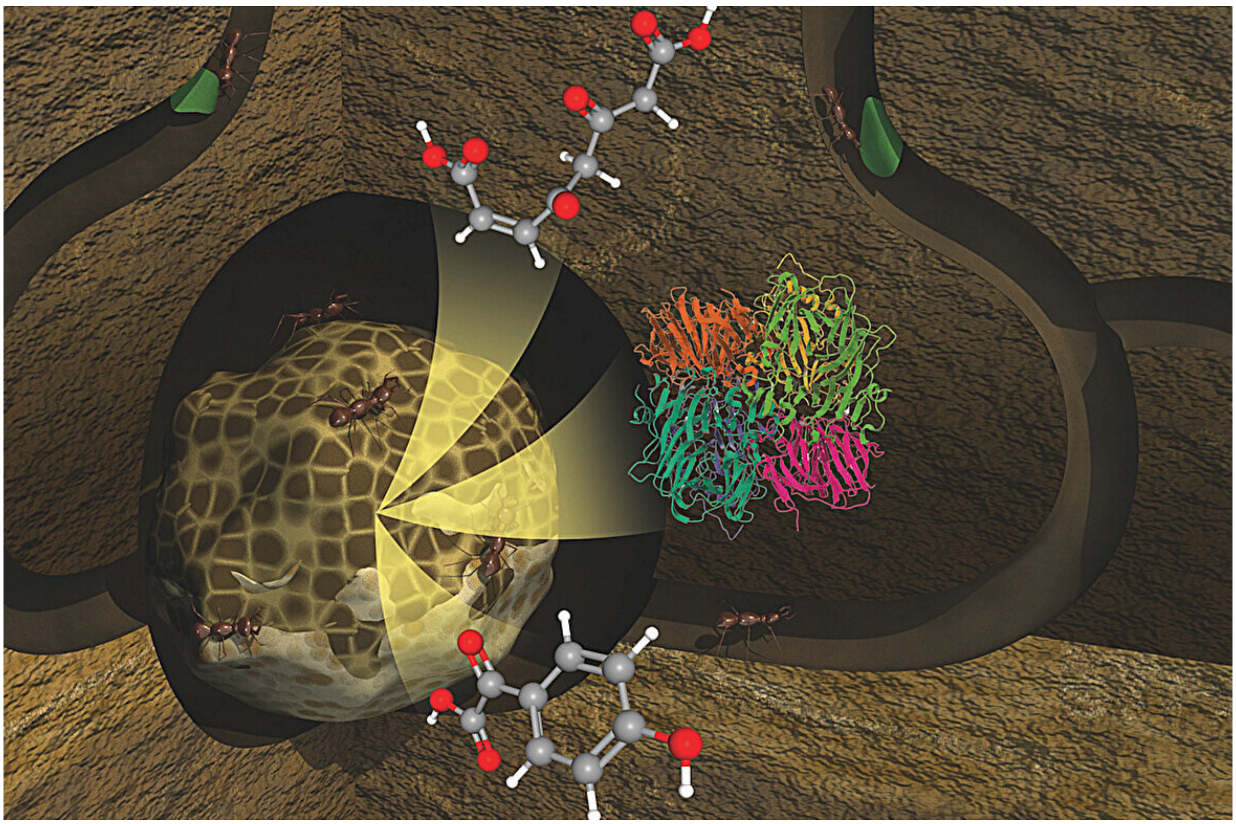
How leafcutter ants cultivate a fungal garden to degrade plants could provide insights into future biofuels

February 1 2024, by Maegan Murray



Scientists have spent decades researching clean and efficient ways to break down plants for use as biofuels and other bioproducts. A species of ants works with a type of fungus to accomplish this naturally. Kristin Burnum-Johnson and her team set out to investigate how this is accomplished at the molecular level. Credit: Mike Perkins and Nathan Johnson | Pacific Northwest National Laboratory

Scientists have spent decades finding ways to efficiently and affordably degrade plant materials so that they can be converted into useful bioproducts that benefit everyday life.

Bio-based fuels, detergents, nutritional supplements, and even plastics are the result of this work. And while scientists have found ways to degrade plants to the extent needed to produce a range of products, certain polymers such as lignin, which is a primary ingredient in the cell wall of plants, remain incredibly difficult to affordably break down without adding pollutants back into the environment. These polymers can be left behind as waste products with no further use.

A specialized microbial community composed of fungus, leafcutter ants, and bacteria is known to naturally degrade plants, turning them into nutrients and other components that are absorbed and used by surrounding organisms and systems. But identifying all components and biochemical reactions needed for the process has remained a significant challenge—until now.

Kristin Burnum-Johnson, science group leader for Functional and Systems Biology at Pacific Northwest National Laboratory (PNNL), and a team of fellow PNNL researchers have developed an imaging method called metabolome informed proteome imaging (MIPI). This method allows scientists to peer deep down to the molecular level and view exactly which base components are part of the plant degradation process, as well as what, when, and where important biochemical reactions occur that make it possible.

Using this method, the team revealed important metabolites and enzymes that spur different biochemical reactions that are vital in the degradation process. They also revealed the purpose of resident bacteria in the system—which is to make the process even more efficient. These insights can be applied to future biofuels and bioproducts development.

The team's research was recently published in *Nature Chemical Biology.*

Symbiotic relationship between leafcutter ants and fungus reveal key to success in plant degradation

For its research, the team studied a type of fungus known for its symbiotic relationship with a species of leafcutter ants—a fungus known as Leucoagaricus gongylophorus. The ants use the fungus to cultivate a fungal garden that degrades plant polymers and other material. Remnant components from this degradation process are used and consumed by a variety of organisms in the garden, allowing all to thrive.

The ants accomplish this process by cultivating fungus on fresh leaves in specialized underground structures. These structures ultimately become the fungal gardens that consume the material. Resident bacterial members help with the degradation by producing amino acids and vitamins that support the overall garden ecosystem.

"Environmental systems have evolved over millions of years to be perfect symbiotic systems," Burnum-Johnson said. "How can we better learn from these systems than by observing how they accomplish these tasks naturally?"

But what makes this fungal community so difficult to study is its complexity. While the plants, fungus, ants, and bacteria are all active components in the plant degradation process, none of them focus on one task or reside in one location. Factor in the small-scale size of the biochemical reactions occurring at the molecular level, and an incredibly difficult puzzle presents itself. But the new MIPI imaging method developed at PNNL allows scientists to see exactly what is going on throughout the degradation process.

"We now have the tools to fully understand the intricacies of these systems and visualize them as a whole for the first time," Burnum-Johnson said.

Revealing important components in a complex system

Using a high-powered laser, the team took scans across 12-micron-thick sections of a fungal garden—the approximate width of plastic cling film. This process helped determine locations of metabolites in the samples, which are remnant products of plant degradation. This technique also helped identify the location and abundance of plant polymers such as cellulose, xylan, and lignin, as well as other molecules in specific regions. The combined locations of these components indicated hot spots where plant material had been broken down.

From there, the team homed in on those regions to see enzymes, which are used to kickstart biochemical reactions in a living system. Knowing the type and location of these enzymes allowed them to determine which microbes were a part of that process.

All of these components together helped affirm the fungus as the primary degrader of the plant material in the system. Additionally, the team determined that the bacteria present in the system transformed previously digested plant polymers into metabolites that are used as vitamins and amino acids in the system. These vitamins and amino acids benefit the entire ecosystem by accelerating fungal growth and plant degradation.

Burnum-Johnson said if scientists had used other more traditional methods that take bulk measurements of primary components in a system, such as metabolites, enzymes, and other molecules, they would simply get an average of those materials, creating more noise and masking information.

"It dilutes the important chemical reactions of interest, often making these processes undetectable," she said. "To analyze the complex environmental ecosystems of these fungal communities, we need to know those fine detail interactions. These conclusions can then be taken back into a lab setting and used to create biofuels and bioproducts that are important in our everyday life."

Using knowledge of complex systems for future fungal research

Marija Velickovic, a chemist and lead author of the paper, said she initially became interested in studying the fungal garden and how it degrades lignin based on the difficulty of the project.

"Fungal gardens are the most interesting because they are one of the most complex ecosystems composed of multiple members that effectively work together," she said. "I really wanted to map activities at the microscale level to better understand the role of each member in this complex ecosystem."

Velickovic performed all the hands-on experiments in the lab, collecting material for the slides, scanning the samples to view and identify metabolites in each of the sections, and identifying hot spots of lignocellulose degradation.

Both Velickovic and Burnum-Johnson said they are ecstatic about their team's success.

"We actually accomplished what we set out for," Burnum-Johnson said.

"Especially in science, that isn't guaranteed."

The team plans to use its findings for further research, with specific plans to study how fungal communities respond and protect themselves amid disturbances and other perturbations.

"We now have an understanding of how these natural systems degrade plant material very well," Burnum-Johnson said. "By looking at complex environmental systems at this level, we can understand how they are performing that activity and capitalize on it to make biofuels and bioproducts."

More information: Marija Veličković et al, Mapping microhabitats of lignocellulose decomposition by a microbial consortium, *Nature Chemical Biology* (2024). DOI: 10.1038/s41589-023-01536-7

Provided by Pacific Northwest National Laboratory