

Inventory of the world belowground: Using DNA to study fungal communities

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This *Pinus banksiana* forest with a rich *Arctostaphylos uva-ursi* and *Vaccinium* sp. understory was a collection site in this study examining how disturbance affects fungal community diversity, while simultaneously testing how sequencing methods compare in characterizing communities of plant root symbionts. Credit: Natalie Scott

Fungi tend to be absent in conversations regarding climate change, deforestation, and environmental pollution, overlooked in favor of large

and conspicuous plants. But plants don't exist in isolation. Beneath the ground, specialized fungi called mycorrhizae form an intricate web in and around roots that is vital to forest health and long-term carbon storage.

A new study published this month in *Applications in Plant Sciences* highlights the negative effects clearcutting has on these symbiotic fungi, showing that even after 17 years of regrowth, formerly deforested areas had less mycorrhizal diversity than intact forests. The study simultaneously offers a roadmap to researchers for determining the best way to analyze mycorrhizal communities when working with DNA.

'The wood-wide web'

In the [boreal forests](#) of sub-Arctic Canada, where co-author Greg Pec has studied [soil microbes](#) for the past ten years, thousands of mycorrhizal fungi species interlace the roots of trees with slender, threadlike hyphae. "If we were able to, in a sense, peel off the organic layer of soil, we would see hyphae just running rampant below ground," said Pec, an assistant professor of biology at the University of Nebraska.

Between 80% and 95% of living vascular plant species form a co-dependent relationship with mycorrhizal fungi, which do much of the heavy lifting in soil ecosystems. Mycorrhizae send out tendrils far beyond and below the reach of roots, from which they absorb water and nutrients for their host plants. In return, the symbiotic fungi live entirely on the sugars plants produce.

But mycorrhizae do far more than aid in the acquisition of nutrients. Their hyphae form connections between [plants](#), allowing them to communicate by transmitting hormones and chemical signals. Pec is particularly interested in how mycorrhizae maintain the health of forest communities and how they respond to disturbance. "There have been

studies indicating they provide defense against pathogens and can absorb heavy metals, making them an invaluable tool for habitat remediation," he said.

Their effectiveness at restoring forest habitats can be hampered by the type and severity of disturbance. Pec and his colleagues wanted to know how mycorrhizae responded to deforestation and chose two sites in Alberta, Canada—one that had been stripped of its vegetation 17 years prior and allowed to regrow and another that had remained untouched—to carry out their study. Although the two forests looked virtually the same above ground, the number of mycorrhizal species found growing on roots was severely impoverished below.

"When we look at forests from a restoration standpoint, we tend to think that five or ten years is a long time," Pec said. "What we're starting to find out, from a soil's perspective, is that soil turnover can take tens or even hundreds of years."

Disentangling mycorrhizal diversity

Analyses like these would have been impossible just a few decades ago. While Pec is primarily interested in the results showing how fungi respond to change, he also studies which methods are best suited to the types of questions researchers are asking.

Before the advent of DNA analysis, the vast majority of fungal diversity remained unknown, locked away beneath the soil. "Above ground, we only see those that are able to reproduce through some type of fruiting body, which is a very small fraction of what's actually underneath," Pec said.

Starting in the 1990s, scientists began isolating and analyzing the DNA from soil and root samples, which they found to be teeming with newly

discovered fungal species.

While analyzing the differences between old and new growth forests, Pec also tested out the efficacy of two widely used DNA sequencing techniques. The older method, called Sanger sequencing, allows researchers to obtain large DNA fragments for one organism per sample. The second, high-throughput sequencing, confers the opposite advantage, providing a large number of small sequences for a virtually unlimited number of organisms in a given sample.

High-throughput sequencing is typically the method of choice when researchers are studying fungal communities because of its ability to capture a wider breadth of diversity. Pec was able to retrieve DNA from more than 300 distinct fungal lineages in soil and root samples using high-throughput sequencing, while Sanger only came up with 28.

Despite the obvious advantage of high-throughput sequencing, scientists still run into a wall of new or unknown species that can be hard to interpret based on the short DNA strands. "The unidentified sequences are a huge issue," Pec said. "Sometimes the best we can say is that it's a fungus."

Although Sanger sequencing can be a slow and laborious process, it's often more reliable when trying to identify individual species. In a field where the unknowns outweigh the answers, Pec maintains that multiple methodologies will need to be leveraged to understand mycorrhizal diversity and the importance of fungi in ecosystems facing environmental change.

"Soil is life," Pec said. "We really need to start focusing on the diversity belowground to take stock of what's coming and what's already here in terms of prolonged drought, the increased severity of insect outbreaks, and more frequent fires."

More information: Jonathan A. Cale et al, Choices on sampling, sequencing, and analyzing DNA influence the estimation of community composition of plant fungal symbionts, *Applications in Plant Sciences* (2021). [DOI: 10.1002/aps3.11449](https://doi.org/10.1002/aps3.11449)

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