FEATURES

Mycologist Toby Kiers marks a fungi sampling plot in Chile's Valdivia National Reserve.

A FUNGAL SAFARI

A new nonprofit has launched an ambitious effort to raise the profile of often invisible soil fungi

By **Gabriel Popkin**, *in Chile's Villarrica National Park*
Photography by **Mateo Barrenengoa L.**

As a motley medley of mycologists climbed the basalt slopes of the Lanín volcano earlier this year, the green foliage at lower elevations gave way to autumnal golds and reds. Chile's famed *Araucaria*—commonly called monkey puzzle trees—soon appeared, their spiny branches curving jauntily upward like so many cats' tails.

Beneath the majestic trees, the scientists were focused on something far less glamorous—indeed, mostly invisible: mycorrhizal fungi, tiny organisms that intertwine with roots of the *Araucaria* and nearly all the other plants in this forest. The multinational research team had come to collect soil samples they hoped would, with help from DNA testing, reveal exactly which fungi live here, and how they support this complex assemblage of flora. By the end of an exhausting day that included bushwhacking through heavy brush, the fungi hunters had filled seven small plastic sacks with dirt from different locations. "I wouldn't be surprised if there are 100 undescribed species" of fungi in each bag, said mycologist Giuliana Furci, founder of the Chilean nonprofit Fungi Foundation and one of the expedition leaders.

The April ascent was also a road test of sorts: the first of many surveys that the Society for the Protection of Underground Networks (SPUN), a new fungus-focused nonprofit, hopes to conduct. It has raised some $3.5 million for an ambitious effort to map the global distribution of mycorrhizal fungi, which can create subterranean networks that are thought to play a key, but often overlooked, role in shaping ecosystems.

"Up to 50% of the living biomass of soils are these networks," says ecologist Toby Kiers of the Free University of Amsterdam, a co-founder of SPUN and one of the leaders of the Chile expedition. "We have to figure out where they are and what they're doing."

SPUN's approach is bold—even bombastic. The project launched last fall with a media-savvy campaign, including slick videos, arguing that society must do more to study and protect fungi to safeguard biodiversity and curb climate change. SPUN researchers describe themselves as "myconauts" heading into the unknown. They wear customized blue jumpsuits emblazoned with "PROTECT THE UNDERGROUND" for publicity photos and when working in the field. ("I really love jumpsuits," Kiers says.) A documentary video crew followed SPUN scientists on their first expedition, into Chile. Celebrities such as primatologist Jane Goodall and best-selling author Michael Pollan have signed on as SPUN advisers.

> **"Up to 50% of the living biomass of soils are these networks"**
> **Toby Kiers**, Free University of Amsterdam

Some researchers, however, harbor doubts that SPUN's mapping effort will have much practical impact, noting that conservationists are already protecting forests and other ecosystems that harbor fungi and store planet-warming carbon. Others question whether the surveys will appreciably add to what scientists already know, in part because SPUN is only studying one segment of the fungal community: those that form associations with plant roots.

Mapping "a single class of microorganism seems to me too limited to come to an understanding of the big picture," says Heribert Hirt, a plant scientist at the King Abdullah University of Science and Technology. "I am rather skeptical that we will really learn a lot from this big science project."

But SPUN's effort to make soil fungi more visible is being welcomed by most mycologists, who often feel as overlooked as the organisms they study. "I don't think anything like this has ever happened before," says Kabir Peay, a mycologist at Stanford University who advises SPUN. "It's amazing there are philanthropists that have the vision and interest to support this type of activity."

APPRECIATED OR NOT, fungi are integral to Earth's ecosystems. They evolved hundreds of millions of years before land plants and animals. By breaking down rock and freeing up nutrients, they helped plants colonize land some 500 million years ago. To this day, most land plants access water and nutrients in part by partnering with mycorrhizal fungi that grow on—and often into—their roots. (Roughly speaking, "mycorrhiza" means "fungal root.") Some plants get up to 80% of their phosphorus—a vital nutrient—from fungi. And some fungi construct intricate underground webs known as mycelium that can stretch for kilometers. "Wherever there are roots," Kiers says, "there are fungi."

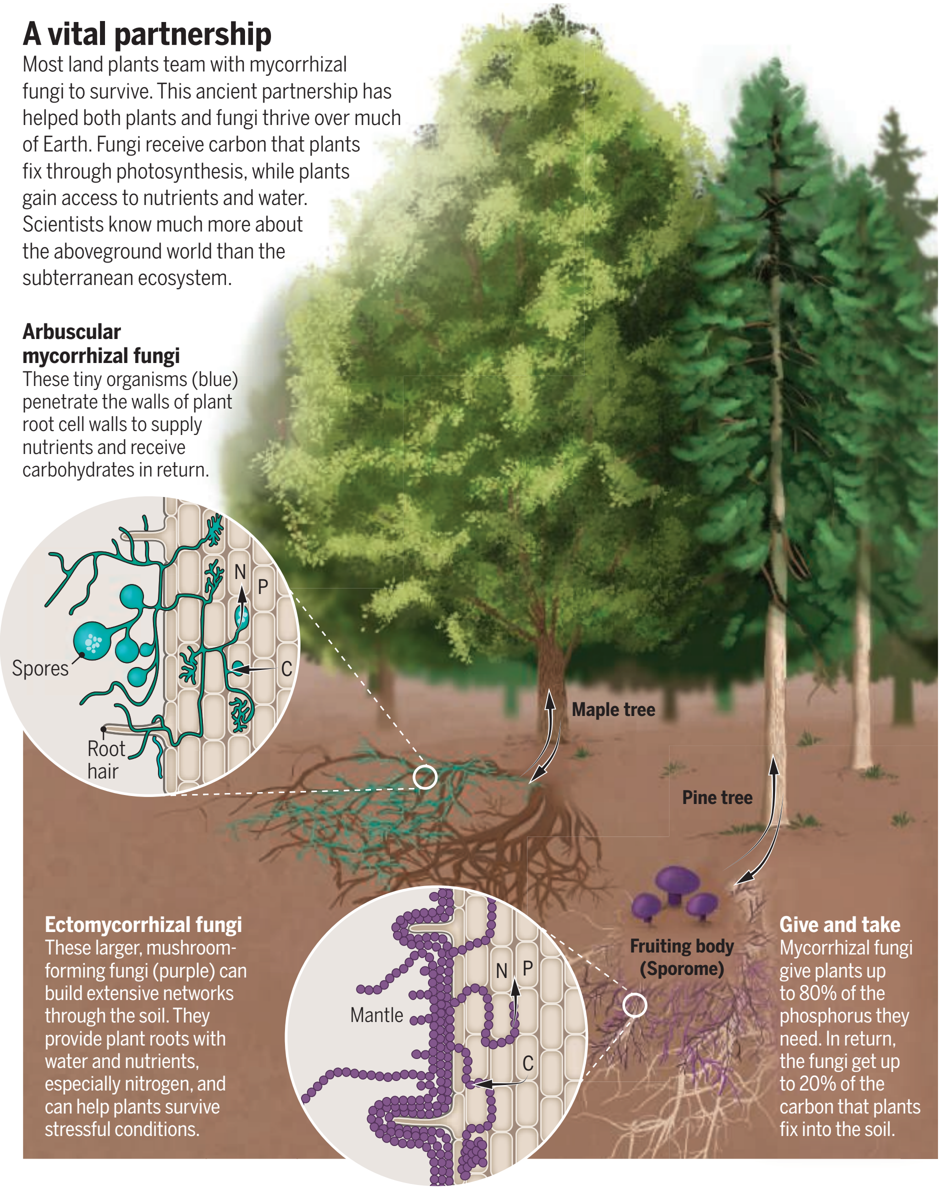
Despite their ubiquity and importance, however, fungi challenge biological paradigms and have defied easy description. The mushrooms that many people think of as classic fungi, for example, are just the spore-forming appendages of larger organisms that are typically hidden from view. Early biologists lumped fungi with plants, even though they don't photosynthesize. Only in 1969 did scientists recognize fungi as a separate kingdom of life. (Furci prefers "queendom.") It's also hard to define an individual fungus: "One" mycelium can hold many cell nuclei that don't always share the same DNA. So is the mycelium an individual, or is each nucleus?

Fungi are often relegated to second-class status within global scientific and conservation agendas. Whereas visually striking and charismatic species such as tigers, whales, and orchids have grabbed attention, fewer than 600 fungal species have had their conservation status assessed. "Fungi are seen as the subservient group" to plants, says Greg Mueller, chief scientist at the Chicago Botanic Garden. But some researchers have responded by flipping the script: Maybe, Mueller says, a bit tongue-in-cheek, "plants just exist to feed fungi."

Kiers, for one, has adopted a fungi-first worldview. She grew up in the United States and fell for fungi during a stay at the Smithsonian Institution's tropical research station in Panama. In her lab in Amsterdam, she uses tools such as microscopy and fluorescence to reveal how nutrients flow through mycelial networks. In one highly cited paper, Kiers' team showed one type of mycorrhizal fungus could reward individual plants that provided it with plentiful sugars by directing other nutrients to those plants' roots, while "punishing" stingier plants by withholding nutrients. Such

Three kinds of ectomycorrhizal fungi found in Chile hint at the immense diversity of these organisms, which associate with trees. From top to bottom: *Ramaria flava*, *Cortinarius lebre*, and *Laccaria tetraspora*.

PHOTOS: MATEO BARRENENGOA L.



A vital partnership

Most land plants team with mycorrhizal fungi to survive. This ancient partnership has helped both plants and fungi thrive over much of Earth. Fungi receive carbon that plants fix through photosynthesis, while plants gain access to nutrients and water. Scientists know much more about the aboveground world than the subterranean ecosystem.

GRAPHIC: N. DESAI/*SCIENCE*

results, Kiers says, demonstrate that fungi can wield real power and agency.

But such experiments drastically simplify the unruly networks that shuttle water and nutrients through natural ecosystems. That disconnect frustrated Kiers. "You're thinking: 'My God, how different is that from what's happening in the real world?'"

That question was in the air when, in September 2020, Kiers met American eco-logist Colin Averill over Zoom. Averill works in the Zürich-based lab of ecologist Thomas Crowther, which specializes in mapping the global distributions of organisms such as trees, nematodes, and mycorrhizal fungi, using computer algorithms to "fill in" areas lacking field data (*Science*, 25 October 2019, p. 412). Kiers was keen to connect insights from her idealized lab experiments to the global scale at which Averill works. The two eventually pitched the Boston-based Grantham Foundation on a global effort to discover and map mycorrhizal fungi, and harness them as a climate solution (see sidebar, p. 147). In November 2021, the foundation gave the researchers $3.5 million to launch SPUN.

THE GROUP'S TASK is daunting. Vast realms of the underground world have hardly been sampled, and scientists estimate that fewer than 10% of fungal species have been formally described—indeed, it could be as little as 1%, according to a recent paper in the journal *Fungal Diversity*.

In part, that's because studying fungi is really hard. They live mostly underground, and many are microscopic. Their most visible and familiar manifestation—the mushroom—is only made by certain types of fungi. By contrast, arbuscular mycorrhizal fungi, which associate with most of the world's plant species, nestle within the cell walls of plant roots. They are so hard to find and identify that scientists have described fewer than 300 kinds. "We can't even talk about diversity in a way that makes sense yet," Kiers says.

Some researchers have been chipping away at the problem. In 2014, a team led by mycologist Leho Tedersoo of the University of Tarfu in Estonia reported in *Science* on an analysis of soil samples from 365 sites on every continent except Antarctica—at the time, an unprecedented sampling effort that one news outlet described as "staggering." The research revealed, among other things, that fungal diversity does not always mirror that of plants. In other words, protecting just the richest aboveground ecosystems might fail to safeguard the full diversity of belowground life.

That survey effort "was just the very tip of the iceberg," says Tedersoo, who now advises SPUN. He subsequently launched a Global Soil Mycobiome consortium and started to email colleagues, begging them to dig up fungi, dry them, and send them to him. The result was a paper published late last year that analyzed samples from 3200 sites holding more than 700,000 "operational taxonomic units"—DNA sequences that could represent fungal species.

Even with that accomplishment, Peay says, "If you took the total volume of soil sampled by all fungal ecologists, it's still teeny." SPUN aims to paint a fuller picture by quickly doubling the number of fungal samples collected from documented locations. The group's leaders will collect some of the material themselves, but they also plan to fund and train a far-flung network of myconauts to sample their own regions.

The SPUN data, which will be combined with Tedersoo's in an open repository, will help fill a key gap, Averill says: "There's no systematic baseline" data on the global distribution and diversity of soil fungi. "We want to build that baseline."

To guide the sampling, Kiers and Averill have harnessed machine learning algorithms developed by Johan van den Hoogen, a researcher in the Crowther lab. The software uses some 10,000 existing fungal records and a bevy of environmental data sets to tease out subtle correlations between where fungi live and variables such as aboveground vegetation, temperature, and rainfall. Those correlations can identify places with conditions favorable to fungi that have not been surveyed using modern DNA analysis.

The potential hot spots identified by SPUN include places such as the high steppes of Mongolia and the lowlands of the Congo River Basin. The modeling also highlighted another candidate: the old-growth forests of Chile.

Mycolgist Giuliana Furci, here with a long-lived Alerce tree in Chile, helped make the South American nation one of the first in the world to legally protect fungi.

SPUN LEADERS CHOSE the South American nation for their inaugural expedition for two reasons. One is that Chile holds ancient and globally unique forests. The Villarrica foothills, for example, are dominated by a mix of *Araucaria* and southern beech trees, whose roots could host unique assemblages of mycorrhizal fungi.

The other is that Chile is home to Furci, who has extensively surveyed the country's mushrooms. She is also the founder of the Fungi Foundation, one of the world's first charities devoted to protecting the underground kingdom. "A sense of injustice" spurred her to start the organization in 2012, Furci says. "At the time, there was nobody advocating for fungi in Chile."

For the trip, Kiers assembled a team that included Furci, independent mycologist Merlin Sheldrake, and his brother Cosmo Sheldrake, a U.K. musician and sound recorder. (Furci and Merlin are both on SPUN's advisory board.) The morning after the researchers trekked across the volcano, they clambered into a van and bumped up a dirt road toward a constellation of sampling spots identified by SPUN's modeling. Pulling over near a gap in a barbed wire fence, the team plunged into a grove of eucalyptus trees—an exotic species imported from Australia—that hid their destination. Every step required negotiating thickets of bamboo and thorny blackberries that caught on hair and clothes. "She emerges from the deep," Furci joked as Kiers used a GPS to navigate through a particularly treacherous stretch. "F*** the computer!" Kiers retorted.

To gather their treasure, the researchers donned blue plastic gloves to avoid contaminating the soil and swung into action. Merlin pounded a roughly quarter-meter-long metal cylinder into the ground with a rubber mallet, then extracted it. Once the dirt core was safely deposited into a plastic ziplock bag, he and Kiers trekked further to collect eight additional samples, forming a three-by-three grid 30 meters on a side.

Once analyzed, these samples could confirm or refute a hypothesis based on the computer modeling: that the eucalyptus grove is a "cold spot" with relatively few fungal species. But the researchers were eager to collect the soil anyway, because they wanted to see whether the roots of the tall, almost comically skinny eucalyptus trees hosted foreign fungi that had hitchhiked in with the Australian trees. Understanding how human-altered ecosystems like this one function is as important as understanding more natural forests, Kiers says.

Mycologists Toby Kiers and Merlin Sheldrake sample along the rocky coast of Chile earlier this year.

The expedition featured some unconventional episodes. Before taking samples on the volcano, for example, the researchers asked Cosmo Sheldrake to play a song, inspired by an Indigenous elder they had met the day before who advised offering music to fungi. The group fell quiet as he produced a penny whistle and played a haunting, ethereal melody followed by *Cooley's Reel*, a popular Celtic tune.

PHOTOS: MATEO BARRENENGOA L.

At another stop, Furci charmed a couple into allowing sampling in their front yard. By the time Kiers and Merlin were done, Furci was handing out licorice and hugs were exchanged all around. She even offered the couple a handful of dirt to smell. "I love it," the man said rapturously.

The next morning, the team headed west toward the Pacific coast to sample in Alerce Costero National Park, home to another old-growth forest dominated by a rare tree species, the alerce. In all, the researchers collected some 30 soil samples in just over a week of fieldwork. They handed them over to César Marín, a mycologist at Santo Tomás University in Chile, for DNA analysis. The results—including some from additional samples collected by Marín—will be fed back into SPUN's modeling in order to improve its predictions.

SPUN'S EFFORTS to map fungi in understudied places reflect a "pragmatic" approach that could help scientists better understand how complex ecosystems like forests work, says mycologist Justine Karst of the University of Alberta, Edmonton. She also lauds the team's efforts to excite the public—something she says most scientists don't prioritize. "I'm looking forward to seeing what they produce."

SPUN, meanwhile, hopes its analyses will also help bolster emerging efforts to protect fungi—and Furci's advocacy work in Chile could provide a template. Largely because of her lobbying, Chilean lawmakers a decade ago passed the world's first law formally protecting fungi nationwide.

Elsewhere, fungi are also beginning to capture official attention. Several European countries have taken steps to protect endangered fungal species, and Estonia has created a small preserve where several red-listed fungi live. (In the United States, by contrast, fungi have relatively low status; just two fungi—both lichens—are protected under the Endangered Species Act.)

Last fall, the International Union for the Conservation of Nature (IUCN), which maintains the global "red list" of threatened and endangered species, explicitly called for fungi to be given the same consideration as plants and animals, as did the conservation organization Re:wild. And in December 2021, the United Nations's Food and Agriculture Organization launched an International Network on Soil Biodiversity that includes research on fungi.

Mueller, who chairs IUCN's fungal conservation committee, and other advocates are now working to have the Convention on Biological Diversity—a multinational pact that helps set the global conservation agenda—explicitly embrace the goal of protecting fungi. And although SPUN "is not the only game in town" when it comes to lobbying for fungi, Mueller believes its well-funded public outreach could aid such efforts. "Having this bright light shining on the issue," he says, "will move a lot of initiatives forward."

Some influential groups, however, have yet to afford fungi as much consideration as flora and fauna. The Nature Conservancy—one of the world's largest conservation groups—"does not specifically target fungi" when prioritizing ecosystems for protection, says David Banks, the group's chief conservation officer. "But because we're working in these bigger [ecosystems], we can capture them." (The conservancy is nevertheless funding a SPUN-led trip to sample mycorrhizal networks on a remote Pacific island.)

Even SPUN supporters acknowledge that a more complete picture of fungi won't automatically lead to better real-world outcomes. "There is a gap between mapping and demonstrating patterns of biodiversity, and demonstrating a connection to conservation," Peay says.

Still, SPUN's leaders believe they can add a new, subterranean dimension to global conservation efforts. Kiers points to recent research suggesting that, over nearly one-third of Earth's land area, aboveground biological diversity doesn't match what's found beneath the surface. That means habitats often seen as relatively species-poor—such as boreal forests and drylands—could harbor far greater underground diversity than is currently recognized. Better maps of fungal diversity, she adds, could aid forestry, farming, and efforts to curb climate change.

Kiers recognizes, however, that the onus is now on SPUN to show its value before its first flush of funding runs out. "There has to be a demonstrable benefit to building an organization dedicated to protecting underground ecosystems," she says. "The clock is ticking." ■

By aiding trees, fungi might help curb warming

Could adding certain kinds of fungi to soil help curb climate change by enabling trees to grow faster and suck more carbon dioxide from the atmosphere? That's a question that researchers with the new Society for the Protection of Underground Networks (SPUN) are trying to answer.

In the spring of 2021, in an abandoned pasture in southwestern Wales, a forestry company planted 25,000 trees over 11 hectares for an experiment designed by mycologist Colin Averill, a co-founder of SPUN (see main story, p. 142). The plantations include sitka spruce, a common timber tree in the United Kingdom, and a mix of native deciduous trees. To half of the seedlings' roots, researchers added mycorrhizal fungi—microorganisms that help provide plants with nutrients by associating their roots—that were sourced from mature forests of the same type. Now, the researchers are waiting to see whether the treated trees grow faster and absorb more carbon than those that didn't get the treatment. A similar trial is ongoing in Yucatán, and a third will be planted this fall in Ireland.

The effort builds on research suggesting the right fungi can give arboreal growth a powerful boost. In a January paper published in *The ISME Journal*, Averill and colleagues reported growth rates of trees in forests across Europe vary by up to a factor of three depending on their fungal partners.

But just how much fungi can supercharge saplings is an open question. Companies have long sold mycorrhizae that tree planters can dab onto roots. But such commercial mixes are not adapted to specific tree species or locations, Averill says, and there is little evidence they help. For success, he believes "you need to get the right organisms in the right place."

The Wales experiment is testing that hypothesis. Averill visited the site in April to measure the trees and found the inoculated saplings are growing faster than ones grown without added fungi. He's awaiting a second year of data before publishing them, "but the effect sizes are large enough that I'd be surprised if they go away."

Even if bespoke fungi enhance tree growth in field studies, however, it's not clear whether they can be deployed cheaply and conveniently enough to broadly appeal to forest owners. Averill will investigate that this fall through a company he's created, Funga, that will inoculate pines being commercially planted in the southeastern United States.

The idea of tailoring fungi to boost tree growth "is something many of us write about in our grants," says Kabir Peay, a mycologist at Stanford University who advises SPUN. "It's nice to see that someone's actually out there trying. *—G.P.*

A fungal safari

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