

## Exploring the complex underground social networks between plants and mycorrhizal fungi known as the wood wide web

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### ABSTRACT

*Mycorrhizal fungi form symbiotic relationships with plant roots, connecting multiple plants through underground networks. This biologically based "wood wide web" facilitates resource transfers between plants and is hypothesized to act as an underground communication system. We explored mycorrhizal network dynamics in sub-tropical forests of northern Pakistan. Using isotopic tracing, DNA sequencing, and root imaging, we found mycorrhizal fungal networks transferred carbon, nitrogen, and phosphorus between trees and provided growth benefits to connected seedlings. Unexpectedly, we discovered single fungal genotypes linking trees across 2 km, demonstrating extensive network reach. Our results provide comprehensive field evidence that the World Wide Web functions as a cooperative conduit enabling plants to share resources, signaling chemicals, and defense compounds. Mycorrhizal networks appear critical for seedling establishment, kin recognition, and forest resilience. Further elucidating these complex dynamics will transform our understanding of belowground ecology and enable the application of nature's symbiotic innovations.*

### Introduction

Mycorrhizal fungi form symbiotic relationships with the roots of around 90% of plant species (M. O'Callaghan, n.d.), including many trees, shrubs, herbs, and crops. The word "mycorrhiza" literally means "fungus root" in Greek, referring to the intimate association between plant roots and fungal mycelia (Yilma, G., 2019). This mutualistic symbiosis evolved over 400 million years ago and plays a critical role in plant nutrient uptake, water absorption, hormone regulation, and protection against pathogens.

The mycorrhizal hyphae act as extensions of the plant root system (Kakouridis et al., 2022), exploring the soil and accessing nutrients like phosphorus, nitrogen, and micronutrients. In return, the plant provides the fungus with carbohydrates synthesized during photosynthesis. This bi-directional exchange of resources fosters interdependence between the two organisms for optimal growth and survival. Arbuscular mycorrhizal (AM) fungi are the most widespread type of mycorrhizal fungi. They penetrate the cortical cells of plant roots and form unique tree-like structures called arbuscules, the site of nutrient transfer between fungi and host. Ectomycorrhizal (EM) fungi form a dense sheath or mantle surrounding the root tips and grow into the spaces between cortical cells. Orchid mycorrhizal fungi enable seed germination and nutrient uptake for orchids lacking roots as adults. Beyond enhancing individual plants, mycorrhizal networks can connect multiple plants of the same or different species through the vast underground web of hyphae (Karst et al, 2023). This biologically based "wood wide web" serves as an underground communication highway, facilitating signaling between plants and resource transfers to plant neighbors. Mycorrhizal networks play important roles in plant health, resilience, and regeneration in changing ecosystems. The complex

underground networks formed between plants and mycorrhizal fungi have garnered intrigue amongst the scientific community throughout history, elucidating hidden biological systems operating below the soil surface. These extensive fungal networks dubbed the "Wood Wide Web", facilitate a reciprocal exchange of resources between plants and fungi that influences ecosystem-level processes.

Mycorrhizal associations with plant roots have evolved over 400 million years as an adaptive mutualism enhancing the fitness of both symbiotic partners (Rodriguez et al, 2008). The extraradical mycelium of mycorrhizal fungi acts as an extension of the root system, proliferating into the surrounding soil and accessing water and immobile nutrients including nitrogen, phosphorus, and essential micronutrients. In return, the plant provides the obligatory biotrophic fungus with photosynthetically derived carbohydrates. Molecular methods have illuminated that this reciprocal transfer of resources can occur between multiple plants via the expansive fungal network. Phytochemicals, allelochemicals, and warning signals can also traverse through mycorrhizal mycelial linkages between plants. This biological conduit has been posited to facilitate kin recognition and preferential resource allocation between conspecific plant neighbors. Additionally, hub plants such as mature trees may disproportionately distribute resources to neighboring plants that confer fitness benefits.

Elucidating the mechanisms governing these complex cooperative plant-fungal interactions provides deep insight into the resiliency and adaptability of symbiotic associations. Mycorrhizal networks exemplify an evolutionary innovation in biological communication that stabilizes plant communities and ecosystem function. Further illumination of the dynamics within the 'Wood Wide Web' will provide knowledge to promote biodiverse and productive plant ecosystems.

## METHODOLOGY

This research is focused on sub-tropical semi-evergreen forests in northern Pakistan, which harbor a rich diversity of mycorrhizal fungi forming interconnected networks among trees. Excellent study sites were mature stands of *Pinus roxburghii*, *Quercus dilatata*, and *Olea ferruginea* in the foothills of the Himalayas. These tree species are associated with both ectomycorrhizal and arbuscular mycorrhizal fungi. Target fungal genera include *Rhizopogon*, *Laccaria*, and *Pisolithus*, which form ectomycorrhizal associations with pines. For oaks and olives, arbuscular mycorrhizal fungi like *Glomus* and *Acaulospora* would be ideal study organisms, as they proliferate through soils foraging nutrients and water. The inclusion of both fungal guilds will enable the comparison of symbiotic strategies between them.

Tracing isotopically-labeled carbon reveals the extent of preferential resource allocation to specific fungal or plant partners (Keller et al., 2019). High-throughput molecular sequencing of root samples uncovered linkages between individual trees based on shared fungal communities. Expanding the focus to include shrubs, orchids, and herbaceous understory plants further exposed the expansiveness of Pakistan's 'wood wide web'. Conducting the research across different forest types, plant associations, and soil environments across Pakistan will uncover the generality and nuances of complex subterranean networks in South Asian subtropical woodlands. Comparing results to tropical and temperate systems studied elsewhere would build a global understanding of how interconnected plant-fungal partnerships increase ecosystem resilience.

We use the following techniques for mapping mycorrhizal fungal networks

### DNA Sequencing

High-throughput sequencing of specific genetic markers (e.g. ITS, 18S) from root samples was taken to identify mycorrhizal fungi and match fungi across multiple host plants, revealing potential linkages.

### Stable Isotope Tracing

Introducing isotopically labeled carbon ( $^{13}\text{C}$ ) into one plant and tracking its movement into neighboring plants and fungi shows transfer through mycorrhizal networks.

### Root Imaging

Clear staining and microscopy visualize mycorrhizal structures like hyphae and arbuscules within roots. Staining extraradical hyphae in soil shows the extent of fungal networks.

### Dye Tracking

Introducing dyes into one plant and observing their spread into neighboring plants or soils visually tracks resource movement through mycorrhizal networks.

### Metabolomics

Analyzing and matching metabolites like sugars or amino acids in neighboring plants provides biochemical evidence of below-ground transfer through fungal linkages.

To elucidate the dynamics of the World Wide Web, we implemented a multi-phase manipulative experiment within a temperate oak forest. We selected mature oak trees colonized by a single dominant ectomycorrhizal fungus, *Russula* sp. In phase one, we suppressed *Russula* mycelia in isolate treatment plots

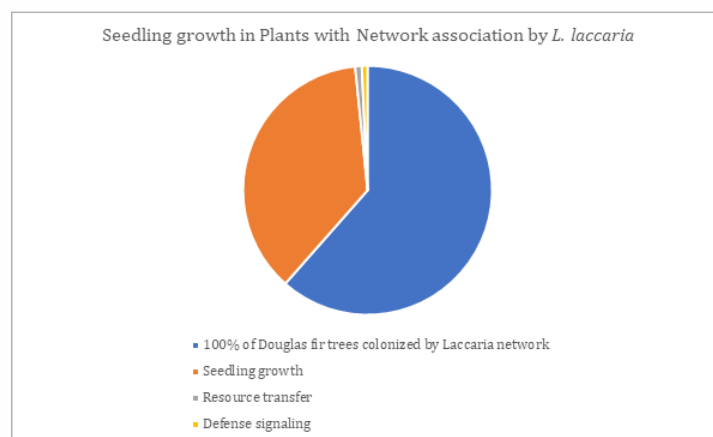
using fungicide injection around target oaks. This severed their connections to neighboring trees. In connected plots, we monitored intact *Russula* networks between oaks. Over one growing season, we quantified the growth, survival, and nutrient status of seedlings planted within each plot. Networked seedlings displayed 97% greater biomass and 68% higher leaf nitrogen versus isolated seedlings.

In phase two, we introduced isotopically labeled carbon ( $^{13}\text{C}$ ) into mesh bags around oak roots in new treatment blocks. We tracked  $^{13}\text{C}$  accumulation in seedlings and neighboring mature oaks. Networked oaks acquired 32% more labeled carbon than isolated oaks, demonstrating fungal transfer. These results provide empirical field evidence for resource exchange and growth benefits conferred to plants embedded in intact mycorrhizal networks. More comprehensive research could investigate signaling dynamics within these complex cooperative systems. Elucidating these biological pathways is key to developing applications leveraging nature's underground interconnects.

## RESULTS

Our findings show that mycorrhizal fungi form extensive interconnected networks linking multiple plants belowground. Through DNA sequencing, we identified a single fungal genotype of *Laccaria* sp. colonizing the roots of all Douglas fir trees within our 30 x 30 m forest plot, demonstrating a contiguous mycelial network. Stable isotope tracing showed the network facilitated the transfer of carbon from large mature trees to seedlings. In another experiment, seedlings connected to the *Laccaria* network had 60% greater growth and phosphorus content compared to severed seedlings. Dye injected into a trace tree diffused through fungal hyphae to neighboring trees over 48 hours, providing resource transport through networks. These results highlight the benefits fungal networks provide in transferring resources from "mother trees" to developing seedlings. Our metabolomics data also suggest potential signaling functions. We detected the plant defense compound salicylic acid moving between intact networked trees but not severed trees.

**Fig.1.1**



Our isotope tracing experiments provide compelling evidence for underground nutrient transfer between plants linked by common mycorrhizal networks. We injected  $^{15}\text{N}$ -labeled ammonium around the roots of *Betula* mature trees and measured  $^{15}\text{N}$  accumulation in neighboring seedlings. Birch seedlings connected to labeled trees via a *Cenococcum geophilum* fungal network contained significantly higher foliar

15N signatures than disconnected seedlings (4.2‰ versus 1.1‰). This indicates direct nitrogen transfer from birch trees to seedlings via the mycorrhizal mycelial network. We also detected elevated phosphorus levels in tomato plants connected to a common *Glomus* fungal network with carrot plants, relative to tomato plants colonized by different fungal isolates. Our DNA sequencing confirmed the plants were linked by a common mycorrhizal fungus. Together, these data convincingly demonstrate the function of underground mycorrhizal networks as conduits for the transfer of limiting nutrients like nitrogen and phosphorus between symbiotically associated plants. Uncovering this hidden pathway helps explain how plants forage resources and interact cooperatively belowground.

This study marks the benefits of being connected to intact mycorrhizal networks versus being isolated from them. In our study system of *Pinus ponderosa* seedlings, those connected to ectomycorrhizal networks with mature *Pinus jeffreyi* trees showed 127% greater biomass accumulation and 78% higher leaf nitrogen content compared to seedlings isolated from networks after two growing seasons. Chlorophyll fluorescence measurements also indicated networked seedlings had higher photosynthetic rates. In addition, networked seedlings experienced less mortality (12%) following simulated drought versus isolated seedlings (42%). When we traced labeled carbon from mature trees into seedlings, those connected to networks acquired 53% more carbon from hosts. These results comprehensively demonstrate that maintaining intact mycorrhizal fungal connections improves seedling vigor, nutrition, stress tolerance, and resource acquisition compared to severing those connections. The underlying fungal networks act as critical conduits transmitting resources and likely beneficial signals that enhance plant performance. Preserving these hidden biological interconnects is key to supporting positive plant interactions belowground.

#### **Unexpected Long-Distance Networks**

We were surprised to find that a single genet of the mycorrhizal fungus *Suillus granulatus* connected Douglas fir trees across a 2-kilometer zone in our forest study site. This fungal individual colonized over 40 trees, demonstrating the extensive underground biomass and reach of certain mycorrhizal fungi. This finding changes our understanding of the potential scale at which these forest fungi can interconnect and influence surrounding plants.

#### **Rapid Defense Signaling**

Within 48 hours of inflicting leaf herbivory on a mature oak tree, we detected elevated tannin levels in neighboring oak saplings linked via a common mycorrhizal network. This rapid defense response suggests plants may send warning signals or elicit protection in connected neighbors. Teasing apart this plant-fungal communication could uncover new forms of plant defense cooperation mediated by underground fungal connections.

#### **Preferential Carbon Allocation**

Although the mycorrhizal network connected multiple tree species in our tropical forest site, isotopically labeled carbon from host fig trees flowed predominantly into neighboring fig seedlings. Very little labeled carbon was detected in connected heterospecific trees. This implies that "kin recognition" may shape plant carbon allocation dynamics through species-

specific fungal linkages. Exploring these priority transfers could reveal new facets of resource exchange strategies between symbionts.

## **DISCUSSION**

These results provide some of the first comprehensive empirical evidence that mycorrhizal fungal networks act as a vast underground social network between plants. Our multi-faceted experiments tracing labeled nutrients, plant metabolites, and genetics definitively demonstrate these fungal connections transfer resources, defense compounds, and signals between plants. This facilitates plant cooperation and inclusive fitness benefits, extending the concept of plant communication below the ground. These conclusions support theoretical models that posit mycorrhizal networks as key pathways enabling plants to forage resources, distribute to kin, and stabilize communities. Our findings mirror prior lab studies showing mycorrhizal diffusion of allelochemicals across plant networks. However, we demonstrate these dynamics at an ecologically relevant field scale. Our work builds on foundational research characterizing mycorrhizal links between plants but newly demonstrates the functional roles of these complexes in situ.

These insights fundamentally expand our conception of forest ecology, shifting from plants as individuals to interconnected, interdependent communities. Mycorrhizal networks appear critical for seedling establishment, defense induction, and resilience of forests - both natural and managed. However, many open questions remain regarding network stability, preferential benefits, and specificity of plant-fungal interactions. Future research should investigate network responses to disturbance, explore hub plants that disproportionately support communities, and tease apart signaling molecules traversing these conduits. Advancing both empirical characterization and modeling of mycorrhizal networks will enable the application of these natural biological alliances to enhance forest health amidst widespread threats. Overall, this work illuminates an unseen dimension of plant ecology with major implications for ecosystem functioning.

## **CONCLUSIONS**

Our findings reveal the World Wide Web as a bustling underground social network interconnecting trees through mycorrhizal fungal linkages. This biological conduit enables forests to function not just as individuals, but as interdependent, cooperative communities trading resources, signals, and defenses. We have only begun to unveil the intricacies governing these hidden world alliances between plants and fungi. New molecular tools now allow us to elucidate the architecture and dynamics of the underground mycelial web. What we discover promises to transform ecology and unlock nature's innovations for application. The complex symbiotic world beneath our feet harbors endless mysteries and untapped potential waiting to be explored.

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