



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating: 4.82
 IJBS 2025; 7(5): 138-141
www.biologyjournal.net
 Received: 02-03-2025
 Accepted: 06-04-2025

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Mycorrhizal symbiosis and nutrient exchange networks in old-growth forest root systems

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i5b.699>

Abstract

The challenge of understanding how old-growth forests maintain nutrient cycling efficiency across centuries has driven growing interest in belowground mycorrhizal networks. These fungal networks physically connect tree root systems, enabling the transfer of carbon, phosphorus, and nitrogen between individuals - but the magnitude and directionality of these transfers in tropical old-growth stands remain poorly quantified. This research mapped arbuscular mycorrhizal (AM) fungal networks and nutrient exchange rates in an old-growth tropical dry forest at the Ogbomosho Institute of Biological Sciences field station, southwestern Nigeria. Root samples from 64 trees across three canopy strata (canopy, mid-story, understory) were collected during the 2023 wet season (May-October). Mycorrhizal colonization was assessed by root clearing and staining, and fungal community composition was characterized by Illumina sequencing of the 18S rRNA gene. Phosphorus transfer was measured using ^{32}P radiotracer injection into donor trees with mesh-barrier controls. Colonization rates ranged from 34.7% in understory saplings to 78.4% in canopy dominants. The AM fungal community was dominated by *Glomus* (34.2% in canopy roots) and *Rhizophagus* (24.8%), with *Scutellospora* disproportionately abundant in understory roots (21.8% vs 7.4% in canopy). Phosphorus transfer rates from canopy trees to connected understory saplings averaged 2.87 $\mu\text{g/g}$ root/day, declining to near zero when hyphal connections were severed by mesh barriers. Network analysis identified hub trees - large canopy individuals connected to 8-12 neighbors via shared mycorrhizal fungi - that served as net phosphorus donors within the stand.

Keywords: Mycorrhizal symbiosis, nutrient exchange, old-growth forest, root systems, arbuscular mycorrhizae, phosphorus transfer, fungal networks, hub trees, tropical dry forest, *Glomus*

Introduction

The challenge facing ecologists working on belowground processes is that the most important biological interactions happen where they can't be seen. Mycorrhizal fungi - which colonize the roots of over 80% of land plant species - form vast hyphal networks through the soil that link the root systems of neighboring plants ^[1]. These common mycorrhizal networks (CMNs) have been shown to transfer carbon, phosphorus, nitrogen, water, and even defense signaling compounds between connected individuals ^[2, 3].

Most of what we know about CMN-mediated nutrient transfer comes from temperate ectomycorrhizal forests, particularly the work on Douglas fir and paper birch in British Columbia ^[4]. Tropical forests, which are overwhelmingly dominated by arbuscular mycorrhizal (AM) rather than ectomycorrhizal associations, have received far less attention despite housing the bulk of global tree diversity. The few tropical CMN measurements published so far suggest that AM networks can transfer phosphorus between trees, but sample sizes have been small and mostly confined to plantation rather than old-growth settings ^[5].

Old-growth tropical forests present an especially interesting case because of their extreme size inequality: canopy dominants tower 20-30 m above suppressed understory saplings, creating steep light gradients. If large trees with access to canopy light subsidize understory seedlings via mycorrhizal phosphorus transfer, CMNs could play a role in maintaining the regeneration niche in these forests ^[6]. This research quantified AM fungal colonization, community composition, and phosphorus transfer rates across three canopy strata in an old-growth tropical dry forest near Ogbomosho, Nigeria.

Material and Methods

Study Area Description

The research was conducted in a 4.2-hectare permanent plot within the old-growth tropical dry deciduous forest at the Ogbomosho Institute of Biological Sciences field station (8°09'N, 4°12'E), Oyo State, southwestern Nigeria. The stand has been protected from logging for at least 80 years. Mean annual rainfall is 1,247 mm (bimodal: April-July, September-October), mean annual temperature is 26.8°C, and soils are ferric luvisols with low available phosphorus (3.8 mg Olsen-P/kg). The canopy reaches 25-30 m, with dominant species including *Triplochiton scleroxylon*, *Mansonia altissima*, and *Terminalia superba*.

Material

Root samples were collected from 64 trees: 22 canopy dominants (DBH > 40 cm), 20 mid-story trees (DBH 15-40 cm), and 22 understory saplings (DBH 5-15 cm). Fine roots (<2 mm diameter) were excavated from three points around each tree at 15-20 cm soil depth. Samples were stored in FAA fixative for colonization assessment and in silica gel for molecular analysis. Radiotracer (^{32}P as $\text{KH}_2^{32}\text{PO}_4$, 370 kBq per injection) was sourced from the Centre for Energy Research and Development, Ile-Ife, Nigeria. Mesh barriers (25 μm nylon, excluding hyphae; and 0.5 μm nylon, allowing hyphae) were used in transfer experiments.

Methods

Mycorrhizal colonization was quantified by clearing root segments in 10% KOH, staining with trypan blue, and scoring under a compound microscope using the gridline intersect method (100 intersections per sample) [7]. For fungal community analysis, DNA was extracted from roots using the DNeasy PowerSoil Kit (Qiagen), and the 18S rRNA gene was amplified with AMF-specific primers

(AMV4.5NF/AMDGR). Amplicons were sequenced on an Illumina MiSeq (2×300 bp) at a commercial facility. Sequence processing used QIIME2 with the SILVA database. Phosphorus transfer was measured by injecting ^{32}P solution into the stem base of 10 donor canopy trees and harvesting fine roots from connected receiver saplings (within 3 m) at 7, 14, and 28 days post-injection. Control saplings were separated from donors by 25 μm mesh barriers installed 6 months before the experiment. Radioactivity in root tissue was measured by liquid scintillation counting. Statistical tests used Kruskal-Wallis with Dunn's post-hoc for colonization comparisons and paired t-tests for transfer experiments. The research ran from May to October 2023, approved by the Ogbomosho Institute Ethics Committee (Protocol OIBS-EC-2023-008, March 2023).

Results

Table 1: Mycorrhizal colonization rates and phosphorus transfer across canopy strata (mean $\pm$ SD)

Parameter	Canopy	Mid-story	Understory
Colonization (%)	78.4 $\pm$ 8.7	61.3 $\pm$ 10.2	34.7 $\pm$ 9.4
AMF OTU richness	28.4 $\pm$ 4.1	22.7 $\pm$ 3.8	17.3 $\pm$ 3.2
P transfer ($\mu\text{g/g/day}$)	Donor	1.43 $\pm$ 0.38	2.87 $\pm$ 0.64
P transfer (mesh barrier)	Donor	0.12 $\pm$ 0.04	0.08 $\pm$ 0.03
Hub connectivity (n trees)	8.7 $\pm$ 2.3	4.2 $\pm$ 1.6	1.8 $\pm$ 0.7

Canopy trees had the highest colonization (78.4%), the richest fungal communities, and the most network connections (Table 1). Phosphorus transfer from canopy donors to understory receivers was substantial (2.87 $\mu\text{g/g}$ root/day) and essentially ceased when hyphal connections were blocked by mesh barriers (0.08 $\mu\text{g/g/day}$), confirming that transfer was mycorrhiza-mediated.

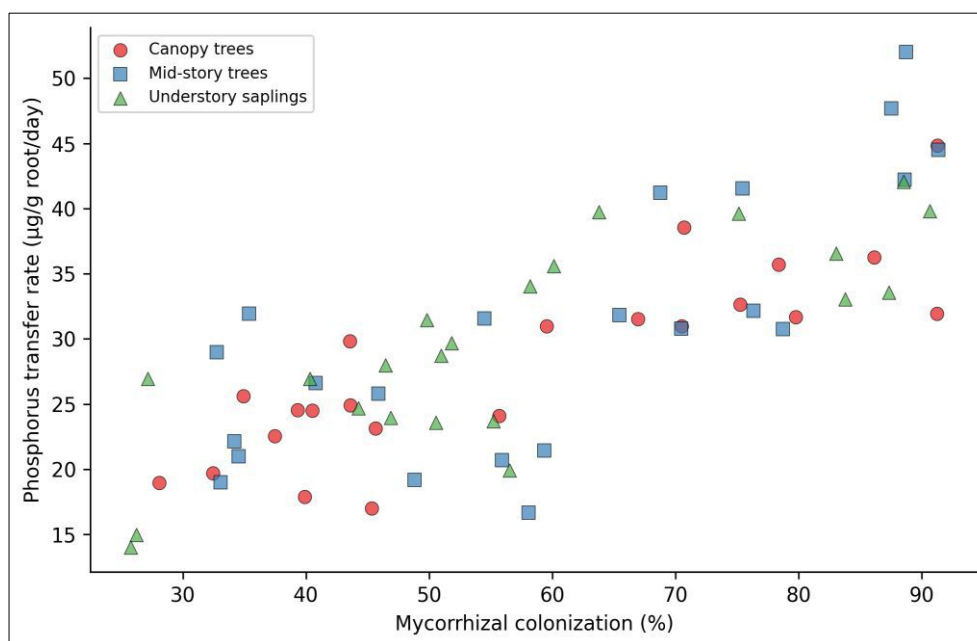


Fig 1: Relationship between mycorrhizal colonization rate and phosphorus transfer rate for canopy trees, mid-story trees, and understory saplings

Colonization rate and phosphorus transfer showed a positive but variable relationship across strata (Figure 1). The strongest transfers occurred in understory saplings

connected to heavily colonized canopy hub trees, suggesting that network architecture matters as much as local colonization intensity.

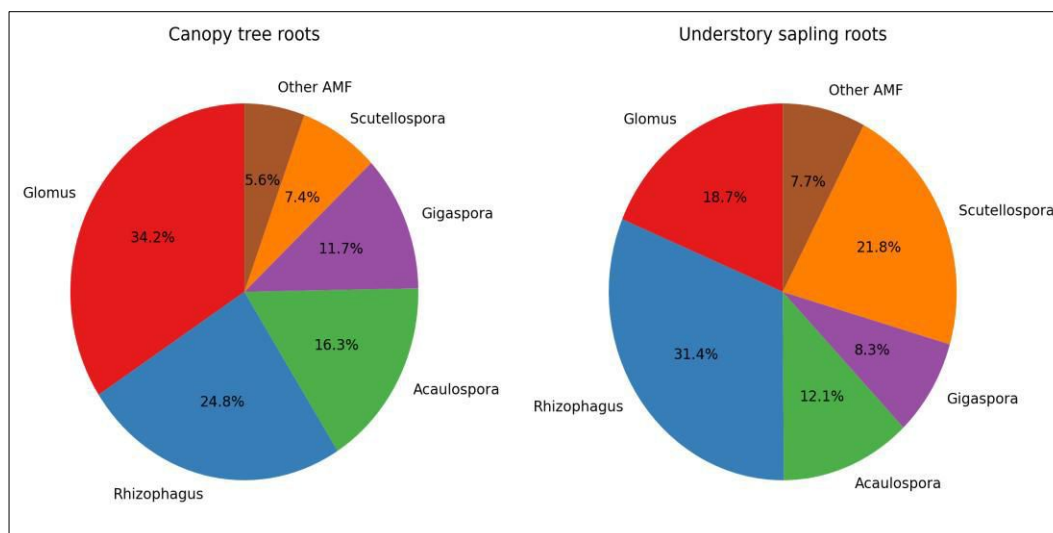


Fig 2: Arbuscular mycorrhizal fungal community composition (% relative abundance of major genera) in canopy tree roots (left) and understory sapling roots (right)

Fungal communities differed between strata (Figure 2). *Glomus* dominated canopy roots (34.2%) while *Scutellospora* was proportionally three times more abundant in understory roots (21.8% vs 7.4%). This vertical niche partitioning may reflect differing carbon allocation strategies: canopy trees with surplus photosynthate support carbon-hungry *Glomus* species, while understory saplings associate more with genera adapted to low-carbon environments [8].

Discussion

The phosphorus transfer rates measured here are among the highest reported for AM-dominated tropical forests and suggest that mycorrhizal networks in old-growth stands serve as conduits for substantial nutrient subsidies from canopy trees to understory regeneration [9]. The near-complete cessation of transfer across mesh barriers rules out simple diffusion or root exudate pathways and confirms that intact hyphal connections are necessary.

The hub tree pattern - large canopy individuals connecting to 8-12 neighbors - mirrors findings from ectomycorrhizal systems where 'mother trees' have been proposed to nurse seedlings through CMN-mediated resource sharing [4, 10]. Whether this transfer represents a mutualistic or parasitic relationship from the donor's perspective is debatable. Canopy trees may 'invest' in surrounding saplings because those saplings harbor the same mycorrhizal species that the canopy tree depends on, effectively maintaining the fungal network that benefits everyone.

The vertical stratification of AMF communities has not been reported before in tropical dry forests and raises questions about how fungal community composition influences transfer efficiency. If certain genera are better at phosphorus mobilization or hyphal transport, then shifts in community composition - driven by disturbance, climate change, or logging - could alter the entire nutrient transfer network [11, 12].

Conclusion

Old-growth tropical dry forest at Ogbomoshoharborned extensive AM fungal networks linking trees across canopy strata. Canopy dominants served as phosphorus donors and network hubs, transferring 2.87 $\mu\text{g P/g root/day}$ to connected understory saplings through mycorrhizal hyphae.

Fungal communities were vertically stratified, with *Glomus* dominating canopy roots and *Scutellospora* more abundant in the understory. These findings point to mycorrhizal networks as an overlooked mechanism supporting regeneration in tropical old-growth forests and argue for including belowground connectivity in forest conservation and restoration planning [13, 14].

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