Diversity of Glomeromycota in orchards of *Persea americana* Mill. with different agronomic management in Xalisco, Nayarit, Mexico†

[Diversidad de Glomeromycota en huertos de *Persea americana* Mill. con diferente manejo agronómico en Xalisco, Nayarit, México]

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SUMMARY

Background. Avocado (*Persea americana*) is a fruit crop of economic importance to Mexican agriculture which is experiencing increasing global demand. Consequently, the cultivated area and the use of chemical fertilizers have expanded. Agrochemicals can negatively affect soil health and reduce both the infectivity and effectiveness of arbuscular mycorrhizal fungi (AMF). Moreover, the composition of the AMF species may shift, favoring species adapted to high-disturbance conditions (ruderal strategy). **Objective**. To estimate the richness and abundance of AMF virtual taxa (VT, a proxy for "species") in two avocado orchards differing in soil management practices. **Methodology**. Soil samples were collected from two orchards: Orchard 1 without agricultural management and, Orchard 2 with agricultural management. The soil was used to increase AMF propagules in trap plants and for physicochemical analyses. DNA was extracted from root segments and soil spores obtained from trap plants. Next, nested PCR using specific primers to amplify the Glomeromycota phylum was performed, then amplicons of ITS rDNA fragments were purified and sequencing in the Illumina MiSeq (2x300) platform. **Results**. A total of 29 virtual taxa were identified, 10 of which were resolved to species level, while the remaining fungal types belong to eight genera. 10 VT were shared between Orchards, six were exclusive to Orchard 1, and 13 to Orchard 2. However, in Orchard 1, 98.33% of the relative abundance was concentrated in only four VT, while in Orchard 2, three VT (98.51%) were dominated. Although richness (q = 0) was higher in Orchard 2 than in Orchard 1, the diversity indices of order 1 (q = 1) and 2 (q = 2) indicated no significant differences between orchards. **Implications.** This study suggests that agrochemicals may act as an environmental filter on AMF community assembly, favoring species with a ruderal strategy that translocate fewer nutrients to host plants. Additionally, new AMF species were recorded for Nayarit (*Dominikia difficilevidera*, *Microkamienskia divaricate*, and *Orientoglomus emiratium*). **Conclusion**. The use of agrochemicals alters the

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composition and abundance of AMF. Furthermore, molecular tools allowed us to increase the number of AMF species recorded in Nayarit.
Key words: abundance; avocado; richness; virtual taxa.

RESUMEN

Antecedentes. El aguacate (*Persea americana*) es un cultivo de importancia económica para la agricultura mexicana y su demanda a nivel mundial se ha incrementado. Consecuentemente, el área cultivada y el uso de fertilizantes químicos también ha aumentado. El uso de agroquímicos puede tener un efecto negativo en la salud de los suelos, y reducir la infectividad y la efectividad de los hongos micorrizógenos arbusculares (HMA). Además, la composición de las especies de HMA puede cambiar y favorecer especies adaptadas a condiciones de disturbio (estrategia ruderal). **Objetivo.** Estimar la riqueza y abundancia de HMA-taxas virtuales en dos parcelas de aguacate con diferente manejo agronómico. **Metodología.** Se colectaron muestras de suelo en dos parcelas: la parcela 1 sin manejo agronómico y, la parcela 2 con manejo agronómico. El suelo fue usado para aumentar los propágulos fúngicos de HMA en cultivos trampa y para análisis fisicoquímicos. El DNA fue extraído de segmentos de raíces y esporas obtenidas de los cultivos trampa usando oligonucleótidos específicos del phylum Glomeromycota. Las amplificaciones se realizaron con un PCR-anidado para obtener fragmentos de la región ITS rDNA, y los amplicones purificados fueron secuenciados en la plataforma Illumina MiSeq (2x300). **Resultados.** Un total de 29 taxa virtuales fueron identificados, 10 de los cuales fueron resueltos a nivel de especie, el resto pertenecen a ocho géneros. Diez taxa virtuales se encontraron en ambas parcelas, seis fueron exclusivos de la parcela 1, y 13 de la parcela 2. Sin embargo, en la parcela 1, 98.33% de la abundancia relativa se concentró en cuatro taxa virtuales, mientras que en la parcela 2, tres taxa virtuales (98.51%) fueron dominantes. Aunque, la riqueza ($q = 0$) fue mayor en la parcela 1 comparado con la parcela 2, los índices de orden 1 ($q = 1$) y 2 ($q = 2$) no indicaron diferencias significativas entre parcelas. **Implicaciones.** Este estudio sugiere que los agroquímicos pueden actuar como un filtro ambiental sobre la comunidad de HMA, favoreciendo especies de hongos con una estrategia ruderal, los cuales transfieren pocos nutrientes a sus plantas hospederas. También, nuevas especies de HMA fueron registradas para Nayarit (*Dominikia difficilevidera*, *Microkamienskia divaricate*, y *Orientoglomus emiratium*). **Conclusión.** El uso de agroquímicos altera la composición y abundancia de HMA. Además, las herramientas moleculares permitieron incrementar el número de especies de HMA registradas en Nayarit.
Palabras clave: abundancia; aguacate; riqueza; taxa virtuales.

INTRODUCTION

The soil environment is biologically diverse and the most complex habitat on earth, approximately containing one half of all living organisms (Anthony *et al*., 2023). Therefore, it has been recognized that the soils are biologically active, and its biodiversity is important in the stability and function of ecosystem processes, including the belowground biotic interactions such as arbuscular mycorrhizal symbiosis (Martin and van der Heijden, 2024). Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota (Hyde *et al*., 2024), are an ancient and monophyletic lineage of obligate biotrophs – that is, they require a living host plant to complete their life cycle. These fungi colonize the roots of around 80% of terrestrial plants and are present in almost all ecosystems (Stürmer *et al*., 2018; Wang and Qiu, 2006). In this symbiosis both partners obtain benefits, plants supply their associated AMF with a microhabitat and essential carbohydrates for fungal survival and growth. In return, AMF develop complex mycelial networks (filamentous aseptate hyphae), which extensively explore the soil to acquire mineral nutrients –such as phosphorus, nitrogen and other immobile nutrients– as well as water. These resources are then translocated to the host plant through specialized symbiotic structures formed within the root cortex (Peterson and Massicotte, 2004). In addition, AMF can provide bioprotection to the plants from biotic and abiotic stresses (Martin and van der Heijden, 2024) and modify the composition and activity of the rhizosphere microbiome, leading to the recruitment of other beneficial microorganisms (Boyno *et al*., 2025).

Beyond promoting plant growth and enhancing stress tolerance, AMF contribute significantly to soil health and environmental sustainability. These fungi produce a glycoprotein called glomalin, which improves soil aggregate stability, thereby strengthening soil structure and reducing the risk of erosion. Glomalin also plays a role in increasing soil organic matter content, enhancing water retention capacity, and boosting the soil's potential for carbon sequestration (Zhang *et al*., 2023; Zhang *et al*., 2022). Therefore, AMF represent a valuable tool for reducing the carbon footprint of agricultural practices and combating climate change.

Intensive agriculture has led to significant soil degradation and contamination. Agrochemicals can negatively affect soil health and reduce the capacity of AMF propagules (spores, extraradical hyphae, and colonized root segments) to colonize host plant roots (infectivity), thereby reducing mycorrhizal-derived benefits such as plant growth and reproduction (effectiveness). In addition, the composition of the AMF species may shift, with species adapted to high-disturbance conditions (ruderal strategy) being

favoured (Cahyaningtyas and Ezawa, 2024; Frew *et al*., 2023; Horsch *et al*., 2023). Such changes can directly impact crop yields (Peng *et al*., 2024; Balderas-Alba *et al*., 2019; Vega-Frutis *et al*., 2018). In Mexico, deforestation due to the conversion of forest into agricultural and pasture lands has been increased significantly (Ramírez-Reyes *et al.*, 2018). For instance, the Food and Agriculture Organization (FAO) of the United Nations estimated that, between 1997 and 2023, 5.4% – equivalent to 3735.6 hectares– of forest land has been lost in Mexico (FAO, 2023).

Despite their broad ecological distribution and fundamental role of AMF in both natural plant communities and cultivated crops, the species-level diversity is relatively low: only around 360 species have been formally described to date (Goto *et al*., 2024). Nevertheless, recent estimates suggest that the actual species richness could reach into the thousands (Lutz *et al.*, 2025). It is important to note, however, that current data on propagule abundance, species richness, and infectivity are subject to significant biases by countries, ecosystems or crops (Boyno *et al*., 2025; Van Nuland *et al*., 2025). AMF spores extracted from soil can serve as useful indicators for assessing species richness across various ecosystems and agroecosystems. Moreover, these spores can be employed as starters in the development of inoculum tailored to specific crop cultivars.

Avocado (*Persea americana* Mill.) has become one of the most commercially valuable drupe-bearing fruit crops worldwide, with global demand steadily increasing over the past two decades. According to the FAO (2024), Mexico is the world's leading avocado producer, accounting for approximately 30% (2.5 million metric tons) of global production. Often referred to as "green gold", avocado cultivation comes at a high cost to biodiversity, soil and hydrological systems, primarily due to deforestation, forest fragmentation, and use of agrochemicals and pesticides (Denvir *et al*., 2022) to maintain the rising demand worldwide. It has been reported that the increase in avocado production in Mexico is largely driven by the expansion of cultivated land, which has increased by 400% over the past 30 years (Charre-Medellín *et al.*, 2021). In Mexico, the states of Michoacán, Jalisco, Estado de México and Nayarit are the main avocado producing regions, with the municipality of Xalisco being the leading producer in Nayarit (SIAP, 2024). However, the expansion of avocado cultivation in this region is encroaching upon the Sierra de San Juan Ecological Reserve, a protected area of high ecological value, recognized for its biodiversity, endemic species, and critical role as a hydrological recharge zone (CEMIC, 2003). Despite this, official deforestation statistics specific to the Reserve remain scarce.

Avocado is considered a highly mycotrophic, drupe-bearing fruit tree, relying on symbiotic associations – particularly with AMF– for optimal nutrient uptake and growth. Studies have reported that AMF can represent up to 50% of the microbial biomass in the avocado rhizosphere (González-Cortés *et al.*, 2012). Therefore, the application of AMF in avocado orchards could offer significant economic advantages by enhancing cost efficiency in agricultural production systems. Additionally, it may reduce the reliance on agrochemicals and pesticides, thereby contributing to environmental sustainability. Although previous studies have documented the presence of genera such as *Acaulospora*, *Glomus* and *Scutellospora* in avocado orchards under different management regimes (Carreón-Abud *et al.*, 2016; Aguirre *et al.*, 2007; González, 2005), most of these studies have relied on morphospecies-based identification. This methodological approach restricts the detection of less abundant or cryptic species, ultimately leading to an underestimation of actual diversity and a potential bias in community composition assessments.

In the present study, we estimated the richness and abundance of arbuscular mycorrhizal fungi virtual taxa (VT, a proxy for "species") in two avocado orchards located in Xalisco, Nayarit, Mexico, that differ in their soil management practices: one with chemical fertilizers and the other without chemical fertilizers. Previous field evidence (see Balderas-Alba *et al*., 2019) showed that root colonization by AMF in avocado trees growing in the agrochemical-free orchard was higher compared to the chemically managed one. Furthermore, under greenhouse conditions, Balderas-Alba *et al*. (2025) observed that avocado plants grown in soil from orchard without chemical management and inoculated with AMF propagules from the same site exhibited higher percentages of all fungal structures (hyphae, vesicles and arbuscules). This reciprocal soil origin and AMF inoculation experiment suggests the presence of locally adapted between soil conditions and indigenous AMF communities. Building upon both field and greenhouse evidence, we asked: what is the richness and abundance of AMF in orchards with and without chemical fertilizers? Given that several studies have shown that crop fertilization can reduce both the richness and abundance of AMF propagules (Peng *et al.*, 2024) and promote ruderal AMF species (Frew *et al*., 2023), we hypothesized that the orchard without soil agricultural management would exhibit higher AMF richness and abundance.

MATERIALS AND METHODS

Study site

Soil sampling was collected in two avocado orchards located within the State Reserve of Sierra de San Juan,

in Xalisco, Nayarit, Mexico. This Reserve is managed by the Government of Nayarit. Orchard 1 (104.94894º W, 21.40142º N) was characterized by the absence of pests and diseases (Luna-Esquivel, pers. comm., September 2017), and by the absence of chemical soil nutrition inputs, except for manual weeding (Luna-Esquivel, pers. comm., September 2017). In contrast, in Orchard 2 (104.93842º W, 21.39864º N), avocado trees are fertilized annually with poultry manure (30 kg per plant $^{-1}$ per year over the past three years) during the dry season, and with a chemical fertilizer mixture containing nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), zinc (Zn) and magnesium (Mg). The amount of chemical fertilizer applied varies according to the foliar nutrient content and soil fertility, but on average, 4 kg of fertilizer mixture is applied per plant each year, distributed in two applications – one in early summer and another in early fall. Orchard 2 is also irrigated once a week using a micro-sprinkler system (60 L per plant, Luna-Esquivel, pers. comm., September 2017). The distance between the two orchards is approximately 1.17 km (Figure 1).

Soil sampling

Soil sampling was carried out during the rainy season (September 2017). In each orchard, two plots measuring 50 m x 50 m (2500 m^2) were established. Within each plot, five random points were selected following the five-gold method (INIFAP, 2012). Soil was collected using a shovel at a depth of 20 cm, and approximately six kg of soil were collected per orchard. The soil samples per orchard were homogenized and subdivided into three pots (per orchard) with ~1.5 kg each one to establish trap cultures (initiated on January 23, 2018). Additionally, one kg of soil from each orchard was used for physicochemical analyses. The results of soil physicochemical parameters are shown in Figure 2 and were determined according to the Official Mexican Norm PROY-NOM-021-RECNAT-2000 (SEMARNAT, 2002). All analyses were conducted at the Laboratorio de análisis de suelo, Instituto de Ecología A.C. (INECOL), Xalapa, Mexico.

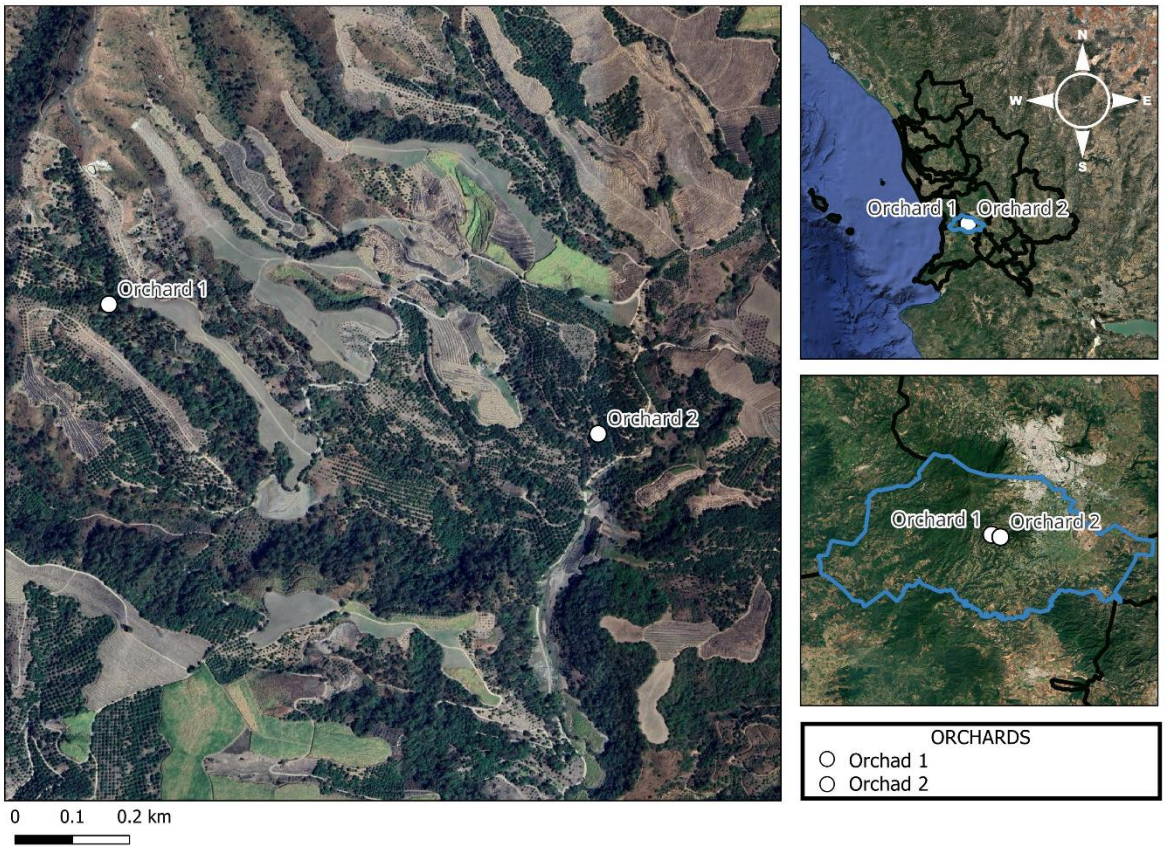

Figure 1. Study orchards located within the Sierra de San Juan State Reserve, Nayarit, Mexico. Map elaborated by Abigail Balderas-Alba

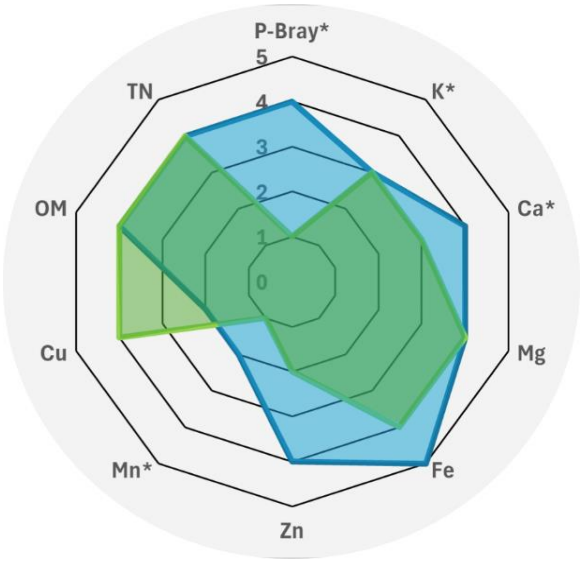

Figure 2. Summary of soil parameters (P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Fe = iron, Zn = zinc, Mn = manganese, Cu = copper, OM, organic matter, TN = total nitrogen) quantified from Orchard 1 (blue) and Orchard 2 (green) located within the Sierra de San Juan State Reserve, Nayarit, Mexico. Numbers indicated: 1) very low or deficient, 2) low or marginal, 3) medium or adequate, 4) high, and 5) very high (following Castellanos *et al.*, 2000). Soil elements marked with an asterisk mean that its minimum scale is 1 and its maximum scale is 3.

Arbuscular mycorrhizal fungi propagation

In each pot containing soil from both orchards, we added seeds from three grass species –*Festuca rubra*, *Cynodon dactylon*, and *Lolium perenne*– with the aim of increasing the number of AMF propagules, enhancing their viability, and obtaining sufficient material for DNA extraction (Vieira *et al.*, 2018). The trap plants were maintained under agriculture shade mesh conditions and irrigated with tap water as needed (approximately three times per week). Two months after germination, 100 mL of Hoagland's nutrient solution without phosphorus was added to each pot.

After five months, the plants were left to dry *in situ* (June 12, 2018). Fifteen days later, a second propagation cycle was initiated, which concluded four months later. At the end of both cycles, dried fragments of colonized roots were collected from each pot, placed in 1.5 mL Eppendorf tubes containing DNAzol™ (ThermoFisher Scientific), and stored at -80 °C (Sangabriel-Conde *et al.*, 2015; Vieira *et al.*, 2018).

Additionally, we extracted AMF spores directly from the dry soil. Soil samples were homogenized according to their origin, i.e., orchard 1 (soil without nutritional management) and orchard 2 (soil with nutritional management), therefore, we had two samples (one per soil orchard). The samples were transferred to the Laboratorio de Ecología Molecular de la Rizosfera en el Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR-Sinaloa). AMF spores were extracted from 33 g of soil per pot using the wet sieving method (Sieverding, 1991). Subsequently, 1,500 viable spores per orchard were isolated, transferred to 1.5 mL Eppendorf tubes, and suspended in 0.5 mL of distilled water.

We used root fragments and spores from three pots in Orchard 1 and two pots in Orchard 2 (since one pot yielded insufficient DNA) with the aim of obtaining a higher DNA yield from, AMF, since the spore community found in the soils could not colonize the roots of the grass used for the trap cultures.

DNA extraction, amplification and sequencing

DNA extraction from roots and spores was performed using DNAzol™ (ThermoFisher Scientific), following the standard protocol suggested by the manufacturer. DNA integrity was verified through agarose gel electrophoresis, and DNA concentration was quantified with a NanoDrop spectrophotometer, according to manufacturer's instructions.

Genomic DNA (gDNA) was used for molecular identification through the nested PCR technique, consisting of three consecutive amplification rounds conducted in a MultiGene™ thermal cycler. In the first round (PCR1) the primer SUAf1-2 and LSUmAr1-4 were used to amplify an approximately 1,800 bp region (Krüger *et al.*, 2009). The PCR1 reaction mixture included: 1 µL of DNA (10 ng), 1 µL of 10x -$MgCl^2$ buffer, 0.3 µL of 50 mM $MgCl^2$, 0.2 µL of each primer mixture from the SSUAf1-2 (SSUAf1: 5' TGG GTA ATC TTT TGA AAC TTY A-3'; SSUAf2: 5' TGG GTA ATC TTR TGA AAC TTC A-3') and LSUmAr1-4 mix (LSUmAr1: 5' GCT CAC ACT CAA ATC TAT CAA A-3'; LSUmAr2: 5' GCT CTA ACT CAA TTC TAT CGA T-3'; LSUmAr3: 5' T GCT CTT ACT CAA ATC TAT CAA A-3'; LSUmAr4: 5' GCT CTT ACT CAA ACC TAT CGA-3') at 10 mM each, 0.2 µL of 10 mM deoxynucleoside triphosphates (dNTPs), and 0.04 µL of Platinum® Taq DNA polymerase (Invitrogen/Life Technologies, Cat. No. 2010497), in a final reaction volume of 10 µL. The thermal cycling conditions were: initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 s; annealing at 50 °C (modified) for 30 s, and extension at 72 °C for 2 min; followed by a final extension 72 °C for 10 min, and a final hold (∞) at 12 °C. Amplified DNA fragments were separated according to their molecular weight using 1% agarose gel electrophoresis in 0.5x Tris-acetate-EDTA buffer (TAE) buffer, stained with

ethidium bromide, and visualized using a Chemidoc imaging system (Biorad).

The PCR2 reaction mixture included: 1 µL PCR1, 1 µL of 10x -$MgCl^2$ buffer, 0.3 µL of 50 mM $MgCl^2$, 0.2 µL of each primer mixture from the SSUAf1-2 (SSUAf1: 5' TGG GTA ATC TTT TGA AAC TTY A-3'; SSUAf2: 5' TGG GTA ATC TTR TGA AAC TTC A-3') and LSUmAr1-4 mix (LSUmAr1: 5' GCT CAC ACT CAA ATC TAT CAA A-3'; LSUmAr2: 5' GCT CTA ACT CAA TTC TAT CGA T-3'; LSUmAr3: 5' T GCT CTT ACT CAA ATC TAT CAA A-3'; LSUmAr4: 5' GCT CTT ACT CAA ACC TAT CGA-3') at 10 mM each, 0.2 µL of 10 mM deoxynucleoside triphosphates (dNTPs), and 0.04 µL of Platinum® Taq DNA polymerase (Invitrogen/Life Technologies, Cat. No. 2010497), in a final reaction volume of 10 µL. The thermal cycling conditions were: initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 s; annealing at 48 °C (modified) for 30 s, and extension at 72 °C for 2 min; followed by a final extension 72 °C for 10 min, and a final hold at 12 °C ∞.

Amplicons obtained from the nested PCR2 (~1500 bp) were used as a template for a third and final PCR (PCR3). This PCR3 was performed using generalist ITS primers that include adapter sequences enabling the amplicons to be recognized by the flow cell channels during bridge amplification in Illumina sequencing. The primers used were: ITS forward: 5' – TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG - TCC GTA GGT GAA CCT GCG G – 3'. ITS Reverse: 5' – GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G – TCC TCC GCT TAT TGA TAT GC– 3'.

These primers have an "extra" sequence that is known as an "adapter" and serve as anchoring sites for binding to flow cells. This primer pair amplifies a region smaller than 600 bp. The PCR3 mixture consisted of: 2 µL of PCR2 product, 5 µL 10x -$MgCl^2$ buffer, 1.5 µL of 50 Mm $MgCl^2$, 1 µL of each ITS primer, 1 µL of 10 mM dNTPs, and 0.2 µL of Platinum® Taq DNA polymerase (Invitrogen/Life Technologies, Cat. No. 2010497), in a total volume of 50 µL. The PCR3 conditions were: initial denaturation at 94 °C for 2 min; followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s (annealing), and 72 °C for 2 min; with a final extension at 72 °C for 10 min and hold (∞) at 12 °C. Next, amplification success was verified by agarose gel electrophoresis as described previously. The PCR3 products were purified using the QIAquick PCR Purification Kit (Qiagen, Cat. No. 28106), and DNA concentrations were quantified with a NanoDrop spectrophotometer.

Finally, the purified amplicons were sent to the Unidad Universitaria de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UUSMB-IBT-UNAM) for sequencing in the Illumina MiSeq (2x300) platform.

Bioinformatic analyses

Bioinformatic analysis of the samples was carried in the UUSMB-IBT-UNAM. First amplicons were merged using Flash (v.1.2.11) software. Next Parallel-META (v2.4.1) was used to perform the annotation and taxonomic classification of the sequences present in each of the samples using an ITS database. Species were reported as virtual taxa and only taxa with two or more reads were reported, singletons were discarded.

Data analyses

All statistical analyses were performed using the R software (R Core Team, 2024). AMF virtual taxa (VT) diversity was determined using the effective number of species proposed by Jost (2006) using the library iNEXT (Hsieh *et al*., 2024; Chao *et al*., 2014).

The effective number of species standardizes richness to a given sample size and allows direct comparison across datasets. For the diversity analyses, we used the diversity indices: $q = 0$, $q = 1$ and $q = 2$, because the sensitivity towards abundant and rare OTUs can be modulated using the scaling parameter q, known as the "order" of diversity (Jost, 2006). The diversity of order zero ($q = 0$) is insensitive to OTU frequencies, thus yielding a richness value. A $q = 1$ is the value that weighs OTUs by their frequency, without disproportionately favoring either rare or abundant ones, and this order of diversity is exactly the exponential of the Shannon index. When the diversity of order q = 2 is applied, the calculation gives greater weight to abundant OTUs, and the formulate corresponds to the inverse of Simpson's index (Alberti and Gilbert, 2019).

To test for differences in sequencing depth among samples, we used normalized read counts expressed as reads per 1000 sequences. This normalization procedure allowed us to compare orchards on a common scale. Therefore, we used a Wilcoxon rank-sum test (non-parametric), since model residuals did not meet the assumption of normality.

RESULTS

A total of 29 virtual taxa (VT) of AMF were identified associated with *P. americana* according to the *Catalogue of Life* (COL Version: 2025-05-13). These taxa belonged to nine genera (*Acaulospora, Archaeospora, Dominikia, Glomus, Microkamienskia, Orientoglomus, Redeckera, Rhizophagus* and *Scutellospora*) distributed in five families

(Acaulosporaceae, Archaeosporaceae, Diversisporaceae, Gigasporaceae, and Glomeraceae) and three orders (Archaeosporales, Diversisporales, and Glomerales). Of the 29 VT, only 10 were identified to species level, and the other fungal types belonged to eight genera (Table 1).

Table 1. Presence/absence of virtual taxa in two avocado orchards. Orchard 1 did not receive agricultural management, while Orchard 2 received agricultural management.

Virtual taxa	Orchard 1	Orchard 2
Acaulospora Appoloni08 Acau 6	X	X
Acaulospora fragilissima	X	X
Acaulospora mellea		X
Acaulospora sp.	X	X
Acaulospora sp. K	X	
Archaeospora Appoloni08 ARCH-5	X	
Archaeospora Kohout14 Arch-1		X
Archaeospora sp.	X	X
Dominikia difficilevidera		X
Glomus Appoloni08 Glom A-20		X
Glomus Konig10 Sequence type 3		X
Glomus OTU41		X
Glomus sinuosum		X
Glomus sp.	X	X
Glomus sp. VTX00113		X
Glomus Wang11 Glo10	X	X
Glomus Wang11 Glo13	X	X
Glomus Wang11 Glo18	X	X
Glomus Wang11 Glo5		X
Glomus Wang11 Glo9		X
Glomus Wubet04 Glom12	X	
Glomus Wubet04 Glom17	X	
Microkamienskia divaricata	X	X
Orientoglomus emiratium		X
Redeckera fulva		X
Rhizophagus fasciculatus	X	X
Rhizophagus irregularis	X	
Rhizophagus irregularis VTX00115	X	
Scutellospora alterata		X

When comparing the AMF community between orchards, there was a high turnover (beta diversity) of VT, with 10 virtual taxa in common, six VT exclusive to Orchard 1, and 13 VT exclusive to Orchard 2 (Figure 3, Table 1). Although our aim was not to compare the AMF community obtained from spores *versus* roots from trap cultures, we found that four VT were detected only from spores (*Archaeospora* Appoloni08 ARCH-5 from Orchard 1, and *Glomus* Wang11 Glo9, *Glomus* sp. VTX00113, and *Redeckera fulva* from Orchard 2), although their relative read abundance was low (below 1%).

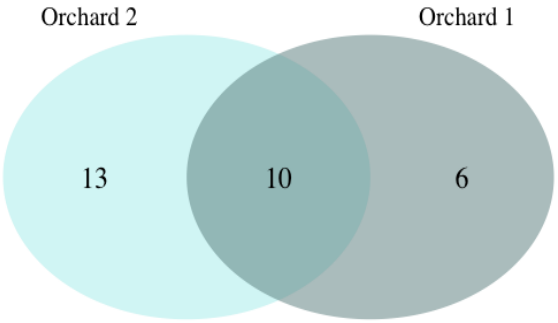

Figure 3. Illustration of shared and exclusive virtual taxa of arbuscular mycorrhizal fungi communities in two avocado orchards under different agronomic management. Orchard 1 without agronomic management, and Orchard 2 with agronomic management.

When we evaluated the relative reads abundance of VT, the patterns differed between orchards. In Orchard 1, the highest relative read abundance corresponded to *Rhizophagus fasciculatus* (59.46%), followed by *Glomus* Wang11 Glo13 (19.95%), *Rhizophagus irregularis* VTX00115 (12.83%), *Microkamienskia divaricata* (6.09%), *Glomus* sp (1.23%), while the remaining VT fungi had relative reads abundance below 1% (Figure 4). In Orchard 2, *Glomus* Appoloni08 Glom A-20 (45.46%), *Archaeospora* sp. (28.03%) and *Archaeospora* Appoloni08 Acau 6 (25.02%) were dominant, whereas the other VT showed relative reads abundance below 1% (Figure 4).

Overall, seven out of the 29 VT accounted for 98.5% of the total relative abundance, and only two VT (*Rhizophagus fasciculatus* and *Glomus* Appoloni08 Glom A-20) together represented 52.46% of the relative abundance across both orchards.

Regarding diversity indices, the richness (q = 0) of VT fungi was higher in Orchard 2 compared with Orchard 1 (Figure 5). However, the diversities of order 1 (q = 1), that gives equal weight to both: the most abundant species and the rarest ones, and of order 2 (q = 2), that will be more biased towards the number of more abundant species, showed that there are not differences between orchards (Figure 5). In the same way, the Wilcoxon rank-sum test (W = 332.5, p = 0.166) was not significant, i.e., showed that the distribution of normalized read counts is the same between Orchard 1 and Orchard 2.

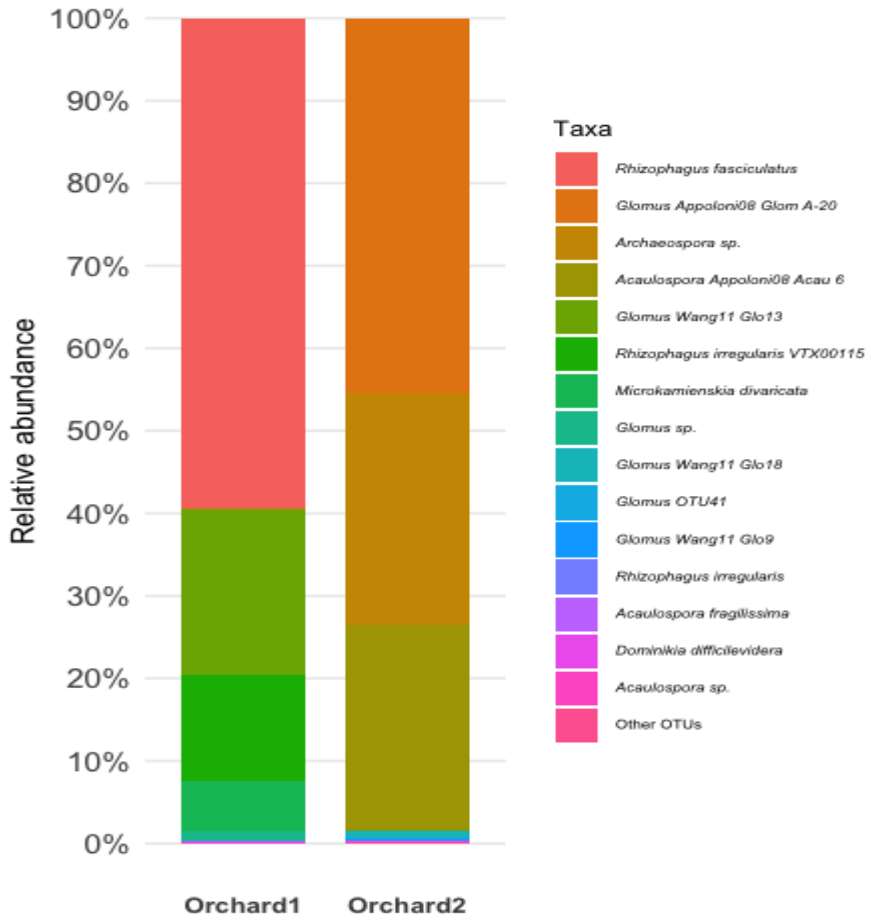

Figure 4. Relative read abundance of AM fungi virtual taxa in Orchard 1 and Orchard 2. Only 15 virtual taxa are shown, "other OTUs" include VT that did not exceed 1% relative abundance in the samples.

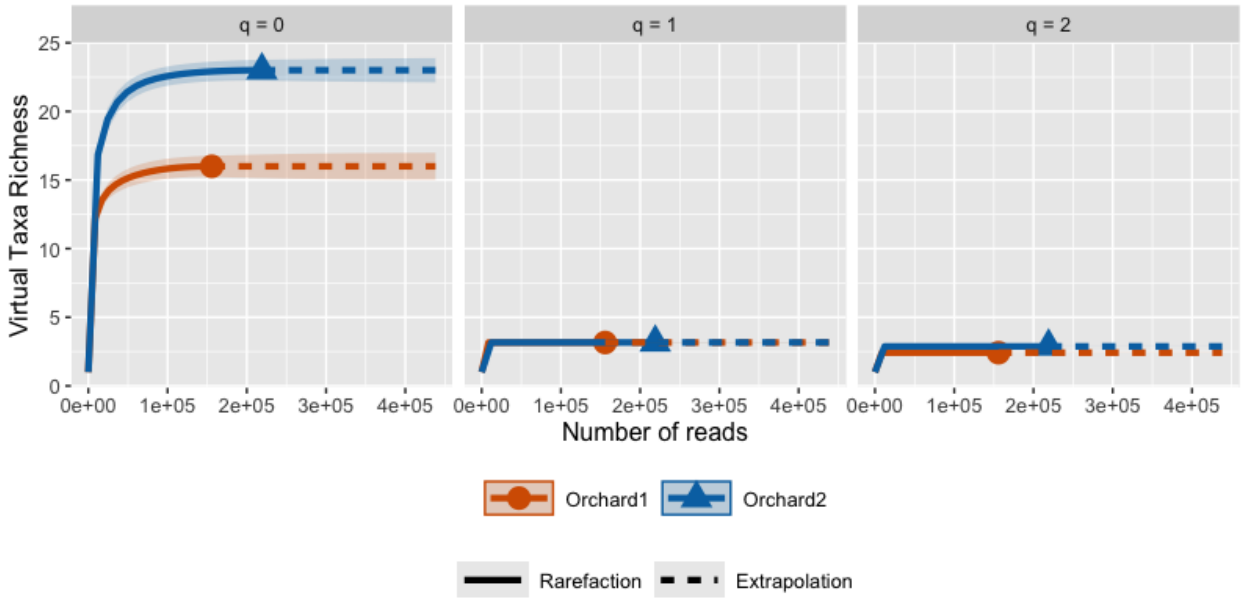

Figure 5. Effective number of AM fungi virtual taxa expressed as diversity indices (q = 0, q = 1 and q = 2). Shaded areas represent 95% confidence intervals. Orchard 1 is shown in orange and Orchard 2 in blue. Extrapolations extend up to twice the number reads recorded.

DISCUSSION

In this study, we aimed to assess the richness and abundance of AMF in two orchards with different soil agricultural management: Orchard 1 without soil nutrition management, and orchard 2 with soil nutritional management. Although previous studies have shown that the addition of agrochemicals to soil can reduce the AMF propagules in agroecosystems (Pagano *et al*., 2023; Martin and van der Heijden, 2024), we found, contrary to our hypothesis, that Orchard 2 had higher richness. However, when abundances were weighted (q = 1 and q = 2), the effective diversity did not increase proportionally in the managed orchard, indicating dominance of a few VT and thus lower evenness. In contrast, the unmanaged orchard exhibited lower richness but greater evenness in the distribution of abundances, suggesting a fungal community structure that is potentially more stable.

Although there were no differences in the effective number of VT between orchards, we found that the composition of VT was different. Of the 29 total VT, 10 were shared, suggesting a low similarity between orchards and, therefore, a high turnover, even though orchards are only 1.17 km apart. Environmental, edaphic factors and plant hosts can filter AMF species based on traits. In Orchard 1, the concentrations of phosphorus (P), calcium (Ca), iron (Fe), zinc (Zn), and manganese (Mn) were higher than in Orchard 2, although the latter received a chemical fertilizer mixture and poultry manure.

In agroecosystems with higher management intensity levels and increasing P availability, plants tend to become more selective towards certain mycorrhizal functional groups (Yan *et al*., 2025; Verbruggen *et al*., 2012; Johnson, 1993) that confer stress tolerance and defence benefits, rather than nutrient acquisition (*community assembly dominated by host filtering*, Frew *et al*., 2023). This filtering process leads to a species-poor community, which could explain the lower diversity found in Orchard 1 compared with Orchard 2. A previous study conducted in the same orchards (Balderas-Alba *et al.*, 2019) showed that the percentage of arbuscular mycorrhizal colonization in avocado plants from Orchard 1 (without management) was highest than in Orchard 2 (with management). This finding may indicate that the composition of AMF species in Orchard 1 has a greater capacity to colonize their avocado host (infectivity). Additionally, Balderas-Alba *et al*. (2025), under experimental conditions, used soil from orchards to grow avocado seedlings that were inoculated with fungal propagules from their respective orchards and vice versa. Overall, the results showed an adaptation to local soils and indigenous AMF communities. Plants growing in soil and inoculum from Orchard 1 exhibited higher benefits in terms of infectivity (greater intraradical colonization) and effectivity (root traits). Moreover, plants growing in soil from Orchard 1 showed a negative correlation between root branching ratio and AMF colonization, suggesting a collaboration gradient, i.e., a beneficial association with mycorrhizal fungi (Matthus *et al*., 2025). Therefore, the studies indicate previously support the idea that the AMF community in Orchard 1 has higher effectivity and infectivity than in Orchard 2. In this way, Frew *et al*. (2023) observed that although AMF VT richness in plant roots became less diverse and more phylogenetically clustered under high P conditions, plants exhibited an increase in total AMF colonization, suggesting that arbuscular mycorrhizal symbiosis may benefit plants in terms of defense and stress resistance.

The addition of agrochemicals to avocado orchards may select AMF families with different life-history traits (*community assembly dominated by competitive exclusion*, Frew *et al*., 2023), potentially affecting the benefits that plants derive from mycorrhizal symbiosis. Orchard 2 harbored more AMF genera (*Dominikia*, *Glomus*, *Microkamienskia* and *Orientoglomus*) and virtual taxa belonging to the family Glomeraceae compared with Orchard 1. Taxa in the Glomeraceae family typically follow a ruderal strategy, adapted to conditions of high disturbance and low stress by prioritizing rapid growth rates, high hyphal turnover, and produce abundant spores, at the cost of low biomass allocation to extraradical hyphae (Cahyaningtyas and Ezawa, 2024; Horsch *et al*., 2023). In addition, spore morphological traits such as smaller size and thicker walls are more frequent in disturbance sites (Lethielleux-Juge, 2025; Hopkins and Bennett, 2023). These life-history traits could explain the higher AMF virtual taxa richness observed in the Orchard 2, however, as noted before, greater AMF richness did not necessarily translate into greater mycorrhizal plant benefits for plants (Balderas-Alba *et al*., 2025). Therefore, it would be of interest to further characterize the AMF community inside avocado roots. An alternative explanation for the higher richness of VT observed in Orchard 2 is that fertilization with commercial poultry manure may have introduced spores from other locations (Bueno and Moora, 2019). Therefore, it would be interesting to analyze the poultry manure for the presence of AMF spores.

To our knowledge, this is the first report of AMF diversity associated to avocado in Nayarit, Mexico, using molecular tools. However, three previous studies in agroecosystems of *Coffea arabica* (Aguilar-Chama and Vega-Frutis, 2023; Luna-Rosales, 2023), *Annona muricata* (González-López *et al*., 2024), and tropical cloud forest (Aguilar-Chama and Vega-Frutis, 2023) based on morphological descriptions, reported 34 species belonging to 12 genera (*Acaulospora*,

Ambispora Claroideoglomus, *Entrophospora*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Rhizoglomus*, *Septoglomus*, *Sclerocystis*, *Scutellospora* and *Septoglomus*). In five morphospecies, identification was only possible at the genus level. In the present study, we found five genera not previously reported in Nayarit (*Archaeospora*, *Dominikia*, *Microkamienskia*, *Orientoglomus*, and *Redeckera*), and only four genera (*Acaulospora*, *Glomus*, *Rhizophagus* and *Scutellospora*) were shared with previous AMF descriptions (González-López *et al*., 2024; Aguilar-Chama and Vega-Frutis, 2023; Luna-Rosales, 2023). This finding indicates greater AMF diversity and suggests that each ecosystem and agroecosystem harbors its own fungal community.

Molecular studies have also led to a major advance in the taxonomy of the phylum Glomeromycota, with new species continuing to be described. In our study, we obtained sequences from the *Dominikia difficilevidera*, *Microkamienskia divaricate*, and *Orientoglomus emiratium* (Corazon-Guivin *et al*., 2019; Blaszkowski *et al*., 2015). It is likely that these species have a worldwide distribution, but they either occur infrequently or have been overlooked or lost during spore extraction from many soils because their spores are extremely small and hyaline. Polo-Marcial *et al*. (2021) published a checklist of AMF in Mexico, and *D. difficilevidera*, *M. divaricate*, and *O. emiratium* were not reported. Therefore, this may represent the first record of these species in Mexico.

Finally, in an agricultural context, the ability of plants to respond to AMF with changes in morphological or performance (e.g., yield) depends on the plant-fungal genotype combination as well as the biotic and abiotic environment (Balderas-Alba *et al*., 2025). However, the genetic and mechanistic bases of these interactions remain largely unknown. Further integration of the closely interlinked life-history traits of AMF with physicochemical parameters of each agroecosystem will provide an ecological framework for understanding how cultivate plants meet their life-cycle requirements and will allow a more accurate prediction of biodiversity patterns in agroecosystems.

CONCLUSIONS

Our results provide convincing evidence that the application of chemical fertilizers, particularly phosphorus, modifies the composition and abundance of AMF associated with avocado orchards soil. Agrochemicals may act as an environmental filter on AMF community assembly, as host plants alter their selectivity for fungal functional groups, prioritizing species associated with lower nutrient acquisition, but potentially enhancing plant stress resistance and defenses. Therefore, AMF species with a ruderal strategy could be favoured under conventional cultivation, as we previously noted. In addition, molecular tools allowed us to increase the number of AMF species recorded in Nayarit associated to avocado orchards soil, including AMF species whose spores are extremely small and hyaline.

It is important to mention that the use of trap cultures to extract the fungal propagules can increase the number of spores and root colonization in the host plants, but may also filter AMF species (Trejo-Aguilar *et al*., 2013). Moreover, commercial poultry manure could contain spores introduced during its preparation, handling or transporting, and we did not analyze its chemical properties. Additionally, we only studied two avocado orchards, and increasing the number of orchards would be desirable, as well as including a comparison with a reference ecosystem to establish a baseline for the AMF diversity originally present.

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Compliance with ethical standards. Consent was obtained from the orchard's owners.

Data availability. The data used to analyze the results are available with Dr. Rocío Vega-Frutis (rocio.vega@uan.edu.mx) upon request.

Author contributions statement (CRediT). A. Balderas-Alba – Fieldwork, AMF propagation, DNA extraction, amplification and writing – original draft preparation. **P.A. Báez-Astorga** – Bioinformatic analysis, writing – review and editing. **I.E. Maldonado-Mendoza** – DNA extraction, amplification, writing – review and editing. **G.R. Peña-Sandoval** – Data analysis, writing – review and editing. **R. Vega-Frutis** – Conceptualization, methodology, supervision, data analysis, writing – review and editing, funding acquisition.

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