FOLIA OECOLOGICA – vol. 52, no. 2 (2025), doi: 10.2478/foecol-2025-0015

Soil mycobiome structure across central and peripherial zones in a pecan nut agroecosystem [*Carya illinoinensis* (Wangenh.) K. Koch]

Judith A. Sánchez-Ledesma[1], Bernardo Águila[2]*, Roberto Garibay-Orijel[2], Cristina García-De la Peña[3], Erika Nava-Reyna[4]

[1]Research Center in Cellular Dynamics, Institute for Research in Basic and Applied Sciences. Autonomous University of the State of Morelos, 62210, Mexico
[2]Institute of Biology, National Autonomous University of Mexico. Outer Circuit s/n, University City, Coyoacán, 04510 Mexico City, Mexico
[3]Faculty of Biological Sciences, Juárez University of the State of Durango. Gómez Palacio, Durango. 35010, Mexico
[4]National Center for Disciplinary Research in Water, Soil, Plant, and Atmosphere Relationships, National Institute of Forestry, Agricultural, and Livestock Research. Right Bank of the Sacramento Channel km 6.5, Gómez Palacio, Durango. 35140, Mexico

Abstract

Sánchez-Ledesma, J.A. Águila, B., Garibay-Orijel, R., García-De la Peña, C., Nava-Reyna, E., 2025. Soil mycobiome structure across central and peripherial zones in a pecan nut agroecosystem [*Carya illinoinensis* (Wangenh.) K. Koch]. *Folia Oecologica*, 52 (2): 149–161.

Using ITS1-based metabarcoding, we investigated the structure of the soil fungal communities in the central and peripheral zones of a 25-hectare pecan nut (*Carya illinoinensis*) orchard located in the arid region of Coahuila, Mexico. While environmental conditions such as soil moisture and temperature varied between zones, physicochemical soil properties (pH, organic carbon, total carbon, organic matter, electrical conductivity, and zinc) remained homogeneous. A total of 4,443 fungal OTUs were detected at 97% similarity. Alpha diversity indices did not differ significantly between zones. The fungal community was dominated by the phyla Ascomycota and Basidiomycota, with Pezizomycetes, Dothideomycetes, and Agaricomycetes as dominant classes. No statistically significant differences in beta diversity or community composition were found between zones (PERMANOVA $p = 0.662$). Redundancy analysis also revealed no clear clustering by zone, though localized differences were observed. Our findings suggest that agronomic management in this system promotes environmental homogeneity, leading to relatively uniform fungal communities. This exploratory study highlights the need for future research incorporating comparisons with adjacent natural ecosystems to better assess spatial patterns and potential edge effects in agroecosystems.

Keywords

agronomic management, arid zones, *Carya illinoinensis*; ITS14, metabarcoding, mycobiome

Introduction

The cultivation of *Carya illinoinensis* (pecan nut) in arid regions of northern Mexico presents important opportunities for sustainable food production and soil biodiversity conservation (Sánchez-Ledesma et al., 2023). Understanding the composition and function of the soil mycobiome in these systems is essential, as fungi play critical roles in soil structure, nutrient cycling, and plant health (Casas Flores, 2012; Zeng, 2023). Fungal communities influence processes such as organic matter decomposition, pathogen suppression, and mycorrhizal symbiosis, which are all vital for orchard productivity and resilience under stressful conditions such as drought and salinity (Tedersoo et al., 2020; Ma et al., 2020).

In ecological theory, the edge effect refers to changes

*Corresponding author:
e-mail: basbajo12@gmail.com

in community structure and function that occur at the boundaries between different habitats (Porensky et al., 2013). The edge effect as a type of spatial structuring, is controlled by the responses of microorganisms to environmental parameters (Aguiar et al., 2023). In agroecosystems, edges may influence microclimatic variables such as light, temperature, and humidity, and may also mediate biotic interactions, including dispersal and competition between fungal taxa (Schmidt, 2019). Studies have shown that edge effects can either promote diversity through habitat heterogeneity or reduce it due to disturbance gradients (Bennett et al., 2022; Johnson et al., 2011).

Despite the increasing attention to microbial ecology in natural ecosystems, relatively few studies have explored fungal communities in orchards or other managed perennial systems, particularly in relation to spatial gradients within the cropping area (Kreitzman, 2020). The genus Carya, including *C. illinoinensis*, has been associated with diverse fungal partners: including arbuscular mycorrhizal fungi (e.g., *Glomus*, *Rhizophagus*) which provide benefits to the tree mainly in the supply of nutrients and helping them to grow in salinity conditions typical of arid zones (Guerrero-Galán et al., 2019; Ma et al., 2021); ectomycorrhizal species (e.g., *Tuber, Lactarius, Russula, Peziza*); as well as saprotrophs which can work as biostimulants in the processes of flower growth, fruit size and fruit set (Alvidrez-Villarreal et al., 2012); and phytopathogenic genera such as *Fusarium* (Gryzenhout et al., 2016; Shi et al., 2022). These communities are shaped by host traits, orchard age, soil chemistry, and irrigation regimes. The incorporation of these soil fungal groups in *Carya illinoinensis* agriculture contributes to the maintenance biodiversity and proper orchards functioning. The interaction between soil microorganisms and agroecosystems involving forest species such as *Carya illinoinensis* positively influences tree development. This interaction significantly impacts agriculture by providing valuable insights into the microbial composition of ecological niches, their metabolic potential, and biological interactions (Rivera-Urbalejo et al., 2021).

However, there is still limited knowledge about spatial variation within orchards, such as differences between central and peripheral zones, may influence fungal community structure, especially in arid systems where microclimatic gradients are accentuated (Eslaminejad et al., 2020; Sharma et al., 2022).

This study explores fungal community composition and spatial structure in the center and periphery of a pecan nut orchard in Coahuila, Mexico. We applied high-throughput DNA sequencing of the ITS1 region to characterize fungal diversity and composition in relation to environmental and physicochemical variables. We aim to determine whether management practices and environmental gradients within the orchard result in spatial differentiation of the soil mycobiome. While our study is exploratory and based on a limited number of composite samples, it provides a first step toward understanding the spatial ecology of soil fungi in *Carya illinoinensis* agroecosystems under arid conditions.

Materials and methods

Study area

This study was conducted in a 25-hectare orchard of *Carya illinoinensis* located in the municipality of Viesca, Coahui-

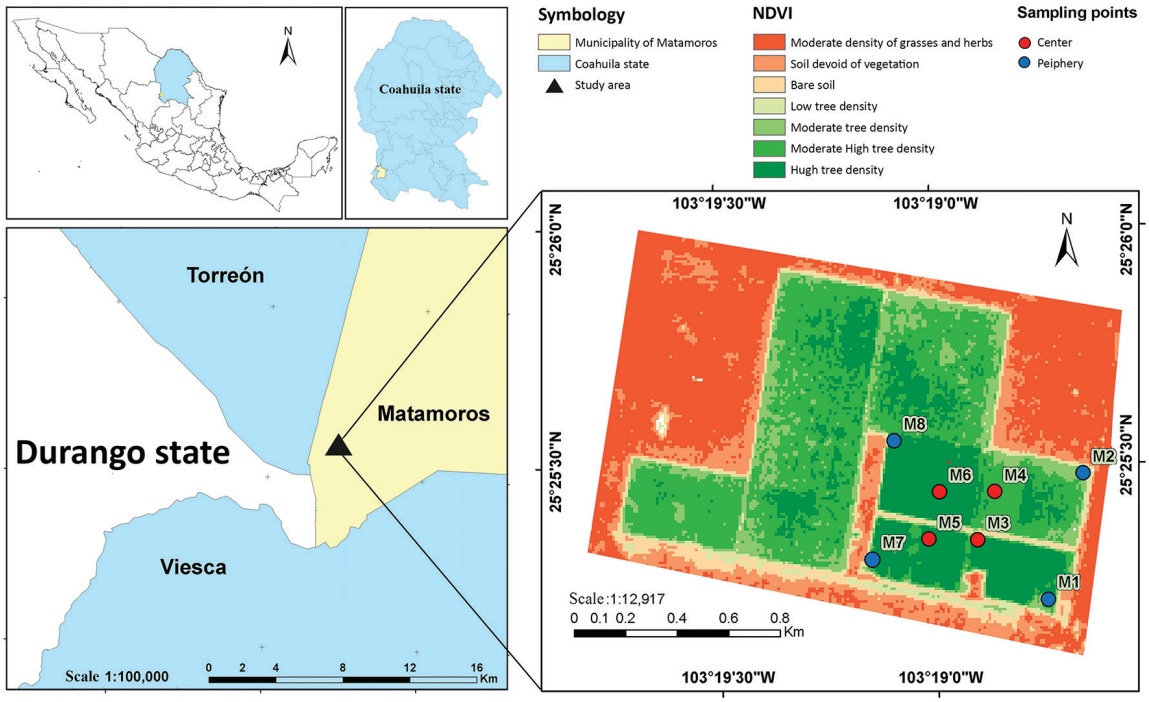

Fig. 1. Geographical location of the study site of pecan nut in Viesca, Coahuila, Mexico. A total of eight samples (composite samples) were sampled; Soils exposed to full sun (Periphery) include samples in blue, while soils on full shadow include samples M3, M4, M5, and M6 (Center) colored in red.

la, Mexico (Fig. 1). It is located at 2.5°25'N, 108°18'W, with an elevation of 1,200 m above sea level. The orchard utilizes a 12 × 12 planting grid, a subsurface drip irrigation system, and has a clay-loam textured soil, with a field capacity of 0.35 m^3 m^{-3} and a permanent wilting point of 0.19 m^3 m^{-3} (Sánchez-Ledesma et al., 2023). The orchard is managed conventionally according to the technological package for pecan nut production (INIFAP, 2002).

Sample collection

A 25-hectare area was surveyed, containing *Carya illinoinensis* trees aged 20–25 years, each producing 15–20 kg of nuts per year. The orchard was divided into two zones: center (shaded soil beneath the tree canopy) and periphery (soil exposed to full sun). Four composite soil samples were collected per zone, resulting in a total of eight composite samples. For each composite sample, soil was collected from bulk soil around the periphery of 10 trees, with four subsamples of ~25 g, taken 1 meter from the tree trunk in each cardinal direction at a depth of 20 cm (Cabrera-Rodríguez et al., 2020). These 40 subsamples were homogenized using a sanitized plastic buckle producing one 1 kg composite sample per site (Supplementary material, Fig. S1). Composite sample was divided for physicochemical analyses, and 0.25 g was placed in PowerLyzer tubes for DNA extraction. All samples were transported to the laboratory at 4 °C. Soils exposed to full sun (Periphery) include samples M1, M2, M7, and M8, while soils on full shadow include samples M3, M4, M5, and M6 (Center).

Physicochemical analysis

For physicochemical analysis, air-dried soils were sieved (2 mm). Zinc (Zn) was determined as a plant nutrient (Bremner, 1965). Soil salinity and pH were determined using the electrode method with water extraction in a 1:5 w/v ratio (Luján Soto et al., 2021). Total carbonates ($CaCO_3$) were determined using the Bernand calcimeter method (Rodríguez, 1996), and total carbon (CT) was determined by the elemental analyzer. Total organic carbon (COS) was obtained by subtracting the concentration of total carbonates from TC, while Soil organic matter (MOS) was determined by the method of Walkley and Black (1934). Measured units are as follows: Carbonates (%), CT (%), COS (%); MOS (%); and Zn (mg kg^{-1}), Humidity (%); Temperature (C°), and CE (dS m^{-1}).

DNA extraction, amplification, and sequencing

A total of 0.25 g of bulk soil was placed in a BashingBead™ cell lysis tube (Zymo ResearchCorp., Irvine, CA, USA), containing 750 µL of lysis/stabilizer solution. Each tube was processed in a cell disruptor (TerraLyzer™) for 30 s, keeping the samples at room temperature. DNA was extracted with a Zymo BIOMICS™ kit (Zymo Research Corp., Irvine, CA, USA). The amount of DNA was measured in a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). Genomic DNA was sent for library preparation and sequencing in Novogene (Novogene Co., Ltd., Beijing, China). The ITS1 region was amplified using the primer pair ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS2 (GCTGCGTTCTTCATCGATGC) (Tedersoo et al., 2014; Delgado-Baquerizo et al., 2016). PCR amplification was conducted with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 2 µM of direct and reverse primers, and about 10 ng of template DNA. Thermal cycling consisted of an initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s and a final elongation at 72 °C for 5 min. PCR products were mixed at equidensity ratios and purified with Qiagen Gel Extraction (Qiagen, Germany). Sequencing libraries were generated using the ADNTruSeq PCR-free sample preparation kit (Illumina, USA) following the manufacturer's recommendations and index codes were added. Library quality was assessed using the Qubit 2.0 fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina NovaSeq 6000 platform (Novogene Co., Ltd., Beijing, China), obtaining 250 bp paired reads, each sample yielded between ~32,000 and ~214,000 high-quality effective reads after chimera removal, with over 96% of bases scoring above Q30.

Bioinformatic processing

Bioinformatic analyses were conducted using the toolkit PipeCraft v0.1.4 (Anslan et al., 2017). Using the vsearch OTU workflow with default values, demultiplexed fastq sequences were reoriented, primers were removed, pair-end sequences were merged, quality filtering was set at a minimal length of 32, for merging min overlap was set at 12 bp, chimeras were removed with the denovo option. ITSs sequences were clustered in OTUs with a 97% cut off. Taxonomy of the OTUs were assigned with BLAST using the UNITE general FASTA release for all eukarionts v 2023-07-18 (Abarenkov et al., 2023), later Fungal OTUs and tables were extracted by taxonomy in the database. Fungal guilds were then assigned to the genera with the Fungal Traits database (Põlme et al., 2020).

Biostatistical analysis

All ecological and statistical analyses were conducted in R (v4.3.1). We used the following packages: Phyloseq (McMurdie and Holmes, 2013) for creating and handling OTU objects, rarefaction, and diversity metrics; vegan v2.3-3 (Oksanen et al., 2016) for multivariate analyses including CAP, ANOVA, PERMANOVA and RDA; ampvis2 (Andersen, 2018) for visualizations including PCA, heatmaps, and Venn diagrams; ggplot2 (Ginestet, 2011) and dplyr (Wickham, 2023) for plotting and data wrangling; corrplot (Wei and Simko, 2021) for correlation matrix visualization; OTU tables were rarefied to even sequencing depth (10k reads) to standardize sampling effort. All downstream analyses were performed on rarefied data. Alpha diversity indices (Richness, Shannon, Simpson, Fisher) and beta diversity (Bray–Curtis dissimilarity) were calculated. Redundancy analysis (RDA) using Hellinger-transformed data was performed to evaluate

Table 1. Table of physicochemical parameters measured in this study (see Materials and Methods section for units)

Name	Location	COS	MOS	pH	Zn	CT	Humidity	Temp	CE	$CaCO_3$
M1	Periphery	2.75	4.73	7.78	4.89	6.52	15	33	595	9.36
M2	Periphery	1.88	3.25	8.07	4.18	5.44	15	34	422	9.76
M3	Center	1.37	2.37	8.31	3.94	4.21	12	30	307.33	7.5
M4	Center	1.97	3.4	8.2	5.23	5.39	12	30	412.33	7.2
M5	Center	2.77	4.77	8.08	4.91	6.75	11	29	501	7.95
M6	Center	1.81	3.12	8.47	3.74	5.22	11	28	383.33	7.75
M7	Periphery	1.37	2.37	8.34	3.7	3.84	15	34	361.67	8.57
M8	Periphery	2.11	3.64	8.22	5.35	5.29	15	35	392.33	7.2

relationships with environmental variables. ANOVA and PERMANOVA were used to evaluate differences within variables and in community composition between center and periphery. Co-occurrence networks were generated using the CoNet plugin in Cytoscape v3.10.2 (Legeay et al., 2020), software was performed, using the top five OTUs within five primary lifestyles present in all sites. All R scripts used for exploring composition, diversity measures, dissimilarity analysis, and multivariate analysis are available at GitHub https://github.com/Burn121212/Fungi_Carya_illinoinensis.

Results

Physicochemical and environmental variables

A table with resulting physicochemical variables is presented (Table 1). The physicochemical variables in soil samples from the two zones did not show statistically significant differences ($p < 0.05$). The Spearman correlation analysis regarding species richness indicated a positive correlation with humidity ($p = 0.591$) and temperature ($p = 0.663$) while, a negative correlation was shown with total carbon (TC) (–0.5) (Table 2).

Table 2. T-test results for comparison of environmental and physicochemical variables between Periphery and Center zones

Variable	t_stat	p_value	Significant
	0.116117	0.911351	–
MOS	0.117785	0.910084	–
pH	–1.108724	0.315206	–
Zn	0.145294	0.889237	–
CT	–0.158165	0.87953	–
Humidity	12.124356	0.001208	*
Temp	7.549834	0.000315	*
CE	0.634623	0.550616	–
$CaCO_3$	1.911284	0.138877	–

Sequence data processing

All samples had >96% of bases with Q30 quality scores, ensuring high confidence in sequence-based community profiling. A total of 1,039,420 quality-filtered ITS1 reads were obtained, with per-sample depths ranging from 32,678 to 214,105 reads. After the Vsearch workflow, a total of 4,442 OTUs were identified at 97% similarity. After rarefaction to 10,000 reads per sample, 1,665 OTUs remained.

Beta diversity and primary lifestyle composition of the mycobiome

A cluster analysis (Beta diversity) using the Bray Curtis model which considers abundances of species (Fig. 2), showed that the lowest dissimilarity, 0.45, was observed between groups M2 and M7 in the periphery, with the main trophic guilds being plant litter saprotrophs and plant pathogens. A second cluster showed a higher dissimilarity level of 0.69 among two samples in the center zone. The main guilds in this cluster were soil saprotrophs, a large number of litter saprotrophs, and ectomycorrhizal fungi. This clustering analysis also indicates similarities among all the analyzed groups, suggesting that the mycobiome community composition was related across both the center and periphery of the orchard. Overall, the primary lifestyles of the mycobiome associated with pecan nut trees were soil saprotroph fungi, a large number of unknown fungi, plant pathogens, and ectomycorrhizal fungi.

Alpha diversity and funga–l family composition

The alpha diversity of fungal communities in the soil of *Carya illinoinensis* orchards was analyzed for each sample, revealing high alpha diversity and taxonomic richness in both zones (Fig. 3). The richness and Shannon indices showed high variation between zones. To further explore whether specific soil conditions were associated with fungal diversity (richness), we performed a Spearman correlation analysis between the environmental and physicochemical variables and the OTU richness (Table 3). In contrast, most physicochemical variables showed weak or no correlation with fungal richness. A detailed table with common alpha diversity indices is presented (Table 4).

Taxonomic annotation using the UNITE database yielded a total of 20 phyla, 67 classes, 159 orders, 358 families, and 735 genera. After rarefaction, 18 phyla, 55 classes, 118 orders, 250 families, and 436 genera remained, representing the retained taxonomic diversity used for downstream analyses. In both zones, the families Pezizaceae, Nectriaceae, and Didymellaceae were predominant, with sample M8 notably containing mostly Peizaceae, although no composition pattern was observed between zones, the families Russulaceae and Saccharomycetales_fam was only found in M3, families Plectosphaerellaceae and GS11_fam were only found in M4, Hydnodontaceae was only found in M5, and Herpotrichiellaceae was only present in M7.

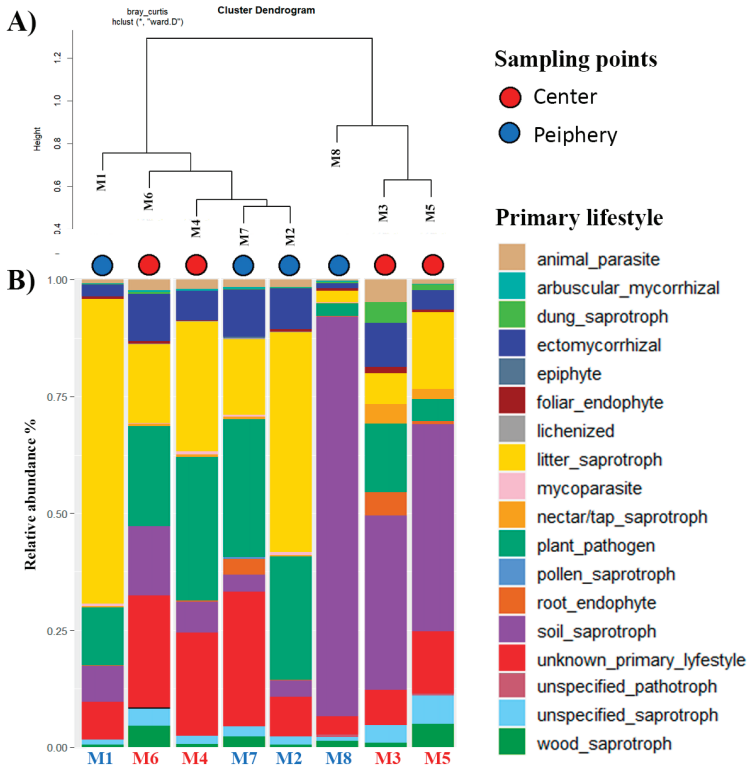

Fig. 2. Analysis of Beta diversity and composition of trophic guilds. 2a. Dissimilarity analysis with the Bray Curtis model of the 8 sampled sites, center (red) and periphery (blue). 2b. Barplots of the fungal primary lifestyles, primary lifestyles representing less than 1% of the relative abundance in each sample were excluded from the graph.

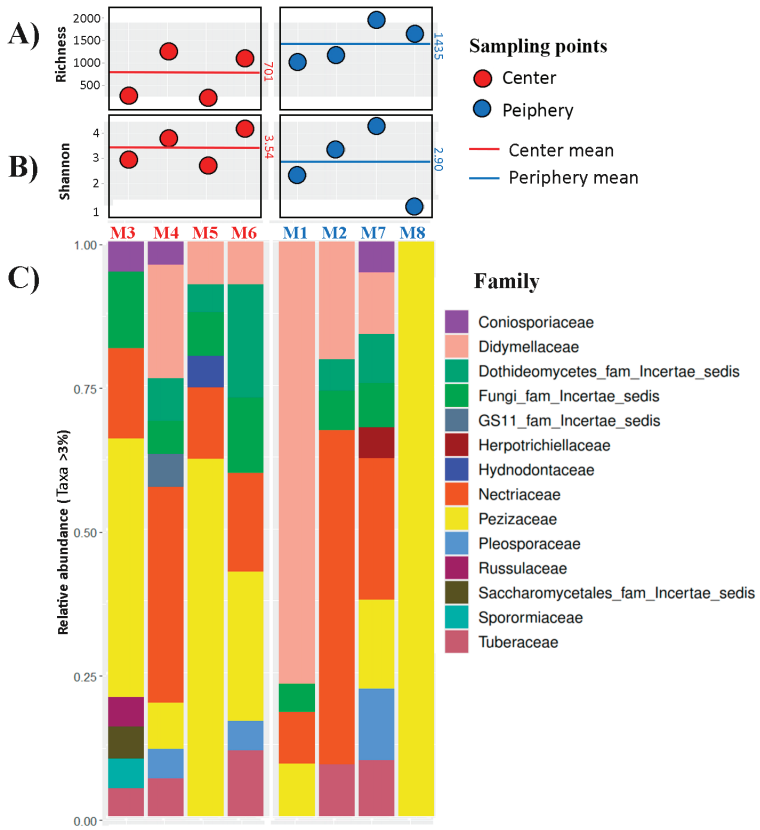

Fig. 3. Alpha diversity and taxonomic composition of the 8 sampled sites, center (red) and periphery (blue). 3a. Observed diversity (richness) for each site. 3b. Shannon diversity index (Evenness for each site). 3c. Barplots of fungal families, families representing less than 3% of the relative abundance in each sample were excluded from the graph.

Table 3. Spearman correlation between environmental and physicochemical variables and species Richness. Bold values indicate variables with a strong positive correlation with richness (rho value).

	COS	MOS	pH	Zn	CT	Humidity	Temp	CE	$CaCO_3$	Richness
COS	1	1	–0.7065	**0.766**	0.898	–0.0646	–0.0363	0.874	0.024	–0.3353
MOS	1	1	–0.7065	0.766	0.898	–0.0646	–0.0363	0.874	0.024	–0.3353
pH	–0.706	–0.7065	1	–0.5238	–0.8571	–0.3343	–0.2289	–0.8809	–0.443	0.309
Zn	0.766	0.766	–0.5238	1	0.595	0.051	0.204	0.523	–0.467	–0.0238
CT	0.898	0.898	–0.8571	0.595	1	–0.1028	–0.1445	0.952	0.299	–0.5
Humidity	–0.0646	–0.0646	–0.3343	0.051	–0.1028	1	0.937	0.102	0.349	**0.591**
Temp	–0.0363	–0.0363	–0.2289	0.204	–0.1445	0.937	1	0	0.139	**0.662**
CE	0.874	0.874	–0.8809	0.523	0.952	0.102	0	1	0.419	–0.3095
$CaCO_3$	0.0240	0.0240	–0.4431	–0.4670	0.299	0.349	0.139	0.419	1	–0.15569
Richness	–0.3353	–0.3353	0.309	–0.0238	–0.5	0.591	0.662	–0.3095	–0.1556	1

Table 4. Alpha diversity indices (Richness, Shannon, and Simpson) of soil fungal communities across sampled sites. Sites M1–M8 are individual sampling locations; bolded sites represent the central zone. The last columns show the average and standard deviation for central and peripheral zones, respectively.

Alph Div	M1	M2	**M3**	**M4**	**M5**	**M6**	M7	M8	**Mean Center**	**SD Center**	Mean Periphery	SD Periphery
Richness	1,008	1,164	265	1,241	217	1,081	1,935	1,635	701	535-3	1,435.5	426.5
Shannon	2.5	3.48	3.09	3.9	2.87	4.28	4.35	1.27	3.54	0.7	2.9	1.3
Simpson	0.66	0.91	0.88	0.95	0.8	0.96	0.96	0.29	0.9	0.1	0.71	0.3

Fungal prevalence analysis

The abundance of the 30 most prevalent fungal species at both conditions is shown (Fig. 4). Among these, four prevalent species were identified in both zones (*Peziza* sp1, *Nothophoma* sp1, *Fusarium* sp1, and *Fusarium equiseti*). The Peziza sp1 was found with a high sequence abundance in sites M3, M5, M6, M1 and M8, with M8 exhibiting the highest abundance.

Ordination methods and multivariate analysis of fungal communities

First, a canonical analysis of principal coordinates (CAP) was performed to explore whether fungal community composition showed spatial structuring in relation to environmental gradients (Supplementary material, Fig. S2). Although the CAP suggested some grouping of samples (particularly M2, M4, and M7 vs. M3 and M5), there was no evidence of a consistent spatial structuring pattern.

Subsequently, a Redundancy Analysis (RDA) was conducted using Hellinger-transformed OTU data to directly evaluate the influence of environmental variables on fungal community composition (Fig. 5). The RDA confirmed similar groupings observed in the CAP but provided a clearer interpretation of variable contributions. For instance, samples M2, M4, and M7 were associated with higher humidity and temperature, while M8 aligned with

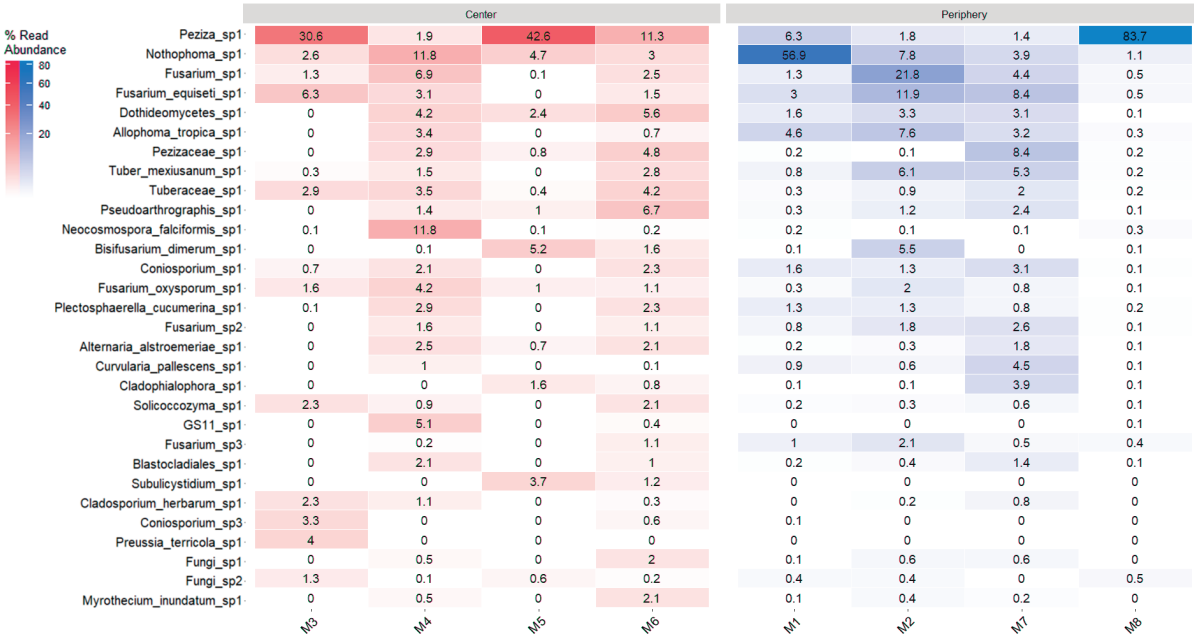

Fig. 4. Heat map of the 30 most prevalent fungi detected in the orchard, ordered by zones.

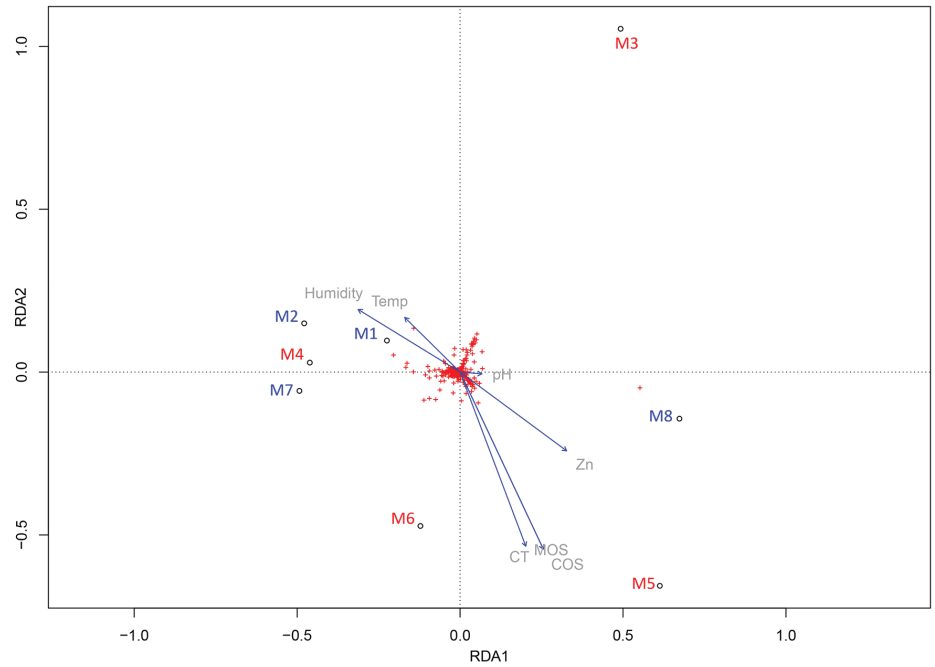

Fig. 5. A Redundancy Analysis (RDA) biplot showing the relationship between soil fungal community composition (Hellinger-transformed OTU data) and environmental variables in a pecan nut orchard. The analysis was constrained by the variables CT, COS, MOS, pH, Zn, temperature, and humidity. Red crosses represent fungal OTUs, while black circles represent individual samples. Blue arrows indicate the direction and strength of the environmental gradients. The length of each arrow reflects the relative contribution of the variable to the explained variance.

Table 5. Results of PERMANOVA (adonis2) testing for differences in soil fungal community composition between central and peripheral zones of the *Carya illinoinensis* agroecosystem

	Df	SumOfSqs	R^2	F	Pr(>F)
Zone	1	0.38375988	0.11568649	0.78492401	0.662
Residual	6	2.9334805	0.88431351	NA	NA
Total	7	3.31724038	1	NA	NA

elevated zinc content. The PERMANOVA analysis using Euclidean distances on Hellinger-transformed OTU data showed that zone explained 11.6% of the total variance in fungal community composition; however, this effect was not statistically significant ($p = 0.662$). This result, together with the ordination analyses, reinforces the conclusion that there is no clear spatial structuring of fungal communities across zones within the orchard (Table 5).

Fungal network co-occurrence analysis

A co-occurrence network analysis was performed to look for potential co-presence and mutual exclusion interactions between the most abundant taxa within the main five primary lifestyles (Fig. 6), which were soil saprotrophs, plant pathogen, ectomycorrhizal, arbuscular mycorrhizal and unknown primary lifestyle. The highest number of ectomycorrhizal fungi were found in the center of the orchard. In addition, *Malassezia* sp1, *Peziza* sp2, *Funneliformis* sp2, and *Fusarium variasi* sp1 were mainly found in the center zone. On the periphery zone, *Glomus* sp1, *Lactarius lacunarum* sp1, *Tuber mexiusanum* sp1, and *Funeliformis* sp1 were identified in greater abundance. The networks showed co-presence between most plant pathogens, unknown primary lifestyles, ectomycorrhizal fungi and arbuscular mycorrhizal, with ectomycorrhizal fungi containing most of the cooccurrences. We found exclusion between soil saprotrophs and unknown primary lifestyles, while plant pathogens showed a remarked exclusion with arbuscular mycorrhizal, *Peziza* sp1, *Peziza* sp2, and *Rhynchogastrema glucoferementans* accounted for most of the mutual exclusions. The network showed multiple potential links between species with different functional traits. Therefore, coexistence and exclusion between species lead to a complex functional network.

Discussion

Homogeneity of fungal communities in the face of differential environmental factors: importance of agronomic management

Environmental variables, such as humidity and temperature, have been reported as the most determinant factors for the differential establishment of fungal communities in soil on a local scale (Melo et al., 2019). However, the temperature and humidity differential caused by vegetation cover between the two zones of the orchard (center and periphery) was not enough to generate spatial structur-

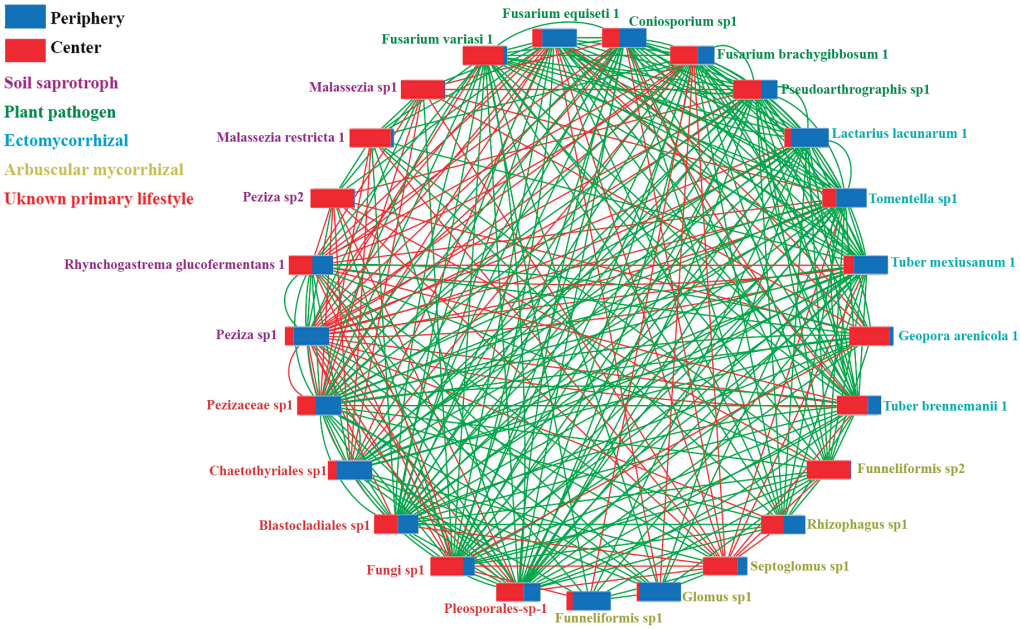

Fig. 6. Cooccurrence network showing the potential interspecific interactions between more abundant taxa of five fungal primary lifestyles. Red lines indicate mutual exclusion between OTUs, while green lines represent co-occurrence. For each OTU, a bar plot displays its relative abundance in the Periphery and Center zones.

ing. A plausible explanation could be the agronomic management of the orchard, which prevents the formation of differentiated communities. This was evident from the fact that no significant differences were found between the two zones in any of the physicochemical variables measured (pH, total carbon, soil organic carbon, soil organic matter and zinc).

Although the PERMANOVA analysis indicated that the variable Zone (center vs periphery) explained 11.6% of the variation in soil fungal community composition, this difference was not statistically significant ($p = 0.662$). This result suggests that the soil mycobiome communities are relatively homogeneous between zones within the *Carya illinoinensis* agroecosystem. The RDA biplot based on Hellinger-transformed data did not reveal a clear separation between central and peripheral sampling zones, which aligns with the PERMANOVA results indicating no significant compositional differences. However, some sites, particularly M3, appeared as outliers in ordination space, suggesting localized environmental conditions or unique fungal community structures. The observed gradient vectors, especially those corresponding to pH, organic matter, and zinc, may help explain this divergence. These findings highlight the potential of constrained ordination to identify microenvironmental drivers of fungal community variation and support the use of RDA as a complementary tool in agroecosystem monitoring.

Agronomic management has been reported as a crucial factor influencing the heterogeneity of the environment and, consequently, the structure and composition of soil communities. Studies on agroecosystems, such as olive (*Olea europaea*), which present arid climatic conditions similar to those of this research, highlight that agricultural management, climate and landscape complexity are key factors for the formation of microbial communities in different agroecological zones (Gkisakis et al., 2016). Therefore, agronomic management should be considered fundamental when assessing biodiversity in agroecosystems.

Dominance patterns and trophic guilds of fungal communities in an agroecosystem: constraints on functional assignment

In our study, the top 30 taxa present in both zones of the pecan nut were identified, showing contrasting distributions of Ascomycota and Basidiomycota, something also reported by other studies in other agricultural crops (Chen et al., 2023; Zhang et al., 2023., Zhang et al., 2024). The dominant families were Pezizaceae, Nectriaceae, and Didymellaceae. Studies conducted in agroecosystems have documented the presence of fungi belonging to the family Pezizaceae. The occurrence and distribution of Pezizales appear to be influenced primarily by edaphic factors, such as soil pH and organic matter content. The Nectriaceae family has been reported to contribute to soil respiration, favoring biomass formation (Yang et al., 2019); especially in moderately saline soils (Yang et al., 2020). On the other hand, Didymellaceae is an important family of soil pathogenic fungi (Ahmadpour et al., 2022; Keirnan et al., 2021; Wei et al., 2021). Phytopathogenic *Fusarium* species were also identified, which coexist with arbuscular mycorrhizae (*Glomus* and *Rhizophagus*), adapting to the ecosystem in a symbiotic manner. *Fusarium* has been reported as a pathogen in pecan orchards, particularly in seedlings, and is associated with infections facilitated by the insect *Euplatypus segnis*. Warm and humid conditions are optimal for its development, and its presence is related to soil factors such as pH and carbon sequestration.

Although the taxonomic composition varied greatly

among the samples and a spatial structural pattern was not observed, a more general pattern was identified when comparing the trophic guilds of the fungal communities. Leaf litter saprotrophs, soil saprotrophs, and a considerable number of species with unknown trophic guilds were identified at most sites. The literature indicates that many leaf litter and soil saprotrophic fungi contribute to soil fertility by decomposing organic matter and releasing essential nutrients, thus favoring the growth of beneficial species (Huang et al., 2022). The presence of a well-managed vegetation cover, even in arid areas, seems to favor these fungi, as it maintains significant levels of organic matter and promotes continuous biological activity. It is important to note that the assignment of trophic functions or guilds presents certain limitations, as it is based on identification to genus level. Therefore, the inferences of this study should be interpreted with caution. Rather than assessing the actual functions performed by the fungi, this analysis focuses on their potential function, assuming that the genera identified have a specific trophic guild. However, there are still a large number of genera that are unknown and for which there is no clear information on their functions (Talbot et al., 2014).

The potential interaction networks of the five most prevalent fungi and of each of the five main trophic guilds were explored. The results indicated that most saprotrophic fungi are concentrated in the central zone and exhibit competitive exclusion interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. Additionally, most co-occurrences were observed between ectomycorrhizal fungi and those with unknown trophic guilds. While no distinct pattern emerged, this analysis contributes to identifying the most prevalent species within each guild and understanding their potential interaction networks within the fungal community. For example, *Malassezia* sp1 and *Peziza* sp1 are the two species that mostly exclude other fungal species, such as those of the orders Pleosporales and Blastocadiales. Pezizales species are particularly abundant in soil with high pH, similar to those of the arid zones studied (Madriz-Valdovinos et al., 2022).

Conclusion

While the number of samples in this study is limited (n = 8), it is important to note that each represents a composite sample derived from multiple soil subsamples per site. This composite sampling strategy, widely validated in soil fungal metabarcoding studies, enhances the representativeness of local community composition while reducing sampling costs. Given the known spatial heterogeneity of soil fungi, composite samples have been shown to effectively capture dominant community signals, making them suitable for comparative assessments across management zones. Although the limited replication may constrain statistical power to detect fine-scale differences or confirm the presence of an edge effect within the agroecosystem. Together, the CAP, RDA, and PERMANOVA results indicate that despite localized environmental gradients, fungal communities remain spatially homogeneous across the orchard, likely due to consistent agronomic management. To comprehensively assess the existence and extent of edge effects, future research should incorporate increased replication and include reference sites outside the agroecosystem, particularly within the surrounding desert matrix. Such comparisons would enable the detection of broader spatial gradients in fungal diversity and provide insights into potential ecological transitions at the agroecosystem–natural habitat interface.

Despite the relatively low number of field samples, the high sequencing depth and base quality (Q30 > 96% in all samples) ensured robust characterization of fungal communities. This supports the reliability of diversity patterns observed and reduces the likelihood that lack of statistical differences between zones is due to technical limitations.

Although a large variation between the central and peripheral orchard zones was expected, agronomic management seemed to minimize the grouping, resulting in similar and homogeneous fungal communities, with high biodiversity in both areas. This highlights the need for management strategies focused on soil fungal activity to ensure the health and productivity of agroecosystems in arid areas. As we move towards more efficient management, it is essential to find a balance between agricultural production and soil biodiversity conservation, considering specific fungal interactions and their ecosystem functions.

Acknowledgements

JASL 510799 thanks CONAHCYT for the grant awarded under the “Convocatoria 2022(1) and 2022(3) de Estancias Posdoctorales por México”. Dr. Ramón Alberto Batista-García, funded the DNA sequencing. To the pecan nut growers for access to the orchards. This research was partially funded by UNAM DGAPA PAPIIT-IV200223 awarded to RGO.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2023. *UNITE general FASTA release for eukaryotes*. Version 18.07.2023. UNITE Community. https://doi.org/10.15156/BIO/2938069

Aguiar, M., Conway, A.J., Bell, J.K., Stewart, K.J., 2023. Agroecosystem edge effects on vegetation, soil properties, and the soil microbial community in the Canadian prairie. *Plos ONE*, 18 (4): e0283832. https://doi.org/10.1371/journal.pone.0283832

Ahmadpour, S.A., Mehrabi-Koushki, M., Farokhinejad, R., Asgari, B., 2022. New species of the family Didymellaceae in Iran. *Mycological Progress*, 21 (2): 28. https://doi.org/10.1007/s11557-022-01800-5

Alvidrez-Villarreal, R., Hernández-Castillo, F.D., Garcia-Martínez, O., Mendoza-Villarreal, R., Rodríguez-Herrera, R., Aguilar, C.N., 2012. Isolation and pathogenicity of fungi associated to ambrosia borer (Euplatypus segnis) found injuring pecan (Carya illinoensis) wood. *Agricultural Sciences*, 3: 405–416. DOI:10.4236/as.2012.33048

Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen,

M., 2018. ampvis2: an R package to analyse and visualize 16S rRNA amplicon data. *BioRxiv*, 299537. https://doi.org/10.1101/299537

Andrades, M., 2012. *Prácticas de edafología y climatología* [Soil science and climatology]. 2nd ed. Universidad de la Rioja, Servicio de publicaciones. [cit. 2024-12-03]. https://www.agapea.com/M-Andrades-Rodriguez/Practicas-de-Edafologia-y-Climatologia-9788488713278-i.htm. (In Spanish).

Anslan, S., Bahram, M., Hiiesalu, I., Tedersoo, L., 2017. PipeCraft: flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. *Molecular Ecology Resources*, 17 (6): e234-e240. https://doi.org/10.1111/1755-0998.12692

Bennett, A. E., Groten, K., 2022. The costs and benefits of plant–arbuscular mycorrhizal fungal interactions. *Annual Review of Plant Biology*, 73 (1): 649–672. https://doi.org/10.1146/annurev-arplant-102820-124504

Blincoe, K., Harrison, F., Damian, D., 2015. Ecosystems in GitHub and a method for ecosystem identification using reference coupling. In *2015 IEEE/ACM 12th working conference on mining software repositories. Florence, Italy, 16-17 May 2015*. Piscataway: IEEE, p. 202–211. DOI: 10.1109/MSR.2015.26

Bremner, J.M., 1965. *Total nitrogen*. *Methods of soil analysis: part 2 Chemical and microbiological properties*. Number 9 in Series Agronomy. Madison: American Society of Agronomy Inc., p. 1149–1178.

Cabrera-Rodríguez, Nava-Reyna, E., Trejo-Calzada, R., García-De la Peña, C., Arreola-Ávila, J.G., Collavino, M.M., Constante-García, V., 2020. Effect of organic and conventional systems used to grow pecan trees on diversity of soil microbiota. *Diversity*, 12 (11): 436. https://doi.org/10.3390/d12110436

Casas Flores, R., 2012. *El suelo de cultivo y las condiciones climáticas* [Crop soil and climatic conditions]. Madrid: Parainfo. 235 p. (In Spanish).

Chen, T., Wang, S., Jiang, X., Huang, Y., Mo, M., Yu, Z., 2023. New species of Didymellaceae within aquatic plants from southwestern China. *Journal of Fungi*, 9 (7): 761. https://doi.org/10.3390/jof9070761

Chimal-Sánchez, E., Senés-Guerrero, C., Varela, L., Montaño, N.M., García-Sánchez, R., Pacheco, A., Montaño-Arias, S.A., Camargo-Ricalde, S.L., 2020. Septoglomus mexicanum, a new species of arbuscular mycorrhizal fungi from semiarid regions in Mexico. *Mycologia*, 112 (1): 121–132. https://doi.org/10.1080/00275514.2019.1671147

Colombo, R.P., Recchi, M., Silvani, V.A., Pérgola, M., Martínez, A., Godeas, A.M., 2018. Detection of arbuscular mycorrhizal fungi associated with pecan (Carya illinoinensis) trees by molecular and morphological approaches. *MycoKeys*, 42: 73–88. https://doi.org/10.3897/mycokeys.42.26118

Delgado-Baquerizo, Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, 7 (1): 10541. https://doi.org/10.1038/ncomms10541

Eslaminejad, P., Heydari, M., Kakhki, F.V., Mirab-Balou, M., Omidipour, R., Muñoz-Rojas, M., Lucas-Borja, M.E., 2020. Plant species and season influence soil physicochemical properties and microbial function in a semi-arid woodland ecosystem. *Plant and Soil*, 456: 43–59. https://doi.org/10.1007/s11104-020-04691-1

Ginestet, C., 2011. ggplot2: elegant graphics for data analysis. *Journal of the Royal Statistical Society Series A: Statistics in Society*, 174 (1): 245–246. https://doi.org/10.1111/j.1467-985X.2010.00676_9.x

Gkisakis, V., Volakakis, N., Kollaros, D., Bàrberi, P., Kabourakis, E.M., 2016. Soil arthropod community in the olive agroecosystem: determined by environment and farming practices in different management systems and agroecological zones. *Agriculture, Ecosystems and Environment*, 218: 178–189. https://doi.org/10.1016/j.agee.2015.11.026

Gryzenhout, M., Khooa, B., Landman, L., 2016. First report of Fusarium boothii from pecan (Carya illinoinensis) and camel thorn (Vachellia erioloba) trees in South Africa. *South African Journal of Botany*, 105: 158–162.

Guerrero-Galán, C., Calvo-Polanco, M., Zimmermann, S.D., 2019. Ectomycorrhizal symbiosis helps plants to challenge salt stress conditions. *Mycorrhiza*, 29: 291–301. https://doi.org/10.1007/s00572-019-00894-2

Huang, W., van Bodegom, P. M., Declerck, S., Heinonsalo, J., Cosme, M., Viskari, T., Soudzilovskaia, N. A., 2022. Mycelium chemistry differs markedly between ectomycorrhizal and arbuscular mycorrhizal fungi. *Communications Biology*, 5 (1): 398. https://doi.org/10.1038/s42003-022-03341-9

Hudson, O., Buchholz, M., Doyle, V., Sundue, M.A., 2019. Multilocus phylogeny of Acrospermaceae: new epibiotic species and placement of Gonatophragmium, Pseudovirgaria, and Phaeodactylium anamorphs. *Mycologia*, 111 (6): 1041–1055. https://doi.org/10.1080/00275514.2019.1668905

INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias), 2002. *Tecnología de producción en nogal pecanero* [Pecan nut production technology]. Ciudad de México: CELALA-CIRNOC-INIFAP. (In Spanish).

Johnson, B.L., Haddad, N.M., 2011. Edge effects, not connectivity, determine the incidence and development of a foliar fungal plant disease. *Ecology*, 92 (8): 1551–1558. https://doi.org/10.1890/10-1072.1

Keirnan, Tan, Y.P., Laurence, M.H., Mertin, A.A., Liew, E.C., Summerell, B.A., Shivas, R.G., 2021. Cryptic diversity found in Didymellaceae from Australian native legumes. *MycoKeys*, 78: 1–20. DOI: 10.3897/mycokeys.78.60063

Kreitzman, M., 2020. *Perennial agriculture: agronomy and environment in long-lived food system*s. PhD thesis. University of British Columbia. 283 p.

Larsson, E., Campo, E., Carbone, M., 2014. Hygrophorus exiguus, a new species in subgenus Colorati section Olivaceoumbrini, subsection Tephroleuci. *Karstenia*, 54 (2): 41–48.

Legeay, M., Doncheva, N.T., Morris, J.H., Jensen, L.J., 2020. Visualize omics data on networks with Omics Visualizer, a Cytoscape App. *F1000Research*, 9: 157. https://doi.org/10.12688/f1000research.22280.2

Luján Soto, R., Martínez-Mena, M., Cuéllar Padilla, M., De Vente, J., 2021. Restoring soil quality of woody agroecosystems in Mediterranean drylands through regenerative agriculture. *Agriculture, Ecosystems and Environment*, 306: 107191. https://doi.org/10.1016/j.agee.2020.107191

Ma, Y., Dias, M.C., Freitas, H., 2020. Drought and salinity

stress responses and microbe-induced tolerance in plants. *Frontiers in Plant Science*, 11: 591911. https://doi.org/10.3389/fpls.2020.591911

Ma, W-Y., Wu, Q.-S.,., Xu, Y.-J., Kuča, K., 2021. Exploring mycorrhizal fungi in walnuts with a focus on physiological roles. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 49 (2): 12363. https://doi.org/10.15835/nbha49212363

Madriz-Valdovinos, Coronado, M.L., Raymundo, T., Gutiérrez, A., Flores, M. S., Esqueda, M. 2022. Pezizales (Ascomycota) associated with pine-oak forest in Yécora, Sonora, Mexico. *Acta Botanica Mexicana*, 129. https://doi. org/10.21829/abm129. 2022.2083

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS ONE*, 8 (4): e61217. https://doi.org/10.1371/journal.pone.0061217

Medel, R., Castillo, R., Marmolejo, J., Baeza, Y., 2013. Análisis de la familia Pezizaceae (Pezizales: Ascomycota) en México [Analysis of the family Pezizaceae (Pezizales: Ascomycota) in Mexico]. *Revista Mexicana de Biodiversidad*, 84: S21-S38. https://doi.org/10.7550/rmb.31741. (In Spanish).

Melo, C., Walker, C., Krüger, C., Borges, P.A., Luna, S., Mendonça, D., Machado, A.C., 2019. Environmental factors driving arbuscular mycorrhizal fungal communities associated with endemic woody plant Picconia azorica on native forest of Azores. *Annals of Microbiology*, 69: 1309–1327. https://doi.org/10.1007/s13213-019-01535-x

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20: 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Oksanen, M.J., 2013. *Package 'vegan'. Community ecology package, version 2 (9)*, p. 1–295.

Palmer, J.M., Lindner, D.L., Volk, T.J., 2008. Ectomycorrhizal characterization of an American chestnut (Castanea dentata)-dominated community in Western Wisconsin. *Mycorrhiza*, 19: 27–36. https://doi.org/10.1007/s00572-008-0200-7

Poletto, T., Muniz, M.F.B., Fantinel, V.S., Harakava, R., Rolim, J.M., 2020. Characterization and pathogenicity of Fusarium oxysporum associated with Carya illinoinensis seedlings. *Floresta e Ambiente*, 27: e20171089. https://doi.org/10.1590/2179-8087.108917

Porensky, L.M., Young, 2013. Edge-effect interactions in fragmented and patchy landscapes. *Conservation Biology*, 27 (3): 509–519.

Rivera-Urbalejo, Vazquez-Sandoval, D., Fernández-Vázquez, J.L., Rosete-Enríquez, M., Cesa-Luna, C., Morales-García, Y.E., Quintero-Hernández, V., 2021. Aportes y dificultades de la metagenómica de suelos y su impacto en la agricultura [Contributions and difficulties of soil metagenomics and its impact on agriculture]. *Acta Biológica Colombiana*, 26 (3): 449–461. https://doi.org/10.15446/abc.v26n3.85760. (In Spanish).

Rodríguez, M.A., 1996. *Prácticas de edafología y climatología* [Practice of soil science and climatology]. Universidad de la Rioja. Servicio de publicaciones. 79 p. (In Spanish).

Rudawska, M., Kujawska, M., Leski, T., Janowski, D., Karliński, L., Wilgan, R., 2019. Ectomycorrhizal community structure of the admixture tree species Betula pendula, Carpinus betulus, and Tilia cordata grown in bare-root forest nurseries. *Forest Ecology and Management*, 437: 113–125. https://doi.org/10.1016/j.foreco.2019.01.009

Sánchez-Ledesma J., Garibay-Orijel, R., Guevara-Guerrero, G., Ávila-Rodríguez, V., Arreola-Ávila, J.G., 2023. Macromicetos asociados con Carya illinoinensis en La Comarca Lagunera, México [Macromycetes associated with Carya illinoinensis in La Comarca Lagunera, Mexico]. *Revista Mexicana de Biodiversidad*, 94: e944074-e944074. https://doi.org/10.22201/ib.20078706e.2023.94.4074. (In Spanish).

Schmidt, M., 2019. *Fragmentation of landscapes: modelling ecosystem services of transition zones*. PhD thesis. Universität Potsdam, Potsdam. https://doi.org/10.25932/publishup-44294

Sharma, S., Singh, P., Chauhan, S., Choudhary, O.P., 2022. Landscape position and slope aspects impacts on soil organic carbon pool and biological indicators of a fragile ecosystem in high-altitude cold arid region. *Journal of Soil Science and Plant Nutrition*, 22 (2): 2612–2632. https://doi.org/10.1007/s42729-022-00831-x

Shi, J.W., Lu, L.X., Shi, H.M., Ye, J.R., 2022. Effects of plant growth-promoting rhizobacteria on the growth and soil microbial community of Carya illinoinensis. *Current Microbiology*, 79 (11: 352.

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Peay, K.G., 2014. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry*, 57: 282–291. https://doi.org/10.1016/j.soilbio.2012.10.004

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Abarenkov, K., 2014. Global diversity and geography of soil fungi. *Science*, 346 (6213): 1256688. DOI: 10.1126/science.1256688

Tedersoo, L., Bahram, M., Zobel, M., 2020. How mycorrhizal associations drive plant population and community biology. *Science*, 367 (6480): eaba1223. DOI: 10.1126/science.aba1223

Viglizzo, E.F., Roberto, Z.E., Filippin, M.C., Pordomingo, A.J., 1995. Climate variability and agroecological change in the Central Pampas of Argentina. *Agriculture, Ecosystems and Environment*, 55 (1): 7–16. https://doi.org/10.1016/0167-8809(95)00608-U

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37 (1): 29–38.

Wei, T., Simko, V.R., 2021. *package "corrplot": Visualization of a Correlation Matrix (Version 0.92)*. Package Corrplot for R Software.

Wei, H., He, X., Riccardo, B., Yang, Y., Yuan, Z., 2021. Stagonosporopsis rhizophilae sp. nov. (Didymellaceae, Pleosporales), a new rhizospheric soil fungus associated with Populus deltoides Marsh. *Phytotaxa*, 491 (1): 23–34. https://doi.org/10.11646/phytotaxa.491.1.3

Wickham, H., 2023. *dplyr: A grammar of data manipulation*. R package version 04.3., p156.

Yang, C., Liu, N., Zhang, Y., 2019. Soil aggregates regulate the impact of soil bacterial and fungal communities on soil respiration. *Geoderma*, 337: 444–452. https://doi.org/10.1016/j.geoderma.2018.10.002

Yang, C., Sun, J., 2020. Soil salinity drives the distribution patterns and ecological functions of fungi in saline-alkali land in the Yellow River Delta, China. *Frontiers in Microbiology*, 11: 594284. https://doi.org/10.3389/fmicb.2020.594284

Zeng, Q., Lebreton, A., Auer, L., Man, X., Jia, L., Wang, G., Gong, S., Sai, W., Lombard, V., Buée, M., Wu, G., Dai, Y., Yang, Z., Martin, F.M., 2023. Stable functional structure despite high taxonomic variability across fungal communities in soils of old-growth montane forests. *Microbiome*, 11 (1): 217. https://doi.org/10.1186/s40168-023-01650-7

Zhang, C., Yang, C., Cai, F., Ma, T., Wei, L., Jin, M., Wang, Y., Qi, N., 2024. Occurrence and identification of two Nothophoma species causing branch canker and leaf brown spot of walnut in China. *Canadian Journal of Plant Pathology*, 46: 367–377. https://doi.org/10.1080/07060661.2024.2317897

Zhang, K., Zentella, R., Burkey, K.O., Liao, H.L., Tisdale, R.H., 2023. Microbial community dynamics responding to nutrient allocation associated with soybean cultivar 'Jake' ozone adaptation. *Science of the Total Environment*, 864: 161008. https://doi.org/10.1016/j.scitotenv.2022.161008

Received, December 16, 2024
Accepted May 24, 2025

Supplementary material

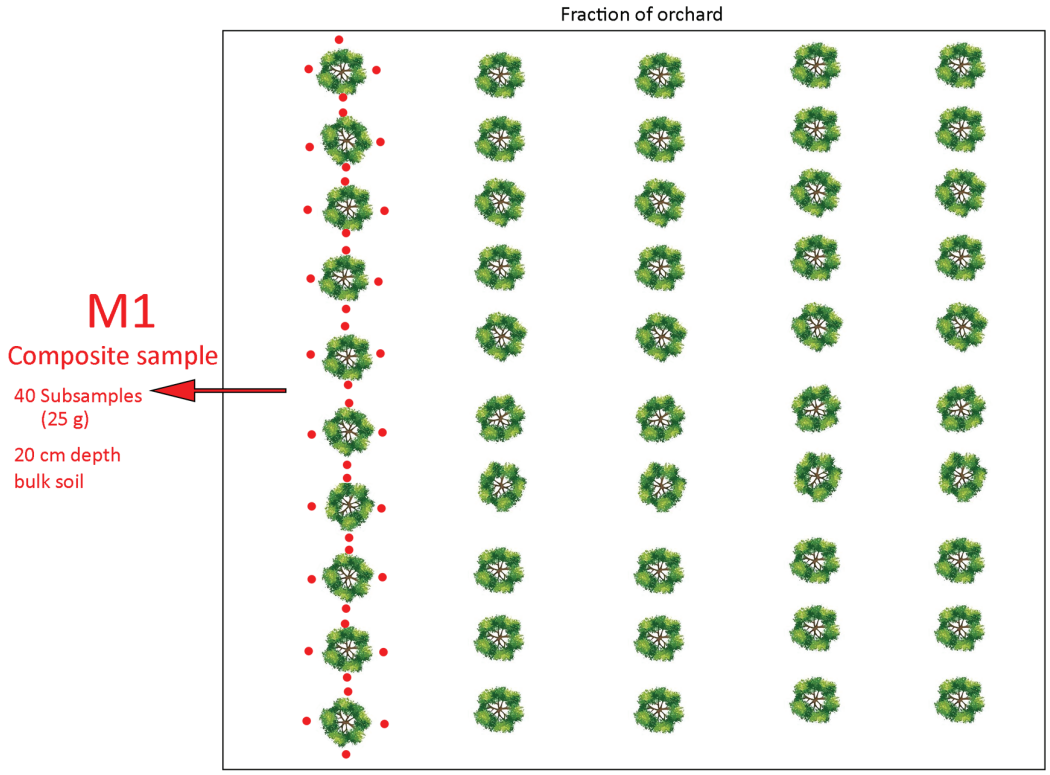

Fig. S1. Composite sample distribution.

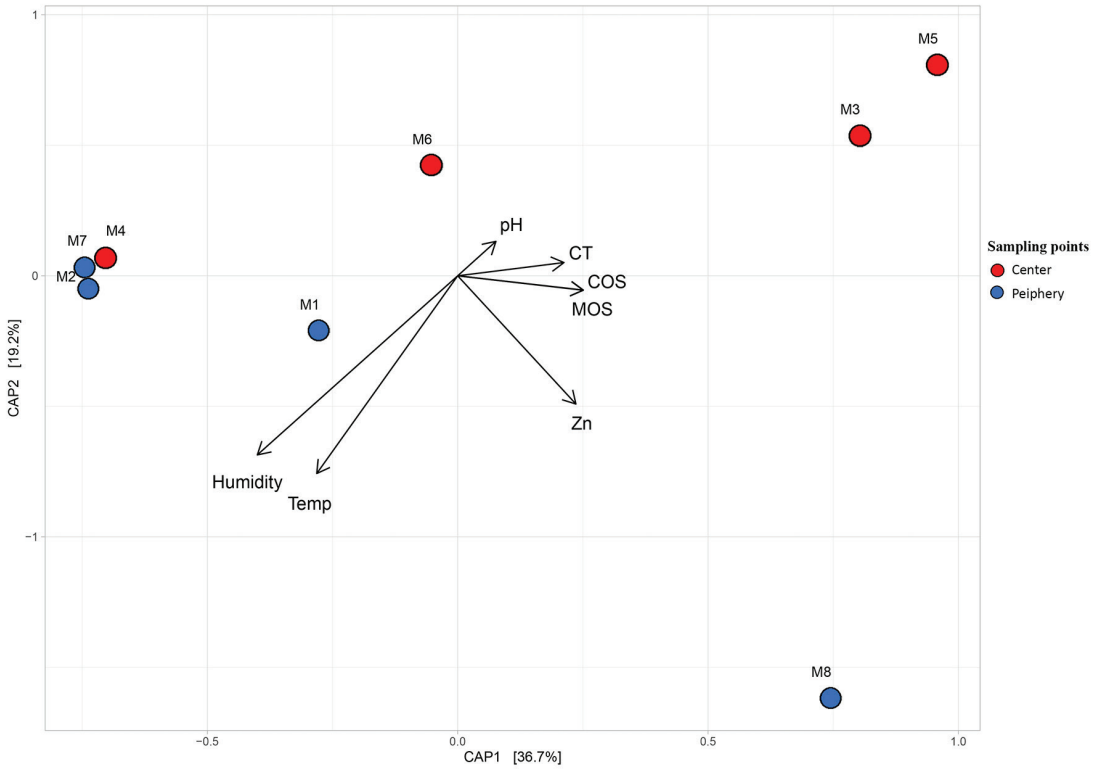

Fig. S2. Graphical representation of principal coordinates canonical analysis (CAP) showing the physicochemical variables pH, TC, COS, MOS, Zn, and environmental variables: humidity, and temperature.