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Fungal diversity in healthy and cladode-thickened *Opuntia ficus-indica* under drought and rainfall conditions: A metagenomic approach

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Abstract. Plants from the Cactaceae family can survive under extreme environmental conditions due to their Crassulacean Acid Metabolism (CAM). However, *Opuntia ficus-indica*, an economically important species of this family, is affected by various pests and diseases, with cladode thickening being one of the most frequent problems. Although some studies have linked this disorder to phytoplasmas and viruses, its etiology remains uncertain. Therefore, exploring other microorganisms that may act as pathogenic agents or serve as indicators of plant health could provide further insight into this disease. The objective of this study was to describe the composition, diversity, and phylogenetic relationships of fungal communities in different compartments of thickened and healthy *O. ficus-indica* plants, through ITS2 region sequencing during the rainy and dry seasons. Thickened and healthy *O. ficus-indica* plants were collected during the rainy and dry seasons in San Miguel Atlamajac, Temascalapa, State of Mexico. Samples from five plant compartments (soil, rhizosphere, root tissue, phyllosphere, and cladode tissue) were analyzed using metagenomics to identify, through the ITS2 region, the fungal communities present in each condition (plant health status and season). Data were processed with the Mothur pipeline to evaluate the fungal diversity and taxonomic composition. Alpha diversity varied across compartments, with soil and rhizosphere harboring the richest and most diverse fungal communities, reflecting the structural heterogeneity that characterizes these environments, whereas cladode tissue showed the lowest diversity values. Fungal communities in the phyllosphere and root tissue were shaped mainly by plant thickening conditions, while cladode tissue, rhizosphere, and soil communities were strongly influenced by seasonal variation. Fungal community composition differed between thickened and healthy plants across all sampled compartments: thickened plants were consistently associated with members of the order Pleosporales, while healthy plants showed a higher relative abundance of Plectosphaerellaceae (order Glomerellales) in belowground compartments, with no clear predominance of a specific fungal group in aerial compartments. Despite these compositional differences, no specific taxon could be directly linked to the etiology of the thickening disorder.

Keywords: *amplicon, bioinformatics, ITS, prickly pear, taxonomy*

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Introduction

In Mexico, prickly pear (*Opuntia ficus-indica*) is one of the crops of greatest cultural importance and makes a significant contribution to the country’s agricultural economy (Sinicropi *et al.*, 2022). Its succulent stems, covered by a thick cuticle with low stomatal density, and the presence of spines, confer remarkable tolerance to extreme conditions such as drought and high temperatures (Davis *et al*., 2019; Sinicropi *et al*., 2022).

Despite its adaptability, the crop is affected by various diseases, including cladode thickening, one of the most common disorders reported in the genus *Opuntia* in Mexico.

In contrast to healthy plants, thickened plants are smaller in size, with rounded, thickened, low-turgid, and chlorotic cladodes (Felker *et al*., 2019). The fruits from thickened plants are round, small, insipid, and display slight chlorotic mottling; they emerge from the flat surface of the cladode rather than from its margins, as normally occurs in healthy plants (Hernández, 1993; Felker *et al*., 2019). Cladode thickening has been studied since 1974 (Pimienta, 1974) and has been primarily associated with phytoplasmas (Choueiri *et al*., 2005; Tessitori *et al*., 2006; Hernández-Pérez *et al*., 2009; Fucikovsky *et al*., 2011; Martínez-Salgado *et al*., 2020) and viruses (Suaste-Dzul *et al*., 2012; Felker *et al*., 2019). In addition to these pathogens, the incidence of the disorder has also been linked to abiotic stress (Valadez-Moctezuma *et al*., 2021). However, no studies have yet confirmed the origin of cladode thickening.

Microbial community composition reflects the environment in which a plant develops, and shifts in its structure can provide insight into the plant's physiological status and surrounding conditions (Scholz *et al*., 2012). Among these communities, fungi play a particularly relevant role, as they can modulate plant growth, hormone balance, and stress tolerance, but may also drive pathological responses such as tissue deformation or chlorosis. Therefore, alterations in fungal community composition could serve as indicators of environmental change or be directly linked to the expression of disease symptoms.

Despite previous reports associating cladode thickening with biotic and abiotic factors, shifts in fungal community composition in the presence of this disorder have not been previously investigated. Seasonal changes, particularly differences between dry and rainy periods, may significantly influence fungal community dynamics; however, the extent to which these communities remain stable across seasons or respond preferentially to plant health status remains unclear. Understanding these associations is essential to generate hypotheses about the potential role of fungal communities in the development or modulation of cladode thickening.

Therefore, the objective of this study was to characterize, through high-throughput amplicon sequencing targeting ITS2, the structure and diversity of fungal communities associated with different compartments of *O. ficus-indica*, and to evaluate whether these communities vary in relation to cladode thickening symptoms and/or seasonal environmental conditions in the State of Mexico, Mexico. To our knowledge, to date, there are no published reports of metagenomic analyses describing the composition of fungal communities associated with cultivated *O. ficus-indica* plants exhibiting cladode thickening.

This study represents an exploratory analysis of the fungal communities associated with thickened and healthy *O. ficus-indica* plants under contrasting seasonal conditions.

Material and Methods

Study area

Sampling was conducted in a commercial orchard of *Opuntia ficus-indica* (cultivar Alfajayucan, white prickly pear) located in the community of San Miguel Atlamajac, Temascalapa, State of Mexico (19°44′58″ N, 98°55′27″ W) (Figure 1A). The orchard has been established for approximately 30 years and covers four hectares, with plants arranged in aligned rows.

Prickly pear production in this region depends exclusively on rainfall, as no irrigation systems are used. According to records from the San Jerónimo Xonacahuacán meteorological station, the rainy season occurs mainly between June and September.

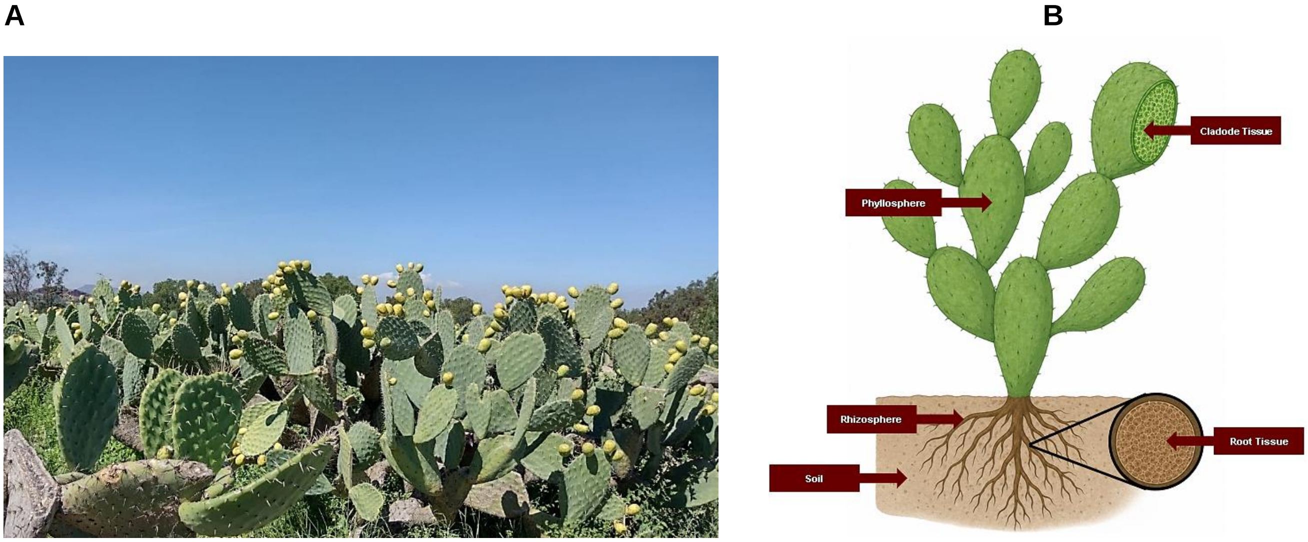

Figure 1. Sample collection site and experimental design. (**A**) Commercial orchard of *Opuntia ficus-indica* in San Miguel Atlamajac, Temascalapa, State of Mexico. (**B**) Schematic representation of the *O. ficus-indica* compartments selected for metagenomic analysis. Photographs and illustration by the authors.

Since its establishment, the agronomic management of the orchard has been extensive: no tillage or soil movement is performed, and cultural practices are limited to the application of cattle manure every two or three years, along with occasional herbicide use. This regime is characteristic of prickly pear production units in the Teotihuacán Valley and has contributed to a pronounced chemical heterogeneity in the soil, which has a loamy texture, alkaline pH, and low salinity. In this region, plants affected by cladode thickening have been documented across orchards of varying ages (5-60 years old) under similar management practices.

Sample collection

During the rainy and dry seasons, samples were collected from three thickened and three healthy *O. ficus-indica* plants, randomly selected from the central area of the orchard, where all plants were subject to uniform management practices and soil conditions. Samples from the three plants within each condition were pooled into a single composite sample per compartment; therefore, no biological replicates were included in the sequencing analysis.

Five ecological compartments were sampled from each plant (Figure 1B): (1) soil, collected at a depth of 20-30 cm; (2) rhizosphere, consisting of soil adhered to the roots; (3) root tissue; (4) phyllosphere, external surface of the cladodes; and (5) cladode tissue. All samples were stored at -20 °C until laboratory processing.

Identification of thickened and healthy plants was based on morphological inspection of the cladodes. As shown in Figure 2 (A and B), thickened plants were characterized by rounded cladodes, some with a heart-shaped form, and fruits emerging from the lateral surface of the cladode; in contrast, healthy plants exhibited oval-shaped cladodes with fruits developing primarily along the edges, as observed in Figure 2 (C and D). These morphological differences remained consistent during both the rainy (Figure 2A and 2C) and dry (Figure 2B and 2D) seasons.

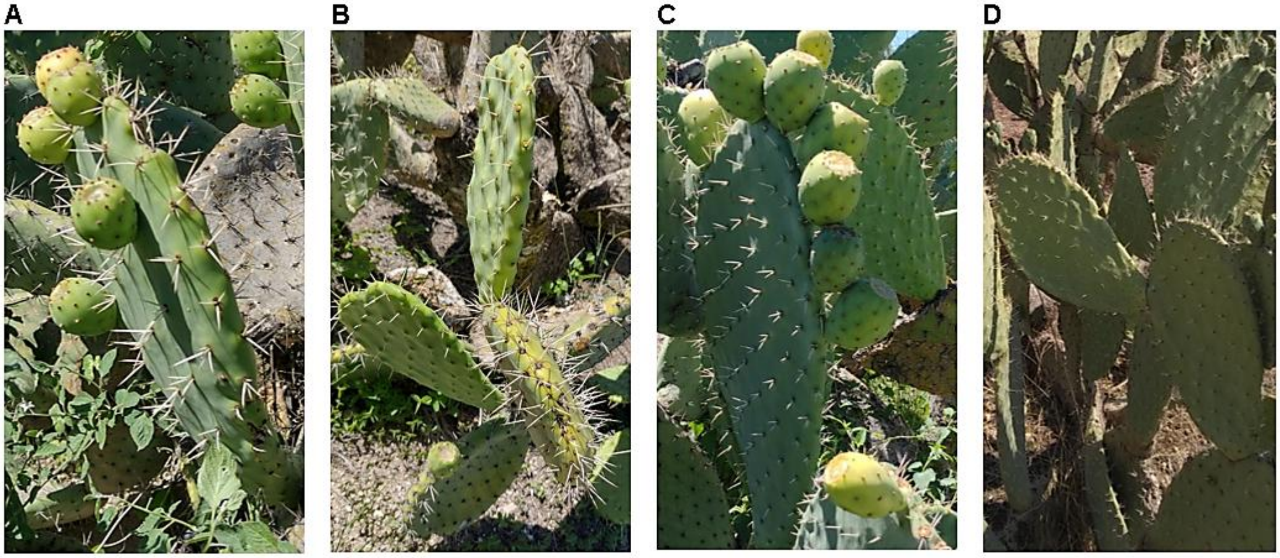

Figure 2. *Opuntia ficus-indica* plants during the dry and rainy seasons in San Miguel Atlamajac, Temascalapa, State of Mexico: (**A**) thickened plant, rainy season; (**B**) thickened plant, dry season; (**C**) healthy plant, rainy season; and (**D**) healthy plant, dry season. Photographs by the authors.

To recover microorganisms from the rhizosphere and phyllosphere, roots and cladodes were washed for 30 min in a phosphate buffer solution (50 mM KH_2PO_4, 50 mM K_2HPO_4, 0.1 % Triton X-100), following the methodology proposed by Desgarennes *et al*. (2014). The resulting suspensions were then centrifuged at 10,700 rpm for 15 min.

DNA Extraction

Phyllosphere

DNA from microorganisms associated with the phyllosphere (external surface) of the cladodes was extracted using the PureLink Microbiome DNA Purification Kit (Invitrogen, USA), with minor modifications to the centrifugation steps. Specifically, the centrifugation steps recommended by the manufacturer at 14,000 rpm for 5 min and 1 min were adjusted to 13,000 rpm for 8 min and 2 min, respectively.

Cladode tissue

DNA from microorganisms residing within the cladode tissue was obtained using the traditional CTAB method described by Weising *et al*. (2005), with slight modifications to the original protocol. A total of 50 mg of sample was macerated in liquid nitrogen, followed by the addition of 900 µL of isolation buffer (2 % w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, 1 % PVP 40,000) with 0.2 % β-mercaptoethanol and 400 µL of chloroform. The solution was incubated at 55 °C for 40 min and centrifuged at 13,000 rpm for 20 min. Subsequently, 600 µL of 100 % isopropanol was added, and the mixture was incubated for 1 h at -20 °C. It was then centrifuged at 13,000 rpm for 15 min, and the resulting pellet was washed with 1 mL of 75 % ethanol and incubated for 10-15 min. Finally, the DNA pellet was resuspended in TE rehydration buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Soil, rhizosphere, and root tissue

DNA from microorganisms associated with soil, rhizosphere (soil adhered to roots), and root tissue were extracted using the ZymoBIOMICS DNA Miniprep Kit (ZymoResearch, USA). DNA quality and concentration were assessed using the QuantiFluor dsDNA System (Promega) and the UVS-99 ATC Gene at an absorbance ratio of 260/280.

Library and sequencing

Libraries were prepared following the Metagenomic Sequencing Library Preparation protocol (Part #15044223 Rev. B) for the Illumina MiSeq system (Illumina Inc., USA), adapted for ITS (Internal Transcribed Spacer) amplicon amplification. The ITS2 subregion of the ITS was amplified using primers ITS3F (5´GCATCGATGAAGAACGCAGC 3´) and ITS4R (5´TCCTCCGCTTATTGATATGC 3´) (White *et al*., 1990). Sequencing was performed by Macrogen (South Korea) using the MiSeq platform (Illumina Inc., USA) and the MiSeq v3 kit, generating 301 bp paired-end reads with an average of approximately 100,000 reads per sample. Negative controls were included in the PCR amplification and library preparation to monitor for potential contamination.

Data quality analysis

Raw reads (Fastq format) were processed on the Galaxy Australia platform (The Galaxy Community, 2024). Read quality was assessed and visualized using FastQC (v0.12.1) and MultiQC (v1.11). Quality trimming was performed with Trimmomatic (v0.36) (Bolger *et al*., 2014) using the following parameters: for “Trailing” and “Leading”, quality thresholds of 20 (Phred score); “Sliding window” of 4 bases with a minimum quality of 20 (Phred score). The minimum average quality “AvQual” was 28 (Phred), and reads shorter than 150 bases were discarded using “Minlen”.

Bioinformatic analysis using the Mothur pipeline

Bioinformatic processing was performed using Mothur v1.39.5 (Schloss *et al*., 2009). The workflow consisted of four main steps: quality control, taxonomic classification, operational taxonomic unit (OTU) clustering, and alpha and beta diversity analyses.

Initial quality filtering was carried out using the following tools: “Make.contigs” to assemble paired-end reads; “Screen.seqs” to remove sequences shorter than 300 bases or longer than 450 bases, as well as those containing ambiguous bases; “Unique.seqs” to identify non-redundant sequences; and “Count.seqs” to track sequence abundances. A second round of “Screen.seqs” was applied to remove sequences that contained more than 8 homopolymers. Unique sequences were identified again using “Unique.seqs”. Subsequently, sequences differing by no more than three bases were merged using “Pre.cluster”. Taxonomic classification was performed with “Classify.seqs” with the UNITE database (Abarenkov *et al*., 2024). OTU clustering at 97 % similarity was performed with “Cluster.split”, and the taxonomy of each OTU was assigned with “Classify.otu”. The number of sequences per sample was obtained with “Count.groups”.

The sequencing coverage estimate was performed using “Rarefaction.single”. Alpha diversity was estimated using “Summary.single” and the Chao1, Shannon Evenness, and Shannon indices. Beta diversity was assessed by constructing dendrograms using “Tree.shared” with the Bray-Curtis index. Taxonomic assignment results were visualized with Phinch (Bik, 2014).

Statistical analysis

Due to the absence of biological replicates, formal statistical comparisons were not made. Therefore, the analysis was limited to descriptive comparisons of fungal community composition between conditions.

Results

Sequencing

The length of the sequencing reads used for downstream analyses was 301 bp. The average number of reads generated per sample was 208,965 (Table 1). The sample with the highest number of reads was the rhizosphere of thickened plants collected during the dry season (255,896 reads), while the sample with the lowest number of reads was the phyllosphere of healthy plants collected during the rainy season (134,180 reads). The total number of reads generated across all samples was 4,179,296.

Data quality analysis

Reads obtained after sequencing were processed and analyzed using the Galaxy Australia platform (The Galaxy Community, 2024). Quality filtering with Trimmomatic yielded an average retention rate of 77 % (Table 1), with an average of 161,756 reads retained per sample and a total of 3,235,122 high-quality reads across all samples.

Overall, samples from thickened plants exhibited a higher number of reads compared to those from healthy plants. Similarly, samples collected during the dry season yielded more reads than those collected during the rainy season (Table 1).

Bioinformatic analysis using Mothur

Sequence processing in Mothur reduced the total number of reads to 1,396,297 (Table 1). Samples from thickened plants generally yielded more reads than those from healthy plants, and samples collected during the dry season had more reads than those from the rainy season.

After OTU clustering at a 97% similarity threshold (Table 1), samples from thickened plants exhibited a higher number of OTUs. Regarding sampling season, no uniform trend was observed: cladode tissue and soil samples contained more OTUs during the rainy season, whereas phyllosphere, rhizosphere, and root tissue samples collected during the dry season contained more OTUs.

Coverage estimation

Coverage curves indicated that sequencing depth was sufficient to capture the majority of fungal diversity present in the analyzed samples (Figure 3). In all cases, sample coverage increased rapidly with sequencing effort, indicating that most of the detectable taxa were already represented at relatively low numbers of reads. This pattern was consistent across all analyzed compartments and sample groups, suggesting that the sequencing effort was adequate.

Table 1. Number and percentage of reads from ITS2 amplicon sequencing before and after quality trimming with Trimmomatic, number of reads retained after Mothur processing, and number of OTUs clustered at 97 % sequence similarity.

Compartment	Library	Reads before Trimmomatic	Reads after Trimmomatic	Surviving (%)	Mothur	OTUs (97 %)
Phyllosphere	**Total**	**744,406**	**597,158**		**269,641**	**2,414**
	CE_R	169,216	135,784	80	59,406	1,175
	CE_D	211,800	169,226	80	77,839	1,015
	CS_R	134,180	105,698	79	44,942	583
	CS_D	229,210	186,450	81	87,454	873
Cladode Tissue	**Total**	**814,760**	**634,494**		**242,393**	**262**
	DCE_R	200,402	151,468	76	46,344	129
	DCE_D	222,764	175,598	79	79,228	67
	DCS_R	188,698	146,408	78	43,608	143
	DCS_D	202,896	161,020	79	73,213	58
Root Tissue	**Total**	**872,618**	**659,856**		**268,592**	**1,418**
	DRE_R	230,250	178,044	77	71,814	294
	DRE_D	225,052	180,042	80	85,961	732
	DRS_R	220,942	174,308	79	67,822	235
	DRS_D	196,374	127,462	65	42,995	605
Rhizosphere	**Total**	**909,714**	**707,110**		**328,042**	**4,693**
	RE_R	239,996	176,354	73	84,910	1,561
	RE_D	255,896	210,658	82	101,877	2,207
	RS_R	182,836	140,646	77	65,625	1,359
	RS_D	230,986	179,452	78	75,630	1,810
Soil	**Total**	**837,798**	**636,504**		**287,629**	**4,037**
	SE_R	185,412	145,448	78	67,875	1,903
	SE_D	251,264	181,244	72	81,179	1,553
	SS_R	180,202	143,322	80	63,860	1,637
	SS_D	220,920	166,490	75	74,715	921

Data corresponds to 20 libraries from different compartments of thickened and healthy *Opuntia ficus-indica* plants collected during the rainy and dry seasons. CE: phyllosphere of thickened plants, CS: phyllosphere of healthy plants, DCE: cladode tissue of thickened plants, DCS: cladode tissue of healthy plants, DRE: root tissue of thickened plants, DRS: root tissue of healthy plants, RE: rhizosphere of thickened plants, RS: rhizosphere of healthy plants, SE: soil of thickened plants, SS: soil of healthy plants. D: dry season, R: rainy season.

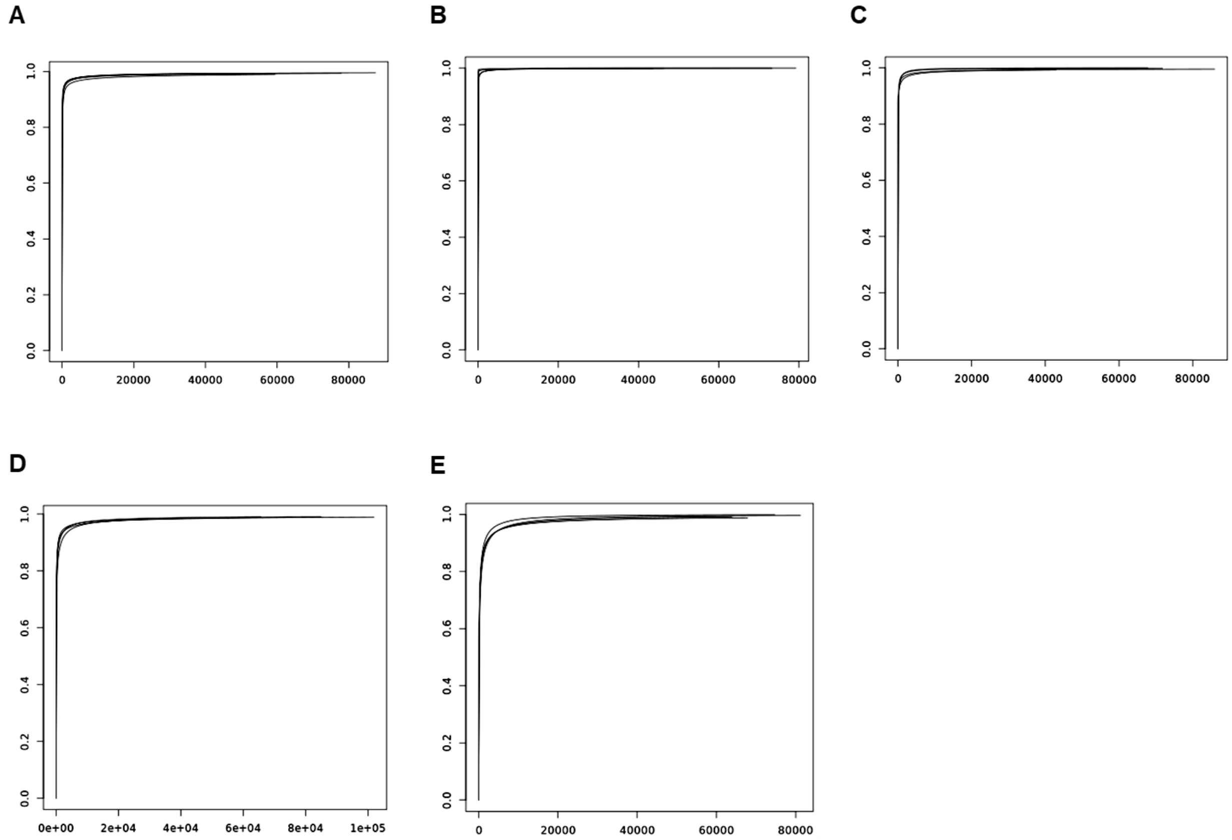

Figure 3. Sample coverage curves showing the relationship between sequencing depth and estimated community coverage for fungal communities associated with the (**A**) phyllosphere, (**B**) cladode tissue, (**C**) root tissue, (**D**) rhizosphere, and (**E**) soil of thickened and healthy *Opuntia ficus-indica* plants collected during the rainy and dry seasons.

Alpha diversity analysis

Alpha diversity encompasses three main components for assessing microbial community structure: richness, evenness, and diversity.

Species richness refers to the total number of taxa present in a community and is estimated using the Chao1 index, which considers both observed and potentially undetected species (Chao, 1984). The highest richness was found in the rhizosphere (RE_D: 4,129; RS_D: 3,173; RE_R: 3,073; RS_R: 2,571), soil collected during the rainy season (SE_R: 3,443; SS_R: 2,483), and in the phyllosphere of thickened plants in both seasons (CE_R: 2,377; CE_D: 2,067). In contrast, the lowest richness corresponded to root tissue during the rainy season (DRE_R: 557; DRS_R: 341) and cladode tissue samples (DCS_R: 192; DCE_R: 156; DCS_D: 77; DCE_D: 74) (Figure 4A).

Evenness indicates how uniformly species proportions are distributed within a community and is evaluated using the Shannon Evenness index (Shannon, 1948). Soil and rhizosphere presented the highest values (0.49-0.74), indicating a more balanced taxonomic distribution, whereas cladode tissue exhibited the lowest evenness among the five analyzed compartments (0.01-0.14) (Figure 4B).

Diversity integrates both richness and evenness to reflect the overall heterogeneity of a microbial community and is calculated using the Shannon-Wiener index (Pielou, 1966). Soil and rhizosphere showed the highest values (3.6-5.4), while cladode tissue displayed the lowest values (0.0-0.7), indicating fewer complex communities (Figure 4C).

Sample	Chao1	Shannon Evenness	Shannon
CE_D	2067	0.47	3.3
CE_R	2377	0.49	3.5
CS_D	1578	0.30	2.0
CS_R	1197	0.38	2.4
DCE_D	74	0.02	0.1
DCE_R	156	0.14	0.7
DCS_D	77	0.01	0.0
DCS_R	192	0.12	0.6
DRE_D	1403	0.40	2.6
DRE_R	557	0.48	2.7
DRS_D	1116	0.39	2.5
DRS_R	341	0.48	2.6
RE_D	4129	0.53	4.0
RE_R	3073	0.49	3.6
RS_D	3173	0.59	4.4
RS_R	2571	0.56	4.0
SE_D	1912	0.74	5.4
SE_R	3443	0.67	5.1
SS_D	1046	0.70	4.8
SS_R	2483	0.64	4.8

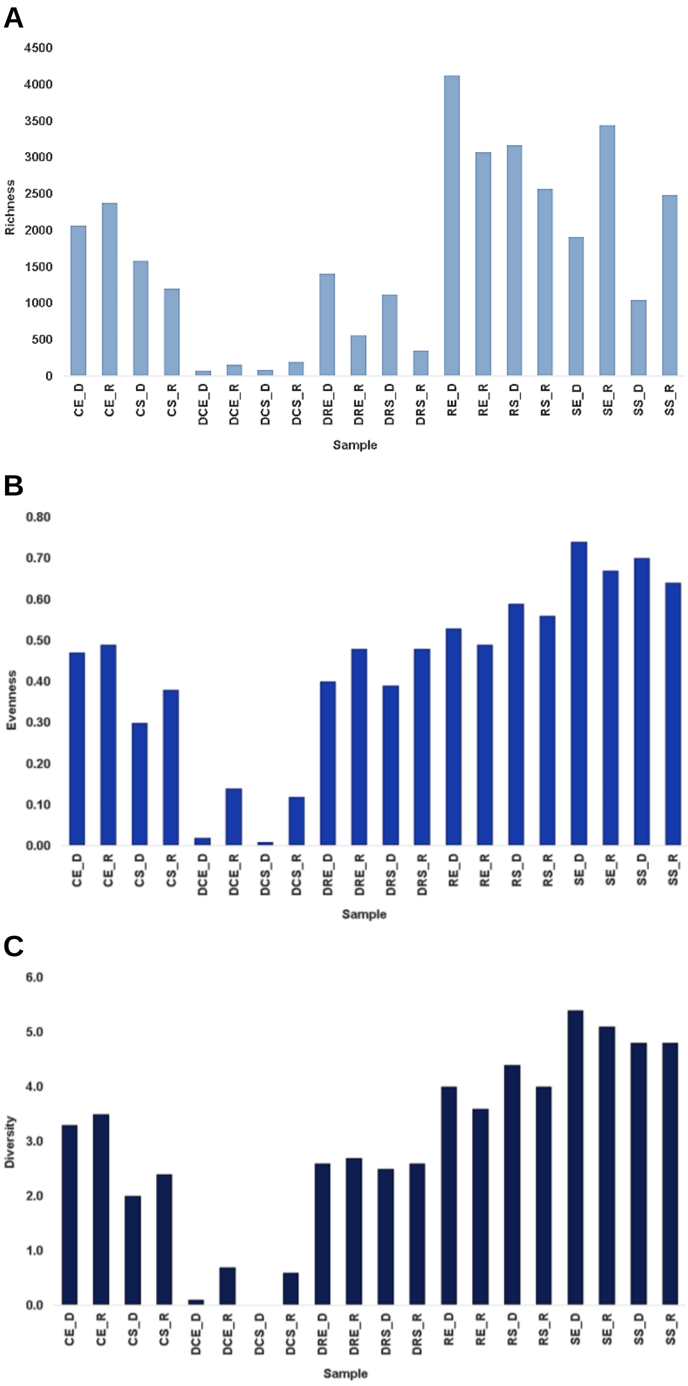

Figure 4. Alpha diversity of fungal communities associated with thickened and healthy *Opuntia ficus-indica* plants collected during the rainy and dry seasons, expressed as (**A**) richness, (**B**) evenness, and (**C**) diversity. CE: phyllosphere of thickened plants, CS: phyllosphere of healthy plants, DCE: cladode tissue of thickened plants, DCS: cladode tissue of healthy plants, DRE: root tissue of thickened plants, DRS: root tissue of healthy plants, RE: rhizosphere of thickened plants, RS: rhizosphere of healthy plants, SE: soil of thickened plants, SS: soil of healthy plants. D: dry season, R: rainy season.

Beta diversity analysis

The beta diversity analysis, evaluated using a Bray-Curtis distance-based clustering dendrogram, allowed visualization of the similarity among fungal communities across the different samples (Figure 5).

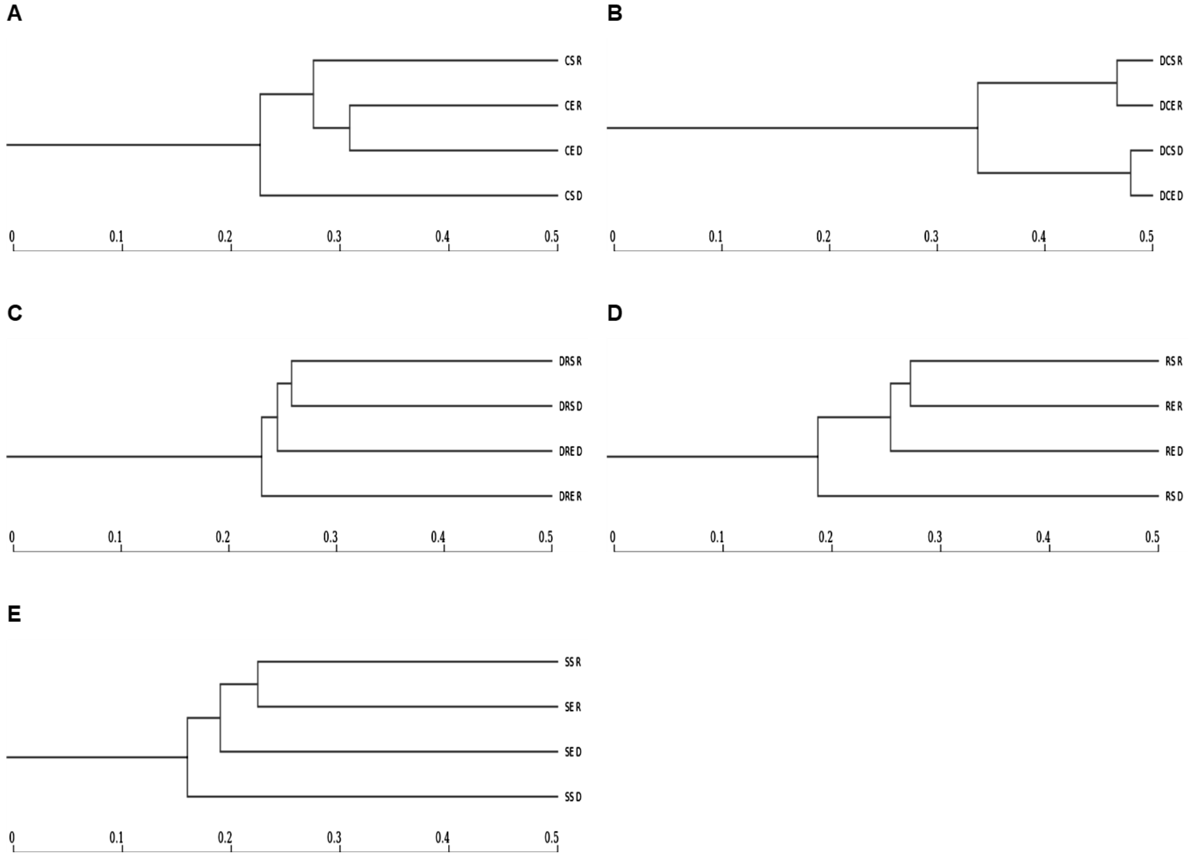

Figure 5. Bray-Curtis clustering dendrogram of fungal communities associated with the (**A**) phyllosphere, (**B**) cladode tissue, (**C**) root tissue, (**D**) rhizosphere, and (**E**) soil of thickened and healthy *Opuntia ficus-indica* plants collected during the rainy and dry seasons. CE: phyllosphere of thickened plants, CS: phyllosphere of healthy plants, DCE: cladode tissue of thickened plants, DCS: cladode tissue of healthy plants, DRE: root tissue of thickened plants, DRS: root tissue of healthy plants, RE: rhizosphere of thickened plants, RS: rhizosphere of healthy plants, SE: soil of thickened plants, SS: soil of healthy plants. D: dry season, R: rainy season.

Phyllosphere communities clustered primarily according to the thickening condition (Figure 5A) (CE_R and CE_D), regardless of the collection season, suggesting that cladode thickening has a stronger influence on fungal composition than seasonality in the case of libraries from thickened plants. Among the four phyllosphere samples, the one collected from healthy plants during the dry season was the most different from the others. In contrast, cladode tissue communities (Figure 5B) (DCS_R and DCE_R) and (DCS_D and DCE_D) clustered mainly according to environmental conditions (rainy or dry season), rather than thickening status, indicating that sampling season had a greater effect on community composition. For root tissue (Figure 5C), the samples also clustered according to plant health condition (DRS_R and DRS_D), such that communities associated with healthy plants were more similar to each other than to the rest of the samples. The root tissue sample from thickened plants collected during the rainy season showed the greatest difference relative to the other samples.

Rhizosphere communities (Figure 5D) collected during the rainy season (RS_R and RE_R) displayed high similarity to each other, regardless of thickening condition. The sample that differed most from the group was the rhizosphere of the healthy plants collected during the dry season. Similarly, soil communities (Figure 5E) also clustered by season with a close relationship observed between SS_R and SE_R, indicating that environmental conditions exert a greater influence on community structure than cladode thickening. The soil associated with healthy plants collected during the dry season was the most distinct from the rest.

Taxonomic identification

The absence of biological replicates, due to the pooling of samples, limits the ability to perform statistical analyses. Consequently, the results should be interpreted as exploratory descriptive patterns of fungal community composition (Figure 6).

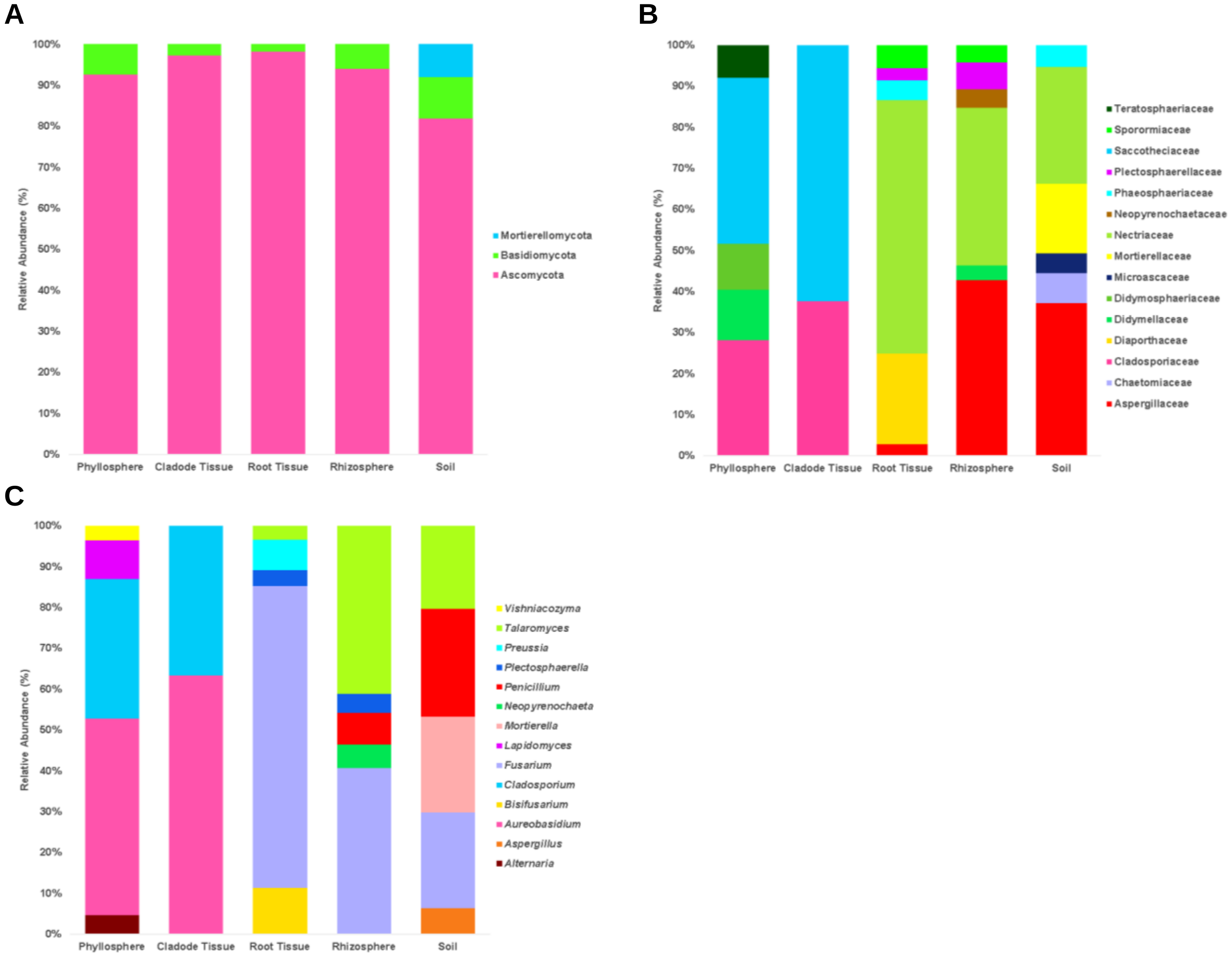

Figure 6. Most abundant fungal (**A**) phyla, (**B**) families, and (**C**) genera based on the number of OTUs detected in libraries derived from different *Opuntia ficus-indica* compartments (phyllosphere, cladode tissue, root tissue, rhizosphere, and soil).

Taxonomic assignment of OTUs was performed using the UNITE database. In the phyllosphere, a total of seven phyla and 156 families were identified; in cladode tissue, two phyla and 56 families; in root tissue, seven phyla and 96 families; in the rhizosphere, 11 phyla and 175 families; and in soil, 11 phyla and 174 families. The most abundant phyla, families, and genera are shown in Figure 6.

Analysis of fungal communities according to cladode thickening condition

Differences in the relative abundance of fungal taxa were observed between samples from thickened and healthy plants (Tables 2 and 3). These patterns were consistent across both rainy and dry seasons.

In the phyllosphere of thickened plants, members of the order Pleosporales were particularly abundant, including the families Roussoellaceae, Didymosphaeriaceae, and Phaeosphaeriaceae, as well as the genera *Paraophiobolus* and *Alternaria*. Across the belowground compartments of thickened plants (root tissue, rhizosphere, and soil), several taxa belonging to the order Pleosporales were consistently detected, including the families Sporormiaceae, Dictyosporiaceae, Phaeosphaeriacea, and Neopyrenochaetaceae, as well as the genera *Preussia*, *Dictyocheirospora*, and *Neopyrenochaeta*. Additionally, phyllosphere, cladode tissue, rhizosphere, and soil of thickened plants were characterized by the frequent presence of the family Cladosporiaceae and the genus *Cladosporium*.

In healthy plants, the family Plectosphaerellaceae from the order Glomerellales was more frequently detected across root tissue, rhizosphere, and soil compartments, while the phyllosphere and cladode tissue did not exhibit a clear predominance of specific fungal groups (Tables 2 and 3).

Analysis of fungal communities according to sampling season

When comparing the composition of fungal communities based on sampling season, variations were observed in the relative abundance of multiple taxa at both the family (Table 2) and genus (Table 3) levels, irrespective of the physiological condition of the plants (thickened or healthy).

In samples collected during the rainy season, the families Phaeosphaeriaceae, Saccotheciaceae, Sulcatisporaceae, Sporormiaceae, and Neopyrenochaetaceae, as well as the genera *Paraophiobolus*, *Kazuakitanaka*, *Preussia*, and *Westerdykella* (order Pleosporales), were more abundant in the phyllosphere, cladode tissue, root tissue, rhizosphere, and soil. In the phyllosphere and cladode tissue, the family Cladosporiaceae and the genus *Cladosporium* predominated. Meanwhile, in the root tissue and rhizosphere, the family Aspergillaceae and the genera *Talaromyces*, *Aspergillus*, and *Penicillium* (order Eurotiales) were more abundant.

In contrast, during the dry season, the families Roussoellaceae, Didymellaceae, Pleosporaceae, Dictyosporiaceae, and Didymosphaeriaceae, along with the genera *Lapidomyces*, *Alternaria*, and *Dictyocheirospora* (order Pleosporales), were more abundant in the phyllosphere and rhizosphere. In the root tissue and rhizosphere, the family Herpotrichiellaceae and the genus *Bisifusarium* predominated. Additionally, in the rhizosphere and soil, the family Filobasidiaceae and the genera *Naganishia* and *Filobasidium* (order Filobasidiales) were more abundant. No representative family or genus was detected in the cladode tissue during the dry season.

Table 2. Relative abundance at the family level of fungal communities associated with different plant compartments of thickened and healthy *Opuntia ficus-indica* plants collected during the dry and rainy seasons.

FAMILY	Phyllosphere				Cladode Tissue				Root Tissue				Rhizosphere				Soil			
	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season
Agaricaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	49.1 %	50.9 %	44.7 %	55.3 %
Aspergillaceae	-	-	-	-	-	-	-	-	74.2 %	25.8 %	73.2 %	26.8 %	65.0 %	35.0 %	62.9 %	37.0 %	43.2 %	56.8 %	56.0 %	44.0 %
Bionectriaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55.7 %	44.3 %	64.4 %	35.6 %
Bolbitiaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	84.9 %	15.1 %	28.4 %	71.6 %
Bulleraceae	57.5 %	42.5 %	74.7 %	25.3 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bulleribasidiaceae	48.1 %	52.0 %	49.8 %	50.2 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceratobasidiaceae	-	-	-	-	-	-	-	-	-	-	-	-	96.9 %	3.1 %	88.9 %	11.1 %	41.4 %	58.6 %	16.8 %	83.2 %
Chaetomiaceae	-	-	-	-	-	-	-	-	-	-	-	-	47.1 %	52.9 %	55.6 %	44.4 %	38.6 %	61.3 %	50.4 %	49.6 %
Cladosporiaceae	60.0 %	40.1 %	72.4 %	27.6 %	73.5 %	26.4 %	96.1 %	3.9 %	-	-	-	-	70.9 %	29.1 %	59.6 %	40.3 %	79.5 %	20.5 %	51.5 %	48.5 %
Clavicipitaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55.6 %	44.4 %	53.2 %	46.8 %
Coniochaetaceae	-	-	-	-	-	-	-	-	-	-	-	-	94.9 %	5.1 %	37.5 %	62.5 %	-	-	-	-
Cunninghamellaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37.6 %	62.4 %	64.2 %	35.8 %
Cyphellophoraceae	-	-	-	-	-	-	-	-	60.5 %	39.5 %	66.8 %	33.2 %	69.9 %	30.1 %	48.1 %	51.8 %	-	-	-	-
Diaporthaceae	-	-	-	-	-	-	-	-	82.5 %	17.5 %	83.3 %	16.7 %	-	-	-	-	-	-	-	-
Dictyosporiaceae	-	-	-	-	-	-	-	-	-	-	-	-	98.0 %	2.0 %	2.7 %	97.3 %	-	-	-	-
Didymellaceae	70.7 %	29.3 %	22.3 %	77.7 %	-	-	-	-	-	-	-	-	38.0 %	62.0 %	7.6 %	92.4 %	82.5 %	17.5 %	29.0 %	71.0 %
Didymosphaeriaceae	96.9 %	3.1 %	42.8 %	57.2 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Entolomataceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.4 %	84.6 %	7.9 %	92.1 %
Filobasidiaceae	54.3 %	45.7 %	46.0 %	54.0 %	-	-	-	-	-	-	-	-	93.7 %	6.3 %	0.8 %	99.2 %	73.7 %	26.3 %	3.2 %	96.8 %
Geastraceae	-	-	-	-	-	-	-	-	-	-	-	-	9.3 %	90.7 %	6.1 %	93.9 %	49.5 %	50.5 %	54.1 %	45.9 %
Glomeraceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	57.8 %	42.2 %	15.0 %	85.0 %
Gymnoascaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	91.6 %	8.4 %	21.4 %	78.5 %
Herpotrichiellaceae	-	-	-	-	-	-	-	-	78.5 %	21.5 %	35.3 %	64.7 %	72.7 %	27.3 %	11.1 %	88.9 %	49.3 %	50.7 %	41.1 %	58.9 %
Microascaceae	-	-	-	-	-	-	-	-	-	-	-	-	37.7 %	62.3 %	28.7 %	71.3 %	47.0 %	53.0 %	46.4 %	53.6 %
Mortierellaceae	-	-	-	-	-	-	-	-	-	-	-	-	18.2 %	81.8 %	15.7 %	84.3 %	41.2 %	58.8 %	22.8 %	77.1 %
Nectriaceae	-	-	-	-	-	-	-	-	66.2 %	33.8 %	36.6 %	63.4 %	54.8 %	45.2 %	38.3 %	61.7 %	53.9 %	46.0 %	67.4 %	32.6 %
Neopyrenochaetaceae	-	-	-	-	-	-	-	-	-	-	-	-	95.2 %	4.8 %	48.9 %	51.1 %	83.9 %	16.1 %	85.1 %	14.9 %
Onygenaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75.2 %	24.8 %	50.8 %	49.2 %
Ophiocordycipitaceae	-	-	-	-	-	-	-	-	-	-	-	-	36.5 %	63.4 %	55.2 %	44.8 %	34.0 %	66.0 %	61.6 %	38.4 %
Pezizaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55.4 %	44.6 %	18.3 %	81.7 %
Phaeosphaeriaceae	76.9 %	23.1 %	62.7 %	37.3 %	-	-	-	-	69.6 %	30.4 %	69.0 %	31.0 %	69.9 %	30.1 %	46.0 %	54.0 %	82.4 %	17.6 %	57.5 %	42.5 %
Phallaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100 %	-	98.2 %	1.8 %
Plectosphaerellaceae	-	-	-	-	-	-	-	-	21.3 %	78.7 %	57.9 %	42.1 %	33.8 %	66.2 %	40.3 %	59.7 %	30.8 %	69.2 %	91.1 %	8.9 %
Pleosporaceae	63.5 %	36.5 %	31.0 %	69.0 %	-	-	-	-	-	-	-	-	-	-	-	-	65.0 %	35.0 %	34.6 %	65.4 %
Roussoellaceae	98.9 %	1.1 %	0.5 %	99.5 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saccotheciaceae	41.2 %	58.8 %	60.3 %	39.8 %	47.7 %	52.3 %	89.2 %	10.8 %	-	-	-	-	-	-	-	-	-	-	-	-
Sporormiaceae	-	-	-	-	-	-	-	-	79.1 %	20.9 %	95.9 %	4.1 %	50.0 %	50.0 %	73.9 %	26.1 %	40.7 %	59.3 %	83.4 %	16.6 %
Sulcatisporaceae	-	-	-	-	-	-	-	-	-	100 %	100 %	-	-	-	-	-	-	-	-	-
Teratosphaeriaceae	69.6 %	30.4 %	21.4 %	78.6 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichomeriaceae	-	-	-	-	-	-	-	-	-	-	-	-	61.5 %	38.5 %	32.4 %	67.6 %	69.5 %	30.5 %	62.6 %	37.4 %

Taxa with abundance > 0.5 % are included.

Table 3. Relative abundance at the genus level of fungal communities associated with different plant compartments of thickened and healthy *Opuntia ficus-indica* plants collected during the dry and rainy seasons.

GENUS	Phyllosphere				Cladode Tissue				Root Tissue				Rhizosphere				Soil			
	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season	Thickened plants	Healthy plants	Rainy season	Dry season
Aaosphaeria	-	-	-	-	-	-	-	-	-	-	-	-	34.0 %	66.0 %	35.9 %	64.1 %	-	-	-	-
Absidia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38.0 %	62.0 %	63.7 %	36.3 %
Acremonium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	53.8 %	46.2 %	25.0 %	75.0 %
Alternaria	64.1 %	35.9 %	31.0 %	69.0 %	-	-	-	-	-	-	-	-	-	-	-	-	69.0 %	31.0 %	57.1 %	42.9 %
Aspergillus	-	-	-	-	-	-	-	-	-	-	-	-	60.8 %	39.2 %	91.2 %	8.8 %	70.5 %	29.5 %	27.9 %	72.1 %
Aureobasidium	42.3 %	57.7 %	58.8 %	41.2 %	47.7 %	52.3 %	89.2 %	10.8 %	-	-	-	-	-	-	-	-	-	-	-	-
Auxarthron	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	83.4 %	16.6 %	47.4 %	52.5 %
Bisifusarium	-	-	-	-	-	-	-	-	99.9 %	0.1 %	0.3 %	99.7 %	94.6 %	5.4 %	7.0 %	93.0 %	92.5 %	7.5 %	89.4 %	10.6 %
Cladosporium	59.2 %	40.8 %	72.4 %	27.6 %	72.4 %	27.6 %	95.9 %	4.1 %	-	-	-	-	70.9 %	29.1 %	59.7 %	40.3 %	79.4 %	20.6 %	51.2 %	48.8 %
Clitopilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.8 %	87.2 %	6.2 %	93.8 %
Conocybe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	81.6 %	18.4 %	88.2 %	11.8 %
Curvularia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	59.9 %	40.1 %	8.9 %	91.1 %
Cyphellophora	-	-	-	-	-	-	-	-	62.8 %	37.2 %	64.4 %	35.6 %	65.7 %	34.3 %	52.5 %	47.5 %	-	-	-	-
Dactylonectria	-	-	-	-	-	-	-	-	-	-	-	-	16.1 %	83.9 %	10.6 %	89.4 %	62.2 %	37.8 %	60.2 %	39.8 %
Dictyocheirospora	-	-	-	-	-	-	-	-	-	-	-	-	99.1 %	0.9 %	1.2 %	98.8 %	-	-	-	-
Exophiala	-	-	-	-	-	-	-	-	-	-	-	-	77.4 %	22.6 %	12.7 %	87.3 %	46.0 %	54.0 %	38.0 %	62.0 %
Filobasidium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	69.9 %	30.1 %	2.8 %	97.2 %
Fusarium	-	-	-	-	-	-	-	-	62.1 %	37.8 %	41.4 %	58.6 %	52.7 %	47.3 %	43.2 %	56.8 %	56.9 %	43.1 %	72.1 %	27.9 %
Fusicolla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	81.0 %	18.9 %	52.1 %	47.9 %
Geastrum	-	-	-	-	-	-	-	-	-	-	-	-	9.2 %	90.8 %	6.1 %	93.9 %	49.3 %	50.7 %	54.2 %	45.8 %
Genolevuria	57.4 %	42.6 %	74.6 %	25.4 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hormiactis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60.4 %	39.6 %	41.7 %	58.3 %
Kazuakitanaka	-	-	-	-	-	-	-	-	-	100 %	100 %	-	-	-	-	-	-	-	-	-
Knufia	-	-	-	-	-	-	-	-	-	-	-	-	61.5 %	38.5 %	32.5 %	67.5 %	-	-	-	-
Lapidomyces	70.2 %	29.8 %	19.4 %	80.6 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortierella	-	-	-	-	-	-	-	-	-	-	-	-	19.2 %	80.8 %	16.4 %	83.6 %	41.2 %	58.8 %	22.1 %	77.9 %
Naganishia	-	-	-	-	-	-	-	-	-	-	-	-	94.7 %	5.3 %	0.7 %	99.3 %	-	-	-	-
Neophaeococcomyces	74.8 %	25.2 %	50.1 %	49.9 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neopyrenochaeta	-	-	-	-	-	-	-	-	-	-	-	-	95.2 %	4.8 %	48.9 %	51.1 %	83.9 %	16.1 %	85.1 %	14.9 %
Paraophiobolus	86.1 %	13.8 %	99.2 %	0.8 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium	-	-	-	-	-	-	-	-	-	-	-	-	46.5 %	53.5 %	66.8 %	33.2 %	45.5 %	54.5 %	39.8 %	60.2 %
Phaeosphaeria	-	-	-	-	-	-	-	-	61.1 %	38.9 %	41.2 %	58.8 %	-	-	-	-	-	-	-	-
Phallus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100 %	-	98.2 %	1.8 %
Pithoascus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65.5 %	34.5 %	32.6 %	67.4 %
Plectosphaerella	-	-	-	-	-	-	-	-	20.9 %	79.1 %	55.9 %	44.1 %	24.3 %	75.7 %	42.2 %	57.8 %	-	-	-	-
Preussia	-	-	-	-	-	-	-	-	85.6 %	14.3 %	96.5 %	3.5 %	94.4 %	5.6 %	69.7 %	30.3 %	-	-	-	-
Sarocladium	-	-	-	-	-	-	-	-	1.7 %	98.2 %	98.2 %	1.7 %	-	-	-	-	-	-	-	-
Talaromyces	-	-	-	-	-	-	-	-	79.0 %	21.0 %	72.7 %	27.3 %	68.8 %	31.1 %	58.8 %	41.2 %	29.1 %	70.9 %	83.8 %	16.2 %
Thelonectria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.8 %	81.1 %	68.8 %	31.2 %
Vishniacozyma	48.1 %	51.9 %	49.5 %	50.5 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Westerdykella	-	-	-	-	-	-	-	-	-	-	-	-	12.7 %	87.3 %	80.2 %	19.8 %	32.5 %	67.5 %	92.8 %	7.2 %

Taxa with abundance > 0.5 % are included.

Discussion

Alpha and beta diversity

To estimate alpha diversity, three indices were used: Chao1, to assess species richness; Shannon Evenness, to estimate evenness; and Shannon, to determine total diversity. The analysis was conducted using fungal communities obtained from 20 samples corresponding to five compartments (phyllosphere, cladode tissue, root tissue, rhizosphere, and soil) of thickened and healthy *Opuntia ficus-indica* plants collected during the dry and rainy seasons.

Overall, the alpha diversity analysis showed that cladode tissue exhibited the lowest richness, evenness, and diversity, followed by root tissue. The phyllosphere displayed intermediate values, higher than those observed in plant tissues. In contrast, rhizosphere and soil samples recorded the highest richness, evenness, and diversity. A similar trend was identified in *Myrtillocactus geometrizans* and *Opuntia robusta*, where soil and rhizosphere samples showed higher alpha diversity than root, stem, and phyllosphere tissues (Fonseca-García *et al*., 2016). Consistently, in *Ephedra przewalskii*, *Nitraria sphaerocarpa*, *Reaumuria soongorica*, *Salsola passerina*, and *Sympegma regelii*, all species characteristic of arid environments, higher alpha diversity values were reported in the root zone than in aerial parts (Zuo *et al*., 2021). A similar pattern was observed in *Agave angustifolia*, where leaf tissue showed the lowest alpha diversity (Contreras-Negrete *et al*., 2025). The lower richness and diversity in root tissue compared to the rhizosphere and soil have also been previously reported in grapevine (Martínez-Diz *et al*., 2019), *Opuntia ficus-indica* (Gargouri *et al*., 2021) and soybean (Popescu *et al*., 2022).

These findings support the hypothesis that microbial communities associated with roots are more diverse than those present in aerial tissues, given that soils constitute ecosystems with greater microbiological diversity (Abdelfattah *et al*., 2018), and that tissues of the same type tend to harbor similar richness levels (Ferreira-Silva *et al*., 2021).

When comparing fungal communities across seasons, most samples collected during the dry season exhibited higher richness, evenness, and diversity. This pattern aligns with previous studies on fungal community responses to precipitation, which suggest that microbial community recovery is favored during drought periods, as these conditions promote the coexistence of species with broad environmental tolerance ranges (Hawkes *et al*., 2011).

Beta diversity analysis was conducted using a cluster dendrogram based on the Bray-Curtis index to evaluate similarity among the microbial communities of the different samples. The results showed that phyllosphere and root tissue communities were primarily grouped according to the thickening condition of the plants. In contrast, cladode tissue, rhizosphere, and soil communities were grouped predominantly by collection season rather than plant health status. Previous studies in pear have also reported the influence of seasonality on the composition of fungal communities (Janakiev *et al*., 2022), as well as the effect of the plant compartment (Zuo *et al*., 2021), agricultural practices, cultivation site, and precipitation (Legeay *et al*., 2024).

Taxonomic identification

Fungal communities associated with cladode thickening

The predominance of fungi belonging to the order Pleosporales in both aerial and belowground compartments of thickened plants suggests that the fungal community may be largely composed of organisms commonly associated with a saprotrophic lifestyle. Members of this order are widely distributed across terrestrial ecosystems and exhibit strong adaptability to diverse environmental conditions and substrates (Wanasinghe and Maharachchikumbura, 2023). Among the families encompassed within this order, members of Phaeosphaeriaceae have been reported to produce antimicrobial compounds (Si *et al*., 2022), suggesting that these fungi could influence microbial interactions within the plant microbiome.

Within this order, *Alternaria* was particularly notable in the phyllosphere of thickened plants. Species from this genus are commonly reported as epiphytes and endophytes in cacti and other succulent plants (Suryanarayanan *et al*., 2005), including *Opuntia humifusa* (Silva-Hughes *et al*., 2015), *Agave tequilana*, *Opuntia robusta*, and *Mammillaria geometrizans* (Camarena-Pozos *et al*., 2021), indicating a strong association with aerial plant surfaces and tissues of cacti. However, *Alternaria* also includes several opportunistic pathogens capable of causing diseases in *Opuntia* species, such as black spot, silver spot, brown spot, and cladode drying (Hernández, 1993; Alvarado and Santín, 2017; Infante *et al*., 2021; Chavarría-Cervera *et al*., 2024). Therefore, its abundance in thickened plants may indicate either increased susceptibility of the host tissue or the colonization of physiologically altered surfaces that facilitate opportunistic fungal establishment.

Several members of Pleosporales were also consistently detected in the belowground environments of thickened plants. Families such as Sporormiaceae and Neopyrenochaetaceae, together with genera including *Preussia* and *Neopyrenochaeta* were consistently detected. Species of *Preussia* have been reported as root endophytes and are considered highly adapted to arid environments (Massimo *et al*., 2015; Ferreira-Silva *et al*., 2021). In addition to their endophytic lifestyle, some species exhibit antimicrobial activity and plant growth-promoting effects (Mapperson *et al*., 2014; Al-Hosni *et al*., 2018), suggesting that they may participate in microbial interactions within the rhizosphere and root tissues. Conversely, species of *Neopyrenochaeta* have been associated with root diseases in various plant hosts (Levic *et al*., 2013; Yang *et al*., 2017), indicating that their higher abundance in thickened plants could reflect shifts toward microbial communities that include opportunistic root-associated pathogens.

Another taxon consistently detected across multiple compartments of thickened plants was Cladosporiaceae and *Cladosporium*. Species of this genus have been reported as epiphytes, endophytes, saprophytes, and occasional pathogens (Laureano-Ahuelicán *et al*., 2021; Cobo-Díaz *et al*., 2022). In different species of *Opuntia* and *Agave*, it has been previously reported as part of the aerial fungal microbiota (Silva-Hughes *et al*., 2015; Bezerra *et al*., 2019; Camarena-Pozos *et al*., 2021). The high abundance of this genus across both aerial and belowground compartments may indicate its capacity to colonize multiple plant-associated niches. Furthermore, some species have been reported as antagonists of plant pathogens (Xu *et al*., 2021), suggesting that their presence could also be related to microbial competition within the plant microbiome.

The abundance of the order Pleosporales in thickened plants may therefore reflect the increased availability of senescent tissues, which constitute suitable substrates for saprotrophic colonization. In this context, orchard age may play a critical role, as older plantings tend to accumulate a higher proportion of physiologically aged individuals exhibiting tissue degradation and weakened defense barriers. These conditions promote the establishment and proliferation of saprotrophic and opportunistic microorganisms. Consequently, long-established orchards may provide a propitious environment for microbial colonization, particularly when combined with inappropriate agronomic management practices, such as those observed in the study region.

In contrast, healthy plants showed a greater abundance of members of the family Plectosphaerellaceae in root and soil compartments. The ecological role of *Plectosphaerella* species is variable and depends on host and environmental conditions. However, several studies have reported a higher abundance of this group in healthy roots compared with diseased tissues (Chen *et al*., 2024; Duan *et al*., 2024), suggesting a possible association with stable root microbial communities. Some species have also been reported as beneficial members of the rhizosphere microbiota, including their ability to suppress plant pathogens or act as biological control agents (Atkins *et al*., 2003; Zhao *et al*., 2025). Thus, the higher abundance of Plectosphaerellaceae in healthy plants could reflect microbial communities potentially associated with more balanced plant-microbe interactions in the root environment.

Seasonal dynamics of the fungal microbiome

During the rainy season, members of the order Eurotiales, particularly the genera *Aspergillus*, *Penicillium*, and *Talaromyces*, were more abundant. Several of these fungi have been previously reported as rhizospheric or endophytic taxa in a variety of plant hosts, including cacti (Batista *et al*., 2016; Silva *et al*., 2022; Oksinska *et al*., 2024). Some of these taxa are known to produce antimicrobial compounds and exhibit antagonistic activity against phytopathogenic fungi, suggesting that their occurrence in the belowground compartments of other cactus species may be related to a potential functional role in the root environment (Tagawa *et al*., 2010; Yamagiwa *et al*., 2011; Romero-Bastidas *et al*., 2025).

Although some *Aspergillus* species are recognized plant pathogens affecting numerous crops (Oksinska *et al*., 2024; Zakaria, 2024), when isolated from *O. ficus-indica* and *Opuntia dillenii,* they exhibited the ability to produce compounds with antibacterial properties (Ratnaweera *et al*., 2015; Bezerra *et al*., 2019), and certain species possess fungicidal and nematocidal properties (Fan *et al*., 2024; García-Conde *et al*., 2024). Similarly, *Talaromyces* is a broadly distributed genus found across diverse habitats. Although generally not considered an important plant pathogen, some species have been reported as postharvest pathogens (Stosic *et al*., 2020; Liu *et al*., 2021), while others exhibit antifungal activity and promote plant growth (Manoch and Dethoup, 2011; Yamagiwa *et al*., 2011; Alghamdi *et al*., 2021). The genus *Penicillium* has been reported as resistant to extreme environmental conditions (Romero-Bastidas *et al*., 2025) and associated with various hosts, including *Opuntia* and *Agave* (Camarena-Pozos *et al*., 2021; Silva *et al*., 2022). Species of this genus are well known for producing antibiotics and extracellular metabolites that suppress plant pathogens (Tagawa *et al*., 2010; Yamagiwa *et al*., 2011). Thus, their prevalence in rhizospheric compartments could contribute to microbial communities that support plant adaptation to environmental conditions, as they have also been reported in roots and soil of other plant species (Batista *et al*., 2016; Wachowska and Rychcik, 2023; Oksinska *et al*., 2024; Romero-Bastidas *et al*., 2025).

Within the order Pleosporales, taxa detected in all compartments during the rainy season included the family Saccotheciaceae and the genus *Westerdykella*. Members of Saccotheciaceae have been reported in the grape carposphere (Iorizzo *et al*., 2024), suggesting that their presence in the phyllosphere may be related to adaptation to aerial plant environments where fungi interact with environmental factors such as humidity and UV exposure. Likewise, species of *Westerdykella* have been associated with plant growth promotion and tolerance to saline conditions (Fuentes-Quiroz *et al*., 2023), indicating that their presence may reflect potential functional contributions to plant adaptation under stressful conditions.

During the dry season, members of the Herpotrichiellaceae, belonging to the order Chaetothyriales, include species capable of tolerating toxic compounds and harsh environmental conditions (Teixeira *et al*., 2017), which may explain their prevalence under the more restrictive environmental conditions characteristic of the dry season. Similarly, species of the genus *Bisifusarium* have been reported from root tissue and rhizosphere environments and are frequently isolated from extreme habitats such as desert soils and plants growing in arid regions (Zhang *et al*., 2025). Although some species exhibit pathogenic potential, most of them are recorded as saprophytes (Yue *et al*., 2024; Badiwe *et al*., 2025) or endophytic (Contreras-Negrete *et al*., 2025; Yang and Wang, 2025). Fungal communities associated with *O. ficus-indica* vary markedly across aridity gradients, exhibiting distinct compositions and interaction networks under different environmental conditions. Members of the order Filobasidiales have been reported to be enriched under semi-arid conditions in rhizosphere samples (Gargouri *et al*., 2021), a pattern consistent with the observations of the present study, suggesting a potential association between these taxa and adaptation to water-limited environments.

In both seasons, members of the order Pleosporales were the most abundant taxa across aboveground and belowground compartments. This pattern has also been reported in other studies on xerophytic plants, where fungal communities are often dominated by this group, suggesting that these taxa may play a key role in maintaining the structure and functional stability of microbial communities in arid-adapted plant systems (Fonseca-García *et al*., 2018; Zuo *et al*., 2021).

Taken together, these results suggest that fungal communities associated with plants may exhibit a high degree of ecological flexibility, with their roles shaped by environmental conditions and host status.

Conclusions

Fungal communities associated with thickened and healthy *Opuntia ficus-indica* plants differed in composition across all sampled compartments and were shaped by both the plant's physiological condition and seasonal variation. Fungal diversity varied markedly among compartments: rhizosphere and soil exhibited the highest levels of richness, evenness, and diversity. The phyllosphere showed intermediate richness, whereas cladode and root tissues displayed the lowest values. Similarly, evenness and diversity were intermediate in root tissue and the phyllosphere, while cladode tissue consistently showed the lowest values. The principal factors that affected the community composition differed by compartment: fungal communities in the phyllosphere and root tissue were predominantly shaped by the plant's thickening condition, whereas those in cladode tissue, rhizosphere, and soil were more strongly influenced by seasonal variation. The increased relative abundance of saprotrophic taxa in thickened plants may reflect tissue senescence as a contributing factor in microbial community structuring, a pattern potentially reinforced by orchard age, as older plantings tend to accumulate altered tissues that favor microbial colonization. Although several fungal families and genera associated with plant responses to biotic and abiotic stress were identified, none provided sufficient evidence to establish a direct causal relationship with cladode thickening in *O. ficus-indica*. To the best of our knowledge, this study represents the first metagenomic characterization of fungal communities in thickened and healthy *O. ficus-indica* plants, conducted during both dry and rainy seasons, providing novel insights into the fungal communities associated with different plant compartments.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

Not applicable.

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