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Bacterial community structure associated with healthy and cladode-thickened of *Opuntia ficus-indica* plants during the dry and rainy seasons

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Abstract. *Opuntia ficus-indica* is renowned for its resilience to adverse environmental conditions. However, it remains susceptible to various diseases caused by both biotic and abiotic factors. Among these diseases, cladode thickening represents a significant agronomic limitation. Although phytoplasmas and viruses have been suggested as potential causal agents, its etiology remains unknown. This study aimed to characterize the structure, diversity, and phylogenetic relationships of bacterial communities across different compartments of cultivated *O. ficus-indica*, comparing healthy and thickened plants during the rainy and dry seasons through 16S rRNA gene amplicon sequencing, to assess the potential role of bacteria in the etiology of this disease. Five ecological compartments were analyzed: soil, rhizosphere, root tissue, phyllosphere, and cladode tissue. The V3-V4 region of the 16S rRNA gene was sequenced from bacterial communities, and bioinformatic analyses were conducted using the Mothur pipeline. Soil and root compartments exhibited the highest alpha diversity. This is due to their role as reservoirs of stable and functionally diverse microbial communities. Aerial compartments, on the other hand, showed lower diversity. The structure of bacterial communities in the phyllosphere, cladode tissue, and rhizosphere varied mainly according to the health status of the plant, while soil and root communities were influenced by environmental conditions. In thickened plants, the bacterial composition was similar across compartments, while in healthy plants, the communities were more distinct and compartment specific. Seasonal dynamics shaped the distribution of bacterial taxa across compartments: fewer taxa were shared during the rainy season, while a greater number of taxa were shared across multiple compartments under dry conditions, suggesting a potential role in adaptation to water-limited environments. Despite these compositional differences, no specific taxon could be directly linked to the etiology of the thickening disorder.

Keywords: *16S rRNA, bioinformatics, metagenomics, next-generation sequencing, prickly pear, taxonomic profile*

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Introduction

The genus *Opuntia* tolerates a wide range of environments because of its Crassulacean acid metabolism (CAM) and various morphological adaptations (Prat *et al*., 2018). This resilience makes it a viable alternative to conventional crops in regions subject to extreme environmental conditions, such as heat and water stress (Sinicropi *et al*., 2022). Mexico is the world's leading producer of prickly pear, accounting for approximately 45 % of global production, followed by Italy (12.2 %) and South Africa (3.7 %) (Andreu-Coll *et al*., 2020). In America, Argentina, Bolivia, Brazil, Chile, Peru, and the United States of America are notable producing countries. In Africa, production is concentrated mainly in Algeria, Ethiopia, Morocco, and Tunisia, while in the Near East, Jordan, Lebanon, Yemen, and Israel

Israel are significant producers. In Europe, besides Italy, production is also reported in Portugal and Spain (Inglese *et al*., 2018).

Despite its tolerance to adverse conditions, *Opuntia* species are also susceptible to diseases caused by biotic and abiotic agents. Among the conditions affecting yield in Mexico, cladode thickening is particularly important. This disorder leads to reduced plant growth and increased cladode thickness, resulting in abnormally rounded cladodes with low turgidity. Fruits produced by thickened plants are characterized by a yellow coloration, small size, rounded shape, and poor organoleptic quality. They grow mainly on the lateral faces of the cladodes rather than on their margins, as they do in healthy plants (Hernández, 1993). Morphological differences between fruits from healthy and thickened plants are shown in Figure 1 (A and B).

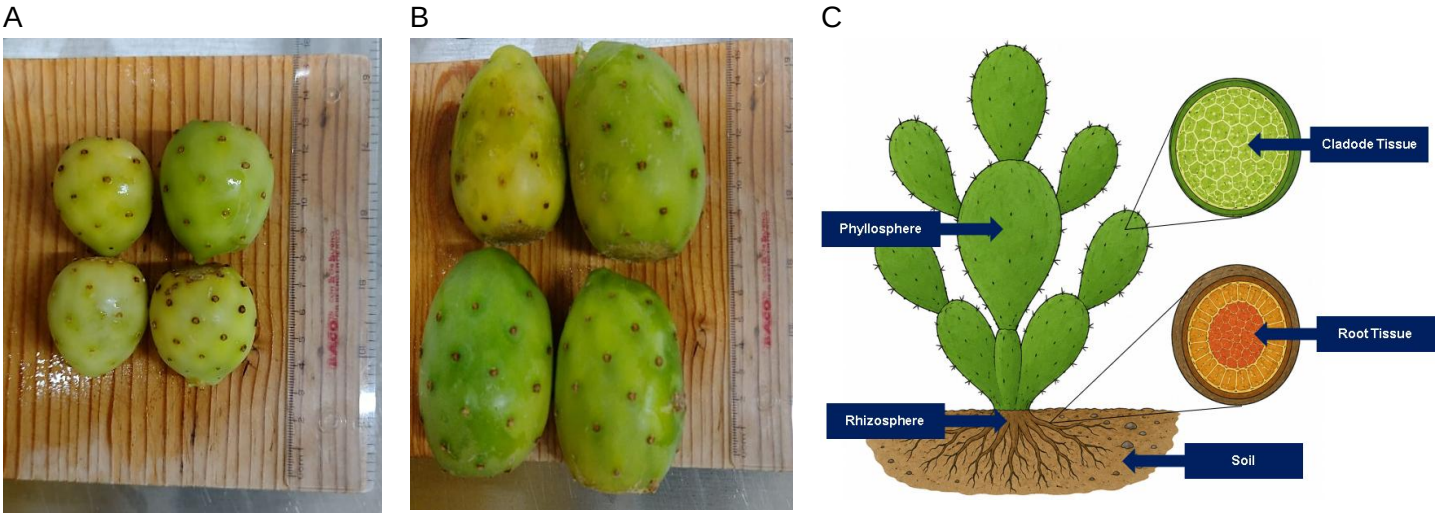

Figure 1. Morphological comparison of *Opuntia ficus-indica* fruits from (**A**) thickened plants and (**B**) healthy plants, illustrating differences in size, shape, and coloration. (**C**) Schematic representation of the five compartments sampled in this study: phyllosphere, cladode tissue, root tissue, rhizosphere, and soil. Photographs and illustrations by the authors.

This condition has been studied by Pimienta (1974) and several potential causal agents have been proposed, including phytoplasmas (Choueiri *et al*., 2005; Tessitori *et al*., 2006; Granata *et al*., 2006; Hernández-Pérez *et al*., 2009; Fucikovsky *et al*., 2011; Martínez-Salgado *et al*., 2020), viruses (Suaste-Dzul *et al*., 2012; Felker *et al*., 2019), and abiotic stress factors (Valadez-Moctezuma *et al*., 2021). However, its etiology has not been conclusively defined.

Characterizing microbial communities and their dynamics offers valuable insight into the environment in which a plant develops. Shifts in microbial abundance and composition under specific conditions may serve as indicators of physiological or pathological alterations (Berg *et al*., 2020; Karray *et al*., 2020). Microbial communities associated with xerophytic plants have been investigated through traditional, molecular, and metagenomic approaches. Among metagenomic methods, amplicon sequencing has become one of the most widely used, enabling the identification of most organisms present in a sample (alive or dead) and the characterization of community diversity through analysis of phylogenetic marker genes. This is accomplished through the integration of next-generation

sequencing platforms, bioinformatic pipelines, and reference databases (Gołezbiewski & Tretyn, 2019; Ghosh *et al*., 2019).

To assess the potential role of bacteria in the etiology of this disease, the objective of this study was to characterize, through the amplicon sequencing of the V3-V4 region of the 16S rRNA gene, the structure, diversity, and phylogenetic relationships of bacterial communities across different compartments of cultivated *O. ficus-indica*, and to evaluate whether these communities vary in relation to cladode thickening symptoms and/or seasonal conditions in the State of Mexico, Mexico. To our knowledge, to date, there are no published reports of metagenomic analyses describing the composition of bacterial communities associated with cultivated *O. ficus-indica* plants exhibiting cladode thickening.

Material and Methods

Study area

The study was conducted in a commercial orchard of *Opuntia ficus-indica*, cultivar Alfajayucan (white prickly pear), established approximately 30 years ago in San Miguel Atlamajac, Temascalapa, State of Mexico (19°44′58″N, 98°55′27″W). The orchard covers 4 ha, with plants arranged in aligned rows. In this region, production relies entirely on rainfall, which occurs predominantly between June and September according to records from the San Jerónimo Xonacahuacán weather station.

Since the orchard's establishment, no tillage or soil movement has been performed, and the agricultural practices are limited to cattle manure application every two to three years and occasional herbicide use. As a result, the soil´s chemical properties in this area are heterogeneous. This regime is representative of most prickly pear production units in the Teotihuacán Valley, where cladode thickening is commonly reported in orchards ranging from 5 to 60 years old. The general edaphic characteristics include a loam texture, alkaline pH, and low salinity.

Sample collection

During the rainy and dry seasons, samples were collected from three healthy and three thickened *O. ficus-indica* plants, randomly selected within the same production unit, where both healthy and thickened plants were present. From each plant, samples were taken from five ecological compartments (Figure 1C): (1) soil, collected at a depth of 20-30 cm around each selected plant; (2) rhizosphere, soil adhered to the roots; (3) root tissue; (4) phyllosphere, external surface of the cladodes; and (5) cladode tissue (Figure 1). All samples were stored at -20 °C until further processing.

The samples from the three plants were pooled to generate a single composite sample. Each composite sample represented a compartment, a condition, and a season, and was considered the experimental unit. Compartments were processed and analyzed independently as distinct sample types representing different niches associated with the plants. This sampling design limited the possibility of performing statistical comparisons among individual plants. The study should be interpreted as an exploratory characterization of bacterial communities associated with the examined conditions.

Plant selection was based on observable morphological characteristics. As shown in Figure 2 (A and B), affected plants exhibited noticeably more rounded and thickened cladodes than healthy plants (C and D). Fruits of thickened plants (Figure 2A) developed on the lateral surfaces of the cladodes rather

than along the edges, as observed in healthy plants (Figure 2C). These morphological differences remained consistent during both the rainy (Figure 2A and 2C) and dry (Figure 2B and 2D) seasons.

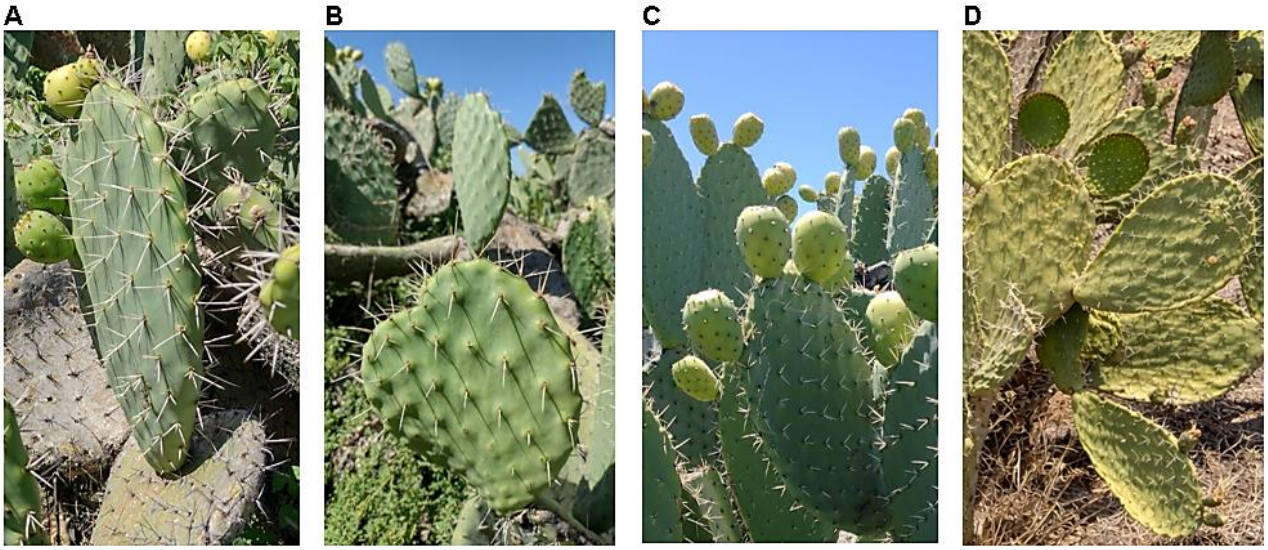

Figure 2. *Opuntia ficus-indica* plants during the dry and rainy seasons in San Miguel Atlamajac, Temascalapa, State of Mexico: (**A**) thickened plant, rainy season; (**B**) thickened plant, dry season; (**C**) healthy plant, rainy season; and (**D**) healthy plant, dry season. Photographs by the authors.

DNA extraction

Phyllosphere

Microbial DNA from the 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 manufacturer's recommended centrifugations 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 extraction from cladode endophytic communities was performed using a modified CTAB method (Weising *et al*., 2005). A total of 50 mg of sample was macerated in liquid nitrogen and mixed with 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) supplemented with 0.2 % β-mercaptoethanol and 400 µL of chloroform. The mixture 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 solution was incubated at -20 °C for 1 hour. Afterward, it was centrifuged at 13,000 rpm for 15 min, and the resulting pellet was washed with 1 mL of 75 % ethanol and left to stand 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 in soil, rhizosphere, and root tissue was extracted using the ZymoBIOMICS DNA Miniprep Kit (ZymoResearch, USA) following the manufacturer´s instructions.

DNA quality and concentration were assessed using the QuantiFluor dsDNA System (Promega) and the UVS-99 ATC Gene spectrophotometer at an absorbance ratio of 260/280.

Library preparation and sequencing

Sequencing libraries were prepared following the Metagenomic Sequencing Library Preparation protocol (Part #15044223 Rev. B) for the Illumina MiSeq system (Illumina Inc., USA). The V3-V4 hypervariable region of the 16S rRNA gene was amplified using primers Bakt_341F (5′ CCTACGGGNGGCWGCAG 3′) and Bakt_805R (5′ GACTACHVGGGTATCTAATCC 3′) (Herlemann *et al*., 2011). 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 both PCR amplification and library preparation to observe 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 30 (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 comprised five main steps, quality control, sequence alignment, taxonomic classification, operational taxonomic unit (OTU) clustering, and alpha and beta diversity analysis.

Initial quality filtering was carried out using the following tools: “Make.contigs” to assemble paired-end reads; “Screen.seqs” to remove sequences shorter than 200 bases or longer than 500 bases, as well as those containing ambiguous bases; “Unique.seqs” to identify non-redundant sequences; and “Count.seqs” to track sequence abundances.

Sequences were aligned using the Silva database (version 4) (Quast *et al*., 2013) with “Align.seqs”. A second round of “Screen.seqs” was applied to remove sequences that ended before position 13,000, were shorter than 150 bases, or contained more than 8 homopolymers. All alignment columns containing the character “.” were removed with “Filter.seqs”, and unique sequences were identified again using “Unique.seqs”. Subsequently, sequences differing by no more than two bases were merged using “Pre.cluster”. Chimeric sequences were identified with “Chimera.vsearch” and removed with “Remove.seqs”.

Taxonomic classification was performed with “Classify.seqs” with the Silva database (Quast *et al*., 2013). The taxa Chloroplast-Mitochondria-Unknown-Eukaryota were removed using “Remove.lineage”. Pairwise distances between aligned sequences were calculated with “Dist.seqs”, and a distance matrix was generated and used for OTU assignment with “Cluster”, at a 97 % similarity threshold. OTU taxonomy was assigned with “Classify.otu” and the number of sequences per sample was obtained with “Count.groups”.

Alpha diversity was estimated using “Summary.single” and the Chao1, Shannon Evenness, and Shannon indices. Beta diversity was assessed by principal coordinates analysis (PCoA) in MicrobiomeAnalyst 2.0 (Lu *et al*., 2023). Each condition was represented by a single composite

sample. Therefore, no biological replication was available, and no significance testing was performed. Consequently, alpha and beta diversity analyses were conducted and are presented only for exploratory and descriptive purposes. Taxonomic assignment results were visualized using Phinch (Bik, 2014).

Results

Sequencing

The length of the sequencing reads used for the analyses was 301 bp. The average number of reads generated per library was 155,530 (Table 1). The library with the highest number of reads corresponded to the cladode tissue from healthy plants collected during the rainy season (184,580 reads), while the library with the lowest number of reads corresponded to the root tissue from thickened plants collected during the dry season (122,574 reads). A total of 3,110,608 reads were generated across 20 libraries.

Data quality analysis

Raw reads were processed on the Galaxy Australia platform (The Galaxy Community, 2024). After quality filtering with Trimmomatic, the average survival rate was 78 %, yielding a mean of 120,760 reads per library (Table 1). The library with the highest number of reads was the cladode tissue from healthy plants collected during the rainy season (140,574 reads), while the root tissue from thickened plants during the dry season had the lowest (94,668). The total number of reads remaining after Trimmomatic was 2,415,208.

As a result of quality filtering, the phyllosphere had the highest number of reads (529,342), followed by rhizosphere (515,488) and soil (480,956), while cladode tissue (461,300) and root tissue (428,122) had the lowest. Libraries from healthy plants generally yielded higher read counts than those from thickened plants, except for soil samples, where higher read counts were obtained from thickened plant libraries.

Bioinformatic analysis with Mothur

Sequences were processed with Mothur (Table 1). Rhizosphere (140,242 reads), soil (128,647), and phyllosphere (111,917) retained the highest number of reads, while root tissue (88,426) and cladode tissue (490) had the lowest. In general, libraries associated with thickened plants retained more reads than those from healthy plants, with some exceptions in cladode tissue and rhizosphere. Regarding the collection season, libraries collected during the dry season showed a higher number of reads than those collected during the rainy season.

After OTU clustering at 97 % similarity threshold (Table 1), belowground compartments exhibited the highest taxonomic richness: soil (7,566 OTUs), rhizosphere (5,766), and root tissue (4,330). In contrast, aerial compartments displayed lower values: phyllosphere (2,955) and cladode tissue (83). Libraries from thickened plants generally contained more OTUs than those from healthy plants, with exceptions in the phyllosphere and rhizosphere. Across most compartments, libraries collected during the dry season contained more OTUs than those from the rainy season.

Table 1. Number and percentage of reads from the V3-V4 region of the 16S rRNA gene 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**	**671,606**	**529,342**		**111,917**	**2,955**
	CE_R	170,080	131,374	77	25,295	1,280
	CE_D	154,014	123,204	80	43,134	1,091
	CS_R	181,102	140,364	78	14,625	1,064
	CS_D	166,410	134,400	81	28,863	1,654
Cladode Tissue	**Total**	**589,238**	**461,300**		**490**	**83**
	DCE_R	126,954	100,684	79	109	31
	DCE_D	136,464	108,354	79	131	42
	DCS_R	184,580	140,574	76	122	30
	DCS_D	141,240	111,688	79	128	19
Root Tissue	**Total**	**546,156**	**428,122**		**88,426**	**4,330**
	DRE_R	131,556	102,806	78	24,559	1,927
	DRE_D	122,574	94,668	77	27,220	1,915
	DRS_R	146,116	114,518	78	13,941	1,415
	DRS_D	145,910	116,130	80	22,706	1,657
Rhizosphere	**Total**	**664,464**	**515,488**		**140,242**	**5,766**
	RE_R	173,660	132,850	77	19,677	2,189
	RE_D	156,244	120,294	77	44,208	2,443
	RS_R	176,410	137,634	78	28,998	2,046
	RS_D	158,150	124,710	79	47,359	2,799
Soil	**Total**	**639,144**	**480,956**		**128,647**	**7,566**
	SE_R	162,060	126,470	78	23,271	2,876
	SE_D	171,258	135,170	79	49,070	4,131
	SS_R	139,656	107,294	77	19,637	2,560
	SS_D	166,170	112,022	67	36,669	3,062

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.

Alpha diversity analysis

Alpha diversity describes the diversity of the species within individual microbial communities, integrating three components: richness, evenness, and diversity (Figure 3).

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Sample	Chao1	Shannon Evenness	Shannon
CE_D	1963	0.60	4.2
CE_R	2438	0.63	4.5
CS_D	2590	0.67	5.0
CS_R	1971	0.71	5.0
DCE_D	81	0.78	2.9
DCE_R	94	0.73	2.5
DCS_D	97	0.59	1.7
DCS_R	59	0.61	2.3
DRE_D	3933	0.67	5.1
DRE_R	3443	0.76	5.8
DRS_D	3963	0.54	4.0
DRS_R	2824	0.73	5.3
RE_D	4784	0.66	5.2
RE_R	4637	0.75	5.7
RS_D	5729	0.67	5.3
RS_R	3874	0.69	5.3
SE_D	7942	0.74	6.2
SE_R	6140	0.77	6.1
SS_D	6183	0.74	5.9
SS_R	5147	0.78	6.1

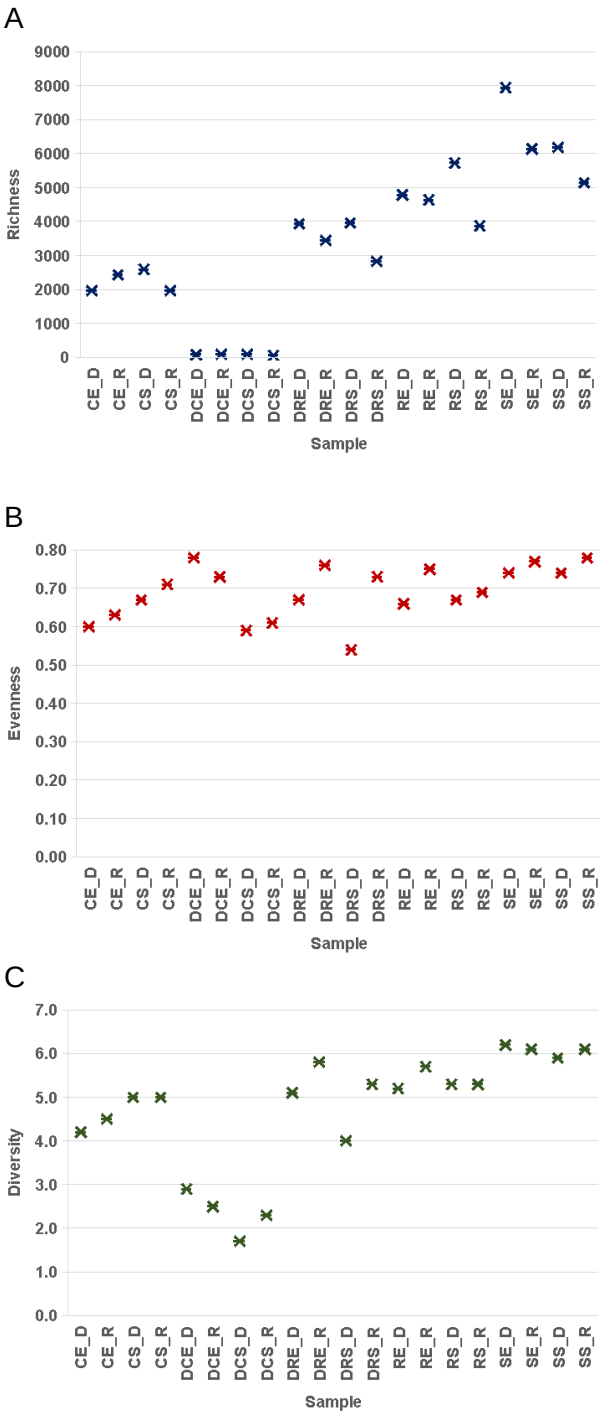

Figure 3. Alpha diversity of bacterial communities associated with healthy and thickened *Opuntia ficus-indica* plants during the rainy and dry seasons: (A) richness, (B) evenness, and (C) diversity. CE: phyllosphere of thickened plants, CS: phyllosphere of healthy plants, DCE: cladode tissue of thickened 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.

Species richness refers to the total number of taxa present in a community and was estimated using the Chao1 index, which accounts for both observed and potentially undetected species (Chao, 2013). Estimated richness across the 20 libraries ranged from 59 to 7,942, with significant differences associated with compartment type and sampling season (Figure 3A). The four soil libraries and the

rhizosphere library from the healthy plants collected during the dry season showed the highest richness values, reflecting greater microbial complexity in these compartments. Cladode tissue consistently exhibited the lowest richness. Overall, belowground compartments were richer than those from aerial compartments.

Evenness describes the relative balance of taxon abundances within a community and was assessed using the Shannon Evenness index (Shannon, 1948). Values ranged from 0.54 to 0.78, indicating a relatively uniform distribution of microbial abundances across most libraries (Figure 3B). The libraries with the highest evenness were DCE_D, SS_R, and SE_R, whereas the lowest evenness was observed in DRS_D, DCS_D, and CE_D. Unlike richness, evenness did not show a clear trend associated with compartment type or sampling season, suggesting that the internal structure of microbial communities remains relatively stable across conditions.

Diversity integrates both richness and evenness and reflects the heterogeneity of the microorganisms composing the community. It was estimated using the Shannon-Wiener index (Pielou, 1966). Values ranged from 1.7 to 6.2, reflecting substantial differences in community complexity among compartments (Figure 3C). The four soil libraries displayed the highest diversity values (≥5.9), indicating highly diverse communities. In contrast, cladode tissue libraries showed the lowest value, consistent with the richness patterns described above.

Beta diversity analysis

Principal coordinates analysis (PCoA) was used to visualize dissimilarity patterns in microbial community composition across compartments and conditions (Figure 4). For the phyllosphere (Figure 4A), the first two axes explained 81.9 % of total variability (axis 1: 50.3 %; axis 2: 31.6 %). Libraries from thickened and healthy plants were clearly separated along axis 1, suggesting that plant health status influences community structure in this compartment. For cladode tissue (Figure 4B), axis 1 and 2 accounted for 88.7 % of total variation (axis 1: 62.1 %; axis 2: 26.6 %). A clear separation between thickened and healthy plants was observed along axis 1, libraries from healthy plants formed a tight cluster, suggesting greater compositional similarity among them. For root tissue (Figure 4C), the two principal axes explained 84.1 % of variability (axis 1: 47.2 %; axis 2: 36.9 %). Libraries from the dry season clustered in the lower portion of the ordination space, while libraries from the rainy season were distributed in the upper portion. This pattern indicates that seasonal environmental conditions are the dominant structuring factor in this compartment. The libraries collected during the rainy season also showed greater internal similarity than those from the dry season. For the rhizosphere (Figure 4D), axes 1 and 2 explained 76.3 % of total variability (axis 1: 39.9 %; axis 2: 36.4 %). Libraries from thickened plants clustered in the upper right quadrant, while those from healthy plants were distributed across the upper left and lower right quadrants, suggesting that plant health status is the primary driver of community structure in this compartment, irrespective of sampling season. For soil (Figure 4E), the first two axes accounted for 93.9 % of total variability (axis 1: 70.3 %; axis 2: 23.6 %). Libraries clustered according to sampling season, indicating that seasonal environmental factors are the dominant influence on soil microbial composition.

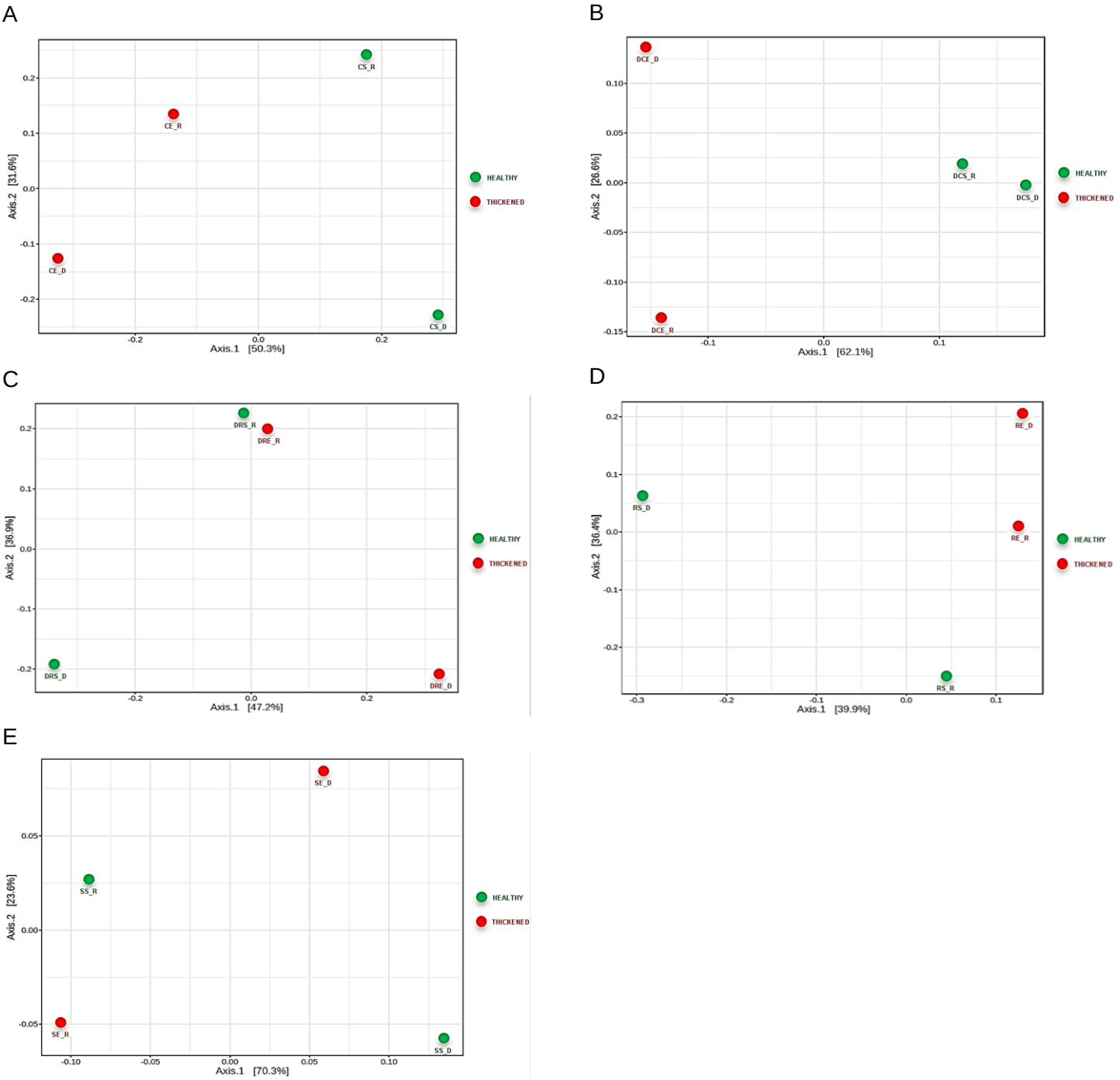

Figure 4. Principal coordinates analysis (PCoA) of microbial communities associated with the (**A**) phyllosphere, (**B**) cladode tissue, (**C**) root tissue, (**D**) rhizosphere, and (**E**) soil of healthy and thickened *Opuntia ficus-indica* plants, collected during the rainy and dry seasons. CE: 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.

Taxonomic identification

Taxonomic assignment of OTUs was performed using the Silva database. In the phyllosphere, a total of 22 phyla and 127 families were identified; in cladode tissue, 12 phyla and 31 families; in root tissue,

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20 phyla and 138 families; in the rhizosphere, 19 phyla and 110 families; and in soil, 19 phyla and 107 families. The most abundant taxa across all 20 libraries are presented in Figure 5.

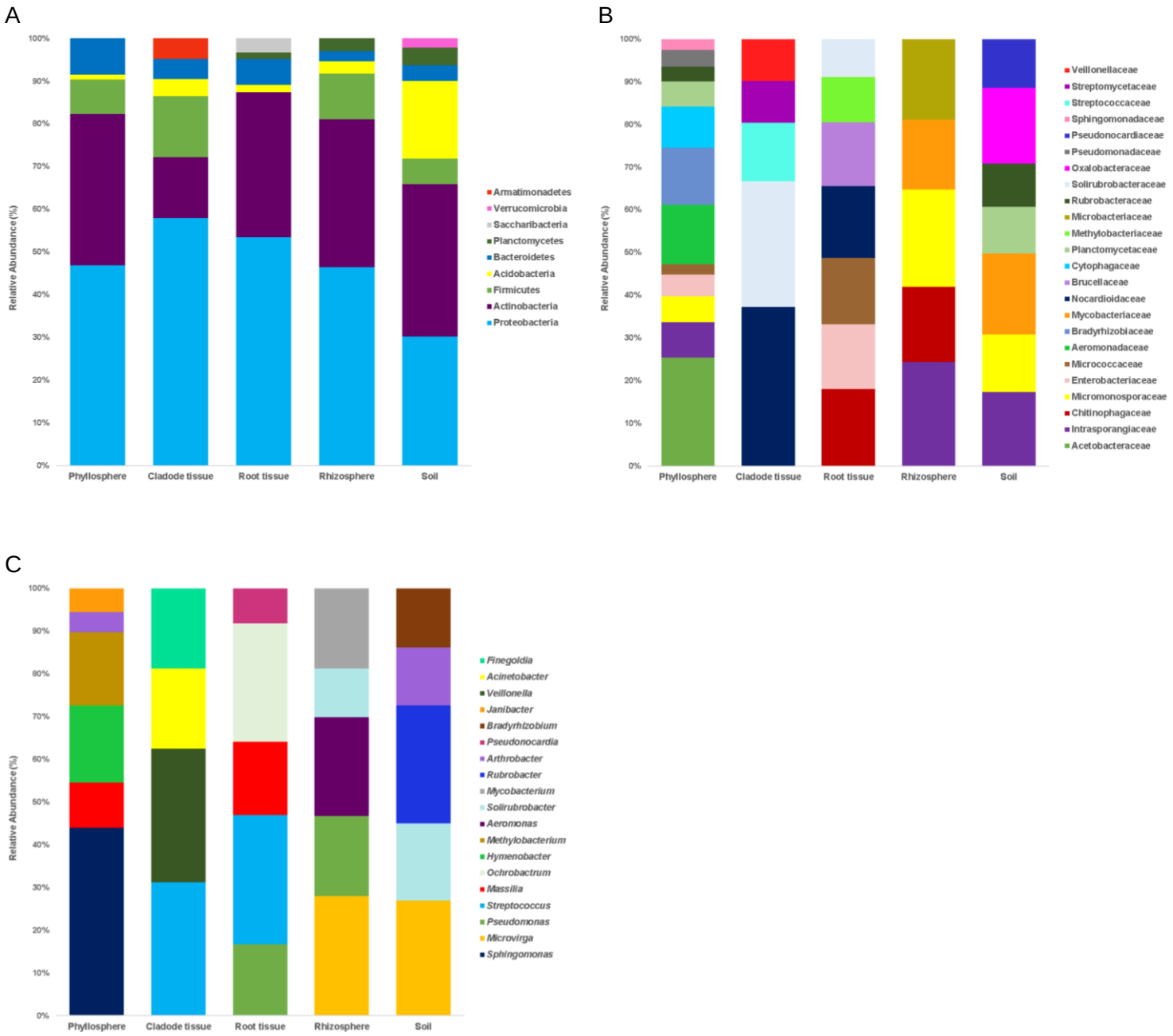

Figure 5. Most abundant bacterial (**A**) phyla, (**B**) families, and (**C**) genera based on the number of OTUs detected across the five *Opuntia ficus-indica* compartments (phyllosphere, cladode tissue, root tissue, rhizosphere, and soil).

Bacterial community composition according to cladode thickening condition

Comparisons based on cladode thickening condition revealed consistent compositional differences, exhibiting distinct taxa associated with either thickened or healthy plants regardless of sampling season (Tables 2 and 3). In thickened plants, Sphingomonadaceae, Methylobacteriaceae, Bradyrhizobiaceae, and Flavobacteriaceae were recurrently enriched across both aerial and

belowground tissues, suggesting a broad association with this condition. Other families showed a more restricted distribution: Actinomycetaceae, Lactobacillaceae, and Streptococcaceae were mainly confined to aerial tissues, while Burkholderiaceae and Hyphomicrobiaceae were detected exclusively in belowground compartments. Healthy plants exhibited a more compartment-specific community organization. Cellulomonadaceae, Intrasporangiaceae, and Promicromonosporaceae were predominantly associated with the phyllosphere and root tissue. Alcaligenaceae and Rubrobacteraceae characterized belowground compartments, while Moraxellaceae was restricted to aerial compartments.

Bacterial community composition according to sampling season

Seasonal effects were shown in consistent differences in bacterial community composition and relative abundance at both the family (Table 2) and genus (Table 3) levels, independent of cladode thickening condition. During the rainy season, Caulobacteraceae and Flavobacteriaceae were detected across both cladode and root tissues. Other families showed a more restricted distribution: Hyphomicrobiaceae was mostly associated with belowground compartments, while Moraxellaceae was confined to the phyllosphere and cladode tissue. During the dry season, a broader set of families was distributed across both aboveground and belowground compartments, including Microbacteriaceae, Micrococcaceae, Oxalobacteraceae, Planctomycetaceae, Pseudomonadaceae, Sinobacteraceae, and Solirubrobacteraceae. Several taxa remained compartment-specific: Brucellaceae, Burkholderiaceae, Rhodospirillaceae, and Rubrobacteraceae were restricted to belowground compartments, whereas Fusobacteriaceae, Actinomycetaceae, and Cellulomonadaceae were detected exclusively in aerial compartments.

Discussion

Alpha and beta diversity

Richness, evenness, and diversity of bacterial communities were estimated using the Chao1, Shannon Evenness, and Shannon-Wiener indices, respectively.

Soil, rhizosphere, and root tissue samples exhibited the highest taxonomic richness, while phyllosphere and cladode tissue communities showed the lowest values. These results are consistent with established patterns in plant microbiome research, where belowground communities typically display greater diversity than those of aerial compartments (Abdelfattah *et al*., 2018). Samples collected during the dry season also showed higher richness than those collected during the rainy season, suggesting that environmental conditions influence taxon accumulation and community diversity. Evenness values were relatively consistent across the 20 libraries; however, communities collected during the rainy season showed greater evenness than those collected during the dry season, indicating that the relative distribution of taxa may be modulated by seasonal environmental conditions. Regarding diversity, cladode tissue communities exhibited the lowest values, while soil communities were the most diverse. Communities collected during the rainy season showed higher diversity than those collected during the dry season. Collectively, these findings highlight the importance of compartment type and environmental conditions in shaping microbial richness, evenness, and overall diversity in *Opuntia ficus-indica*.

Table 2. Relative abundance at the family level of bacterial communities associated with five compartments of healthy and thickened *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
Acetobacteraceae	77.3 %	22.7 %	55.9 %	44.1 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Actinomycetaceae	-	-	-	-	100 %	-	-	100 %	-	-	-	-	-	-	-	-	-	-	-	-
Aeromonadaceae	59.8 %	40.1 %	63.0 %	37.0 %	-	-	-	-	-	-	-	-	6.3 %	93.7 %	17.7 %	82.3 %	-	-	-	-
Alcaligenaceae	-	-	-	-	-	-	-	-	7.3 %	92.7 %	50.6 %	49.4 %	18.2 %	81.8 %	49.4 %	50.5 %	-	-	-	-
Bradyrhizobiaceae	-	-	-	-	85.7 %	14.3 %	57.1 %	42.9 %	68.1 %	31.9 %	48.6 %	51.4 %	-	-	-	-	51.1 %	48.9 %	52.5 %	47.5 %
Brucellaceae	-	-	-	-	-	-	-	-	1.7 %	98.3 %	2.1 %	97.9 %	-	-	-	-	-	-	-	-
Burkholderiaceae	-	-	-	-	-	-	-	-	91.8 %	8.2 %	7.4 %	92.6 %	-	-	-	-	-	-	-	-
Caulobacteraceae	-	-	-	-	66.7 %	33.3 %	66.7 %	33.3 %	75.9 %	24.1 %	66.1 %	33.9 %	-	-	-	-	-	-	-	-
Cellulomonadaceae	12.9 %	87.1 %	10.9 %	89.1 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chitinophagaceae	56.3 %	43.7 %	43.8 %	56.2 %	-	-	-	-	77.1 %	22.9 %	52.4 %	47.5 %	46.9 %	53.1 %	44.0 %	56.0 %	68.9 %	31.1 %	42.4 %	57.6 %
Comamonadaceae	-	-	-	-	-	-	-	-	67.8 %	32.2 %	51.0 %	49.0 %	32.3 %	67.7 %	30.2 %	69.8 %	63.6 %	36.4 %	31.5 %	68.5 %
Cytophagaceae	64.7 %	35.3 %	35.7 %	64.3 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enterobacteriaceae	-	-	-	-	57.9 %	42.1 %	94.7 %	5.3 %	0.5 %	99.5 %	1.1 %	98.9 %	-	-	-	-	-	-	-	-
Flavobacteriaceae	92.7 %	7.3 %	86.4 %	13.6 %	-	-	-	-	87.8 %	12.2 %	77.3 %	22.7 %	-	-	-	-	-	-	-	-
Fusobacteriaceae	-	-	-	-	100 %	-	-	100 %	-	-	-	-	-	-	-	-	-	-	-	-
Geodermatophilaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	56.4 %	43.6 %	35.7 %	64.3 %
Hyphomicrobiaceae	-	-	-	-	-	-	-	-	62.2 %	37.8 %	58.0 %	42.0 %	-	-	-	-	56.6 %	43.3 %	53.0 %	47.0 %
Intrasporangiaceae	30.7 %	69.3 %	80.9 %	19.1 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactobacillaceae	96.9 %	3.1 %	5.7 %	94.3 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methylobacteriaceae	91.6 %	8.4 %	38.5 %	61.5 %	-	-	-	-	71.0 %	29.0 %	66.4 %	33.6 %	51.9 %	48.1 %	29.3 %	70.7 %	63.0 %	37.0 %	40.6 %	59.4 %
Microbacteriaceae	74.6 %	25.4 %	45.7 %	54.2 %	-	-	-	-	78.7 %	21.2 %	47.6 %	52.4 %	46.8 %	53.2 %	25.3 %	74.7 %	47.4 %	52.6 %	34.3 %	65.7 %
Micrococcaceae	33.6 %	66.4 %	29.1 %	70.9 %	-	-	-	-	78.6 %	21.4 %	41.5 %	58.5 %	58.4 %	41.6 %	23.9 %	76.1 %	47.4 %	52.6 %	34.3 %	65.7 %
Micromonosporaceae	-	-	-	-	-	-	-	-	68.9 %	31.1 %	53.0 %	47.0 %	-	-	-	-	-	-	-	-
Moraxellaceae	16.7 %	83.3 %	78.4 %	21.6 %	-	100 %	100 %	-	-	-	-	-	-	-	-	-	-	-	-	-
Mycobacteriaceae	96.4 %	3.6 %	3.5 %	96.5 %	-	-	-	-	61.8 %	38.2 %	57.7 %	42.3 %	37.1 %	62.9 %	59.2 %	40.8 %	62.0 %	38.0 %	49.3 %	50.7 %
Nocardiaceae	52.3 %	47.7 %	97.2 %	2.8 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nocardioidaceae	39.2 %	60.8 %	28.1 %	71.9 %	50.0 %	50.0 %	100 %	-	57.1 %	42.8 %	67.9 %	32.1 %	44.8 %	55.2 %	37.0 %	63.0 %	56.5 %	43.5 %	41.6 %	58.4 %
Oxalobacteraceae	46.0 %	54.0 %	41.6 %	58.4 %	-	-	-	-	95.5 %	4.5 %	9.2 %	90.8 %	88.3 %	11.7 %	9.1 %	90.9 %	-	-	-	-
Peptostreptococcaceae	-	-	-	-	-	-	-	-	-	-	-	-	65.7 %	34.3 %	67.6 %	32.4 %	-	-	-	-
Phyllobacteriaceae	-	-	-	-	-	-	-	-	47.6 %	52.4 %	45.7 %	54.2 %	-	-	-	-	-	-	-	-
Planctomycetaceae	-	-	-	-	50.0 %	50.0 %	-	100 %	57.4 %	42.6 %	36.1 %	63.9 %	48.8 %	51.2 %	23.3 %	76.7 %	58.2 %	41.8 %	31.5 %	68.5 %
Planococcaceae	-	-	-	-	-	-	-	-	-	-	-	-	57.1 %	42.9 %	42.8 %	57.2 %	55.2 %	44.8 %	46.1 %	53.8 %
Promicromonosporaceae	-	-	-	-	-	-	-	-	31.9 %	68.1 %	72.3 %	27.7 %	-	-	-	-	-	-	-	-
Propionibacteriaceae	69.1 %	30.9 %	26.3 %	73.7 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonadaceae	34.0 %	66.0 %	25.8 %	74.2 %	80.0 %	20.0 %	40.0 %	60.0 %	87.8 %	12.2 %	18.7 %	81.3 %	93.2 %	6.8 %	9.0 %	91.0 %	-	-	-	-
Pseudonocardiaceae	3.1 %	96.9 %	1.0 %	99.0 %	-	100 %	100 %	-	55.3 %	44.7 %	50.8 %	49.2 %	-	-	-	-	-	-	-	-
Rhizobiaceae	-	-	-	-	-	-	-	-	75.3 %	24.7 %	50.4 %	49.6 %	-	-	-	-	-	-	-	-
Rhodospirillaceae	-	-	-	-	-	-	-	-	-	-	-	-	47.9 %	52.1 %	18.0 %	82.0 %	60.7 %	39.3 %	43.7 %	56.3 %
Rubrobacteraceae	-	-	-	-	-	-	-	-	-	-	-	-	30.5 %	69.5 %	8.1 %	91.9 %	47.9 %	52.1 %	14.3 %	85.7 %
Sinobacteraceae	-	-	-	-	100 %	-	-	100 %	70.6 %	29.4 %	25.5 %	74.5 %	-	-	-	-	52.7 %	47.3 %	44.2 %	55.8 %
Solirubrobacteraceae	29.6 %	70.4 %	19.4 %	80.6 %	-	-	-	-	-	-	-	-	49.8 %	50.2 %	19.3 %	80.6 %	54.4 %	45.6 %	34.3 %	65.7 %
Sphingobacteriaceae	93.5 %	6.5 %	10.2 %	89.8 %	-	-	-	-	67.6 %	32.4 %	55.8 %	44.2 %	59.1 %	40.9 %	47.3 %	52.7 %	71.9 %	28.1 %	50.4 %	49.6 %
Sphingomonadaceae	80.1 %	19.9 %	28.8 %	71.2 %	50.0 %	50.0 %	75.0 %	25.0 %	75.8 %	24.2 %	54.2 %	45.8 %	59.1 %	40.9 %	47.3 %	52.7 %	71.9 %	28.1 %	50.4 %	49.6 %
Streptococcaceae	-	-	-	-	100 %	-	20.0 %	80.0 %	-	-	-	-	-	-	-	-	-	-	-	-
Streptomycetaceae	-	-	-	-	-	-	-	-	43.3 %	56.7 %	64.2 %	35.8 %	43.2 %	56.8 %	45.5 %	54.5 %	56.9 %	43.1 %	44.6 %	55.4 %
Thermomonosporaceae	-	-	-	-	-	-	-	-	56.2 %	43.8 %	73.8 %	26.2 %	-	-	-	-	-	-	-	-
Veillonellaceae	-	-	-	-	80.0 %	20.0 %	20.0 %	80.0 %	-	-	-	-	-	-	-	-	-	-	-	-
Xanthomonadaceae	-	-	-	-	-	-	-	-	53.1 %	46.9 %	63.4 %	36.6 %	24.1 %	75.9 %	49.0 %	51.0 %	-	-	-	-

Taxa with abundance > 0.5 % are included.

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Table 3. Relative abundance at the genus level of bacterial communities associated with five compartments of healthy and thickened *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
Achromobacter	-	-	-	-	-	-	-	-	2.2 %	97.8 %	50.2 %	49.8 %	18.2 %	81.8 %	49.4 %	50.5 %	-	-	-	-
Acinetobacter	-	-	-	-	-	100 %	100 %	-	-	-	-	-	-	-	-	-	-	-	-	-
Actinomyces	-	-	-	-	100 %	-	-	100 %	-	-	-	-	-	-	-	-	-	-	-	-
Actinoplanes	-	-	-	-	-	-	-	-	70.8 %	29.2 %	84.9 %	15.1 %	-	-	-	-	-	-	-	-
Aeromicrobium	-	-	-	-	-	-	-	-	70.7 %	29.2 %	77.4 %	22.6 %	-	-	-	-	-	-	-	-
Aeromonas	59.8 %	40.1 %	63.0 %	37.0 %	-	-	-	-	-	-	-	-	6.3 %	93.7 %	17.7 %	82.3 %	-	-	-	-
Arthrobacter	23.2 %	76.8 %	22.0 %	78.0 %	-	-	-	-	80.2 %	19.8 %	39.8 %	60.1 %	63.8 %	36.2 %	15.2 %	84.8 %	47.6 %	52.4 %	29.3 %	70.7 %
Bradyrhizobium	-	-	-	-	-	-	-	-	71.8 %	28.2 %	44.1 %	55.9 %	-	-	-	-	50.7 %	49.3 %	52.3 %	47.7 %
Brevundimonas	-	-	-	-	50.0 %	50.0 %	100 %	-	-	-	-	-	-	-	-	-	-	-	-	-
Burkholderia	-	-	-	-	-	-	-	-	91.8 %	8.2 %	7.4 %	92.6 %	-	-	-	-	-	-	-	-
Cellulomonas	12.9 %	87.1 %	10.9 %	89.1 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chryseobacterium	98.2 %	1.8 %	91.6 %	8.4 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Devosia	-	-	-	-	-	-	-	-	49.1 %	50.9 %	70.7 %	29.3 %	-	-	-	-	-	-	-	-
Finegoldia	-	-	-	-	66.7 %	33.3 %	33.3 %	66.7 %	-	-	-	-	-	-	-	-	-	-	-	-
Flavobacterium	-	-	-	-	-	-	-	-	89.4 %	10.6 %	79.7 %	20.2 %	-	-	-	-	-	-	-	-
Friedmanniella	72.9 %	27.1 %	26.1 %	73.9 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusobacterium	-	-	-	-	100 %	-	-	100 %	-	-	-	-	-	-	-	-	-	-	-	-
Hymenobacter	66.7 %	33.3 %	34.0 %	66.0 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Janibacter	30.7 %	69.3 %	80.9 %	19.1 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kribbella	-	-	-	-	-	-	-	-	35.9 %	64.1 %	71.0 %	29.0 %	25.6 %	74.4 %	47.3 %	52.6 %	-	-	-	-
Massilia	46.1 %	53.9 %	41.7 %	58.3 %	-	-	-	-	98.8 %	1.2 %	4.4 %	95.6 %	88.2 %	11.8 %	9.1 %	90.9 %	-	-	-	-
Methylobacterium	91.6 %	8.4 %	38.5 %	61.5 %	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Microvirga	-	-	-	-	-	-	-	-	-	-	-	-	52.0 %	48.0 %	28.2 %	71.8 %	62.9 %	37.1 %	40.7 %	59.3 %
Mycobacterium	96.4 %	3.6 %	3.5 %	96.5 %	-	-	-	-	62.5 %	37.4 %	57.7 %	42.3 %	37.1 %	62.9 %	59.2 %	40.8 %	62.0 %	38.0 %	49.3 %	50.7 %
Nevskia	-	-	-	-	100 %	-	-	100 %	-	-	-	-	-	-	-	-	-	-	-	-
Nocardioides	64.0 %	36.0 %	20.4 %	79.6 %	-	-	-	-	68.9 %	31.1 %	64.5 %	35.5 %	-	-	-	-	-	-	-	-
Ochrobactrum	-	-	-	-	-	-	-	-	-	100 %	-	100 %	-	-	-	-	-	-	-	-
Pedobacter	-	-	-	-	-	-	-	-	73.0 %	27.0 %	49.3 %	50.7 %	-	-	-	-	-	-	-	-
Promicromonospora	-	-	-	-	-	-	-	-	32.2 %	67.8 %	72.0 %	28.0 %	-	-	-	-	-	-	-	-
Pseudomonas	29.0 %	71.0 %	17.3 %	82.7 %	-	-	-	-	87.8 %	12.2 %	18.7 %	81.3 %	93.2 %	6.8 %	8.9 %	91.1 %	-	-	-	-
Pseudonocardia	-	-	-	-	-	-	-	-	54.5 %	45.5 %	60.5 %	39.5 %	-	-	-	-	-	-	-	-
Rhizobium	-	-	-	-	-	-	-	-	65.5 %	34.5 %	69.8 %	30.2 %	-	-	-	-	-	-	-	-
Rubrobacter	-	-	-	-	-	-	-	-	-	-	-	-	30.5 %	69.5 %	8.1 %	91.9 %	47.9 %	52.1 %	14.3 %	85.7 %
Skermanella	-	-	-	-	-	-	-	-	-	-	-	-	51.7 %	48.3 %	12.4 %	87.6 %	60.6 %	39.4 %	44.1 %	55.8 %
Solirubrobacter	29.6 %	70.4 %	19.4 %	80.6 %	-	-	-	-	-	-	-	-	49.8 %	50.2 %	19.3 %	80.6 %	54.4 %	45.6 %	34.3 %	65.7 %
Sphingomonas	81.3 %	18.7 %	26.8 %	73.2 %	-	-	-	-	84.6 %	15.4 %	55.7 %	44.3 %	59.2 %	40.7 %	47.0 %	53.0 %	-	-	-	-
Sporosarcina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55.1 %	44.9 %	46.0 %	54.0 %
Streptococcus	-	-	-	-	100 %	-	20.0 %	80.0 %	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces	-	-	-	-	-	-	-	-	46.4 %	53.6 %	68.9 %	31.1 %	-	-	-	-	-	-	-	-
Veillonella	-	-	-	-	80.0 %	20.0 %	20.0 %	80.0 %	-	-	-	-	-	-	-	-	-	-	-	-
Virgisporangium	-	-	-	-	-	-	-	-	82.9 %	17.1 %	24.2 %	75.8 %	-	-	-	-	-	-	-	-

Taxa with abundance > 0.5 % are included.

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Microbial distribution is not uniform but varies temporally and spatially in response to environmental conditions. Temporal variation shows shifts in community composition associated with seasonal or environmental fluctuations (Torres-Cortés *et al*., 2012), while spatial variation arises from differences in microbial composition attributable to the sampling site (Coleman-Derr *et al*., 2016), host species (Fonseca-García *et al*., 2016), plant organ (Karray *et al*., 2020), or even distinct zones within a single organ (Berg *et al*., 2020).

Comparable patterns have been reported by Fonseca-García *et al*. (2018) for several xerophytic plant species, including *Agave tequilana*, *Agave deserti*, *Agave salmiana*, *Myrtillocactus geometrizans*, and *Opuntia robusta*, in which root and soil communities exhibited the highest alpha diversity, followed by phyllosphere samples, while aerial tissue showed the lowest values. This general trend, in which belowground compartments commonly display greater richness, evenness, and diversity than aerial compartments, is well documented across diverse plant systems (Aguirre-Garrido *et al*., 2012; Abdelfattah *et al*., 2018; Olimi *et al*., 2022).

Beta diversity analysis revealed that phyllosphere, cladode tissue, and rhizosphere communities grouped mainly according to plant health condition, while root tissue and soil communities were more influenced by environmental conditions at the time of sampling, regardless of cladode thickening. This pattern is consistent with previous findings demonstrating that sampling season can modulate bacterial community structure in plant tissues, as reported for Mexican agaves (Coleman-Derr *et al*., 2016).

Analogous dynamics have been described in grapevine wood microbiota, where significant compositional shifts in bacterial communities were associated with “esca disease”, a destructive trunk disease in which bacterial composition differs markedly between necrotic and non-necrotic tissue (Bruez *et al*., 2020). These findings reinforce the notion that biotic factors, such as disease condition, and abiotic factors, such as seasonality, exert a decisive influence on microbial community structure. The marked contrast between soil and aerial compartment communities observed here is consistent with previous reports (Dong *et al*., 2019). Lower temperatures, reduced water availability, and elevated UV radiation act as strong environmental filters in aboveground habitats, likely explaining the lower diversity observed in aerial microbial communities compared to soil microbial communities (Gómez-Godínez *et al*., 2025). Root activity plays a key role in the belowground diversity: the release of root exudates modulates microbial colonization in the rhizosphere, and under dry conditions, certain microorganisms maintain close associations with roots, potentially contributing to host drought tolerance (Ullah *et al*., 2019). These findings indicate that the diversity and composition of bacterial communities are shaped by a complex interplay of biotic and abiotic factors.

Taxonomic identification

Bacterial communities associated with thickened and healthy plants

The differences observed between thickened and healthy plants reflect a reorganization of overall microbiome structure rather than compositional changes confined to individual compartments. In thickened plants, the predominance of certain bacterial families across both aerial and belowground compartments suggest that communities become more similar throughout the plant, with a reduced differentiation among plant niches. Healthy plants, by contrast, showed a more compartment-specific organization, with distinct taxa consistently associated with particular niches such as the phyllosphere, root, or soil. These patterns suggest that in the presence of cladode thickening, there is a homogenization of bacterial composition across plant compartments.

The families enriched in thickened plants are widely reported in plant-associated environments. Members of Sphingomonadaceae, Methylobacteriaceae, and Bradyrhizobiaceae have been documented in studies that examine changes in the microbial community under changing plant conditions, including abiotic stress and pathogen presence (Innerebner *et al*., 2011; Ardanov *et al*., 2012; Purahong *et al*., 2018; Wang *et al*., 2024). Flavobacteriaceae are a common constituent of root-associated microbiomes (Kolton *et al*., 2016; Olimi *et al*., 2022) and have also been reported to enhance plant defense mechanisms against diseases (Carrión *et al*., 2019; Huang *et al*., 2022). Lactobacillaceae and Streptococcaceae were mainly confined to aerial tissues of thickened plants. Members of Lactobacillaceae have been reported to exhibit pathogen-inhibiting activity (Saragoça *et al*., 2024), while Streptococcaceae have been associated with diseased plants in other species, including *Gossypium hirsutum* L. and *Euterpe oleracea* (Moura *et al*., 2018). Burkholderiaceae and Hyphomicrobiaceae were detected exclusively in belowground compartments; the ecological diversity of Burkholderiaceae is broad, including plant-associated, beneficial, and pathogenic organisms (Pal *et al*., 2022).

In healthy plants, the association of Promicromonosporaceae with root tissue is consistent with previous studies in *O. ficus-indica* (Karray *et al*., 2020). Moraxellaceae and Intrasporangiaceae both showed specificity for aerial compartments; Moraxellaceae is a recognized constituent of aerial microbial communities across diverse crops (Erlacher *et al*., 2014; Dong *et al*., 2019; Olimi *et al*., 2022), and Intrasporangiaceae, restricted to the phyllosphere, has been reported as a common member of the leaves with a potential role in disease response and bioremediation (Santana *et al*., 2016; Li *et al*., 2023).

Overall, these results indicate that cladode thickening is associated with the homogenization of bacterial communities in the plant, as well as with changes in the relative abundance of bacterial families. However, the etiology of this condition does not appear to be associated with any bacterial taxonomic profile. While biotic agents have been proposed as causal factors, abiotic conditions may also play a contributing role. In this context, differences in the accumulation of nutritional elements have been reported between thickened and healthy cladodes, with distinct macro and micronutrient profiles observed in each condition (Valadez-Moctezuma *et al*., 2021). Given that the studied orchard has received no tillage, fertilization, or cultural practices since its establishment, nutritional imbalances resulting from this low-input management could represent a predisposing condition for cladode thickening, particularly considering that soil management has been shown to modulate microbial community functions in *Opuntia* agroecosystems (Gómez-Godínez *et al*., 2025).

Bacterial community composition under dry and rainy conditions

Changes in the composition and distribution of the bacterial community in different plant compartments were associated with seasonal variation, indicating that temporal environmental changes play an important role in the microbiome structure of *O. ficus-indica*, regardless of cladode thickening status. Several members of the genus *Flavobacterium* have been reported to contribute to plant health and development through growth promotion, disease suppression, and tolerance to abiotic stressors such as drought and salinity (Seo *et al*., 2024). Moraxellaceae has been described as a common constituent of cactus microbiomes, particularly in the phyllosphere and root tissues (Fonseca, 2016). In this study, both taxa were predominantly detected in samples collected during the rainy season, suggesting a potential association with higher relative humidity conditions.

The dry season was characterized by a broader distribution of bacterial families across compartments. Microbacteriaceae is widely documented in phyllosphere communities across diverse plant species (Junker *et al*., 2011), and its relative abundance has been observed to increase under reduced precipitation (Allard *et al*., 2020). Micrococcaceae and Rhodospirillaceae are frequent members of rhizosphere microbiomes in plants from arid environments, including the Atacama Desert (Fuentes *et al*., 2020), consistent with their occurrence under water-limited conditions. Members of Oxalobacteraceae have been reported to tolerate both abiotic and biotic stressors, including dry and heavy metal exposure (Ofek *et al*., 2012), and are commonly found in root tissue and rhizosphere communities from desert environments such as the Sonoran Desert (Nagy *et al*., 2005), potentially through interactions with root exudates (Yu *et al*., 2021).

Planctomycetaceae, enriched under dry conditions, comprises environmentally widespread bacteria documented across a broad range of ecosystems (Wiegand *et al*., 2018). The genus *Pseudomonas* (Pseudomonadaceae) includes species with plant-associated functions, including growth promotion, nitrogen fixation, and the production of pathogen-suppressive compounds (Trujillo *et al*., 2007; Pérez *et al*., 2015). Increased abundance of Solirubrobacteraceae under water-limited conditions has also been reported (Metze *et al*., 2023; Goemann *et al*., 2024), and the genus *Solirubrobacter* in particular has been associated with the biosynthesis of metabolites linked to environmental stress tolerance, including drought (Jara-Servin *et al*., 2024).

Burkholderiaceae has been identified as a frequent member of cactus microbiomes in both phyllosphere and root tissues (Fonseca, 2016). Furthermore, studies on *O. ficus-indica* have shown that increasing aridity is associated with higher abundances of Rhodospirillaceae and Rubrobacteraceae in the rhizosphere, suggesting a role in drought adaptation (Karray *et al*., 2020).

Plant-associated bacterial assemblages can contribute to plant performance through mechanisms such as stress tolerance. These interactions are likely shaped by both environmental conditions and host-specific traits, which together determine microbial activity and community composition. In this context, *O. ficus-indica* appears to harbor a stable and functionally relevant core microbiome capable of responding to variable environmental conditions. Soil management practices have additionally been shown to influence microbial community functions within *Opuntia* agroecosystems (Gómez-Godínez *et al*., 2025).

Conclusions

Bacterial communities associated with thickened and healthy *Opuntia ficus-indica* plants were shaped by both the thickening condition and seasonal variation. Belowground compartments exhibited greater richness, evenness, and diversity, highlighting soil as a key reservoir of stable and functionally diverse microbial communities. In contrast, the phyllosphere and cladode tissue showed less diversity. The structure of the bacterial community in the phyllosphere, cladode tissue, and rhizosphere was strongly influenced by plant health status, while communities in soil and root tissue were shaped mainly by environmental conditions. This pattern suggests that belowground communities provide compositional stability under changing conditions, whereas aerial and rhizosphere communities are more responsive to host physiological state. Several bacterial genera associated with biotic and abiotic stress were identified across compartments; however, none showed a conclusive causal association with this disease in the present study. Cladode thickening may influence the reorganization of microbiome structure: thickened plants exhibited greater compositional similarity across compartments, suggesting

a homogenization of microbial communities, whereas healthy plants maintained a more compartment-specific community organization. The collection season determined the distribution of bacterial taxa in the plant compartments. During the rainy season, fewer taxa were shared among compartments. On the other hand, under dry conditions, there was greater similarity in the taxa present in multiple compartments, suggesting a possible role in adaptation to water-limited environments. This study represents the first metagenomic characterization of bacterial communities associated with healthy and thickened *O. ficus-indica* plants across five ecological compartments during both the dry and rainy seasons.

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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