Microbial Ecology (2024) 87:181
https://doi.org/10.1007/s00248-025-02496-2

RESEARCH

Agricultural Practices and Environmental Factors Drive Microbial Communities in the Mezcal-Producing *Agave angustifolia* Haw

Gonzalo Contreras-Negrete[1] · Alfonso Valiente-Banuet[2] · Francisco Molina-Freaner[2,4] · Laila P. Partida-Martínez[3] · Antonio Hernández-López[1]

Received: 9 October 2024 / Accepted: 13 January 2025

Abstract

Mezcal, a traditional Mexican alcoholic beverage, has been a vital source of livelihood for indigenous and rural communities for centuries. However, increasing international demand is exerting pressure on natural resources and encouraging intensive agricultural practices. This study investigates the impact of management practices (wild, traditional, and conventional) and environmental factors on the microbial communities associated with *Agave angustifolia*, a key species in mezcal production. High-throughput sequencing of the 16S rRNA and ITS2 gene regions revealed distinct prokaryotic and fungal community structures across different plant compartments (endosphere, episphere, and soil), identifying 8214 prokaryotic and 7459 fungal ASVs. Core microbial communities were dominated by Proteobacteria, Actinobacteria, Ascomycota, and Basidiomycota. Alpha diversity analyses showed significant increases in prokaryotic diversity from the endosphere to soil, while fungal diversity remained stable. Notably, conventional management practices were associated with reductions in beneficial microbial taxa. Environmental factors such as precipitation and temperature significantly influenced microbial diversity and composition, especially in the rhizosphere. Beta diversity patterns underscored the strong impact of plant compartment, with management practices and aridity further shaping microbial communities. These results reveal the intricate interactions between management practices, environmental conditions, and microbial diversity, providing valuable insights for the sustainable cultivation of *A. angustifolia*.

Keywords Agricultural practices · Microbiome · Domestication · Agave · Spirits

Introduction

Plant-associated microorganisms play a central role in vital functions such as germination, growth, and development of plants [1]. For plant species, particularly those with widespread distribution, microbial communities are influenced by a combination of intrinsic factors (such as genotype and phenotype) and extrinsic factors (including climate, geography, soil type, and seasons). Additionally, the type of holobiont sample crucially shapes microbial diversity and composition under natural conditions [2–5]. In contrast, plants subjected to artificial selection often exhibit significant changes in microbiome diversity and composition. These changes are frequently associated with morphological and physiological alterations in managed genotypes (e.g., *Phaseolus* spp., barley, wheat, soybean) [6–8], particularly in root traits such as the root endosphere and rhizosphere [6, 7, 9].

Positive interactions between the host and associated microbiome (*eubiosis*) for both prokaryotic and fungal

✉ Gonzalo Contreras-Negrete
congo@cieco.unam.mx

✉ Antonio Hernández-López
ahernandez@enes.unam.mx

1 Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores Unidad León, Universidad Nacional Autónoma de México, León, Guanajuato, México

2 Departamento de Ecología de La Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico City, México

3 Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Irapuato, Irapuato, México

4 Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Hermosillo, Sonora, México

Published online: 30 January 2025

communities significantly enhance beneficial interactions such as diazotrophic functions, CO oxidation, and nutrient availability, as well as tolerance to drought, salinity, and cold [10–12]. These benefits are particularly evident in arid and semi-arid plants such as agaves, including autotrophy, biofilm formation, and water and nutrient retention [2, 13] together with protection against UV radiation, increasing the plant's host resistance [14]. However, imbalances in the host-associated microbiome (*dysbiosis*) associated with management practices could cause plants to lose healthy functions such as digestion of complex polysaccharides, plant cell wall degrading enzymes, nitrogen fixation, and protection against soil pathogens, [15–17], low activity of bio-fertilization, bio-stimulation and biocontrol [18].

The *Agave* genus, which is influenced by both natural and artificial factors, provides a compelling example of these processes. Comprising around 210 species primarily distributed in arid and semiarid regions across Mexico and the southern United States [19–21], *Agave* species have been historically utilized for diverse purposes, including human and animal food, biofuel, fiber, medicine, and the production of fermented beverages and spirits [21]. In these species, domestication syndromes characterized by traits such as stem gigantism, higher carbohydrate concentration, reduced leaf dentition, and lower levels of saponins to facilitate harvesting have been identified [21]. The significant increase in *Agave* spirit production since the nineteenth century reflects recent management practices, which, along with the long-life cycle of *Agave*, accounts for the early stages of domestication seen in cultivars and landraces [21, 22]. However, the establishment of large monocultures, particularly when the *Agave* species involved can produce clones, results in a significant loss of genetic diversity, making these monocultures highly vulnerable to pathogens and pests. The loss of soil quality, high incidence of pests, and extensive use of agrochemicals, including herbicides, further contribute to environmental contamination and the reduction of associated microbial diversity and functions [2]. These microbial associations are key to promoting the health and growth of *Agave* plants. The relationships are maintained through the mutualistic interactions facilitated by mycorrhizae and other microorganisms, indicating that both the plants and their associated microbial communities are part of an interconnected system, coexisting through fungal and bacterial-mediated interactions. Therefore, one alternative for achieving sustainable mezcal production in arid and semi-arid regions is the rational use of microbial consortia adapted to these extreme habitats and their specific hosts [23, 24]. This approach leverages microbial biodiversity, incorporating it into integrated and sustainable production systems. This study evaluated the effects of agroecological management versus monoculture and wild populations on the microbial communities associated with *A. angustifolia*, the most widely used mezcal-producing species in Mexico, across three key production regions in the north, central, and southern parts of the country.

Materials and Methods

Sample Collection and Processing

Agave angustifolia Haw is the most widespread species of the genus in Mexico, occurring from northern Mexico in the states of Chihuahua and Sonora to Nicaragua in Central America. It inhabits a wide variety of plant communities, such as coastal dunes, low deciduous and sub-deciduous forests, xerophytic scrub, and pine-oak forests. Likewise, throughout its distribution, it is used in a variety of ways as a spirit drink, to obtain fiber, and as a medicinal plant [19, 21]. In 2022, we conducted a comprehensive collection of samples from 13 sites across the Mexican states of Sonora, Nayarit, Jalisco, and Oaxaca, encompassing most of the geographic distribution of *A. angustifoli*a. These sites were chosen to represent a spectrum of agricultural management practices. Specifically, the sites were categorized based on their management status into three distinct groups: wild, traditional, and conventional. This categorization is referred to as "status" throughout this document (see Fig. 1a; Table 1). Wild sites were natural populations untouched by human agricultural activity, providing a baseline for natural conditions. Traditional sites include age-old farming practices that typically involve low input and organic methods, silviculture plantations, often passed down through generations. Conventional sites used modern agricultural techniques, including chemical fertilizers, pesticides, and clonally generated monocultures, reflecting current industrial farming standards. Each collection site was classified according to the De Martonne aridity index (hereafter IAM) following Rahimi et al. [25]. The De Martonne aridity index is based on the aridity index $I = P/(T + 10)$, where T is the mean temperature (°C), and P is the mean annual precipitation in mm of each collection site [25]. The sampling sites were assigned to AR, arid; SAM, semiarid Mediterranean; SH, subhumid; HUM, humid conditions according to the IAM (see Table 1).

Samples were collected from three healthy plants at each collection site. For each sampled individual, four sub-samples were collected, representing the endosphere (leaves and root endosphere), the episphere (phyllosphere and rhizosphere) and the bulk soil. Two leaves for the leaf endosphere and phyllosphere and root tissue for root endosphere and rhizosphere were collected. Rhizosphere (microbial communities within the first 2 mm from the roots) and phyllosphere (leaf surface microbial communities) samples were prepared by washing collected roots and leaves with sterilized epiphyte buffer (50 mM KH_2PO_4, 50 mM K_2HPO_4, 0.1% Triton

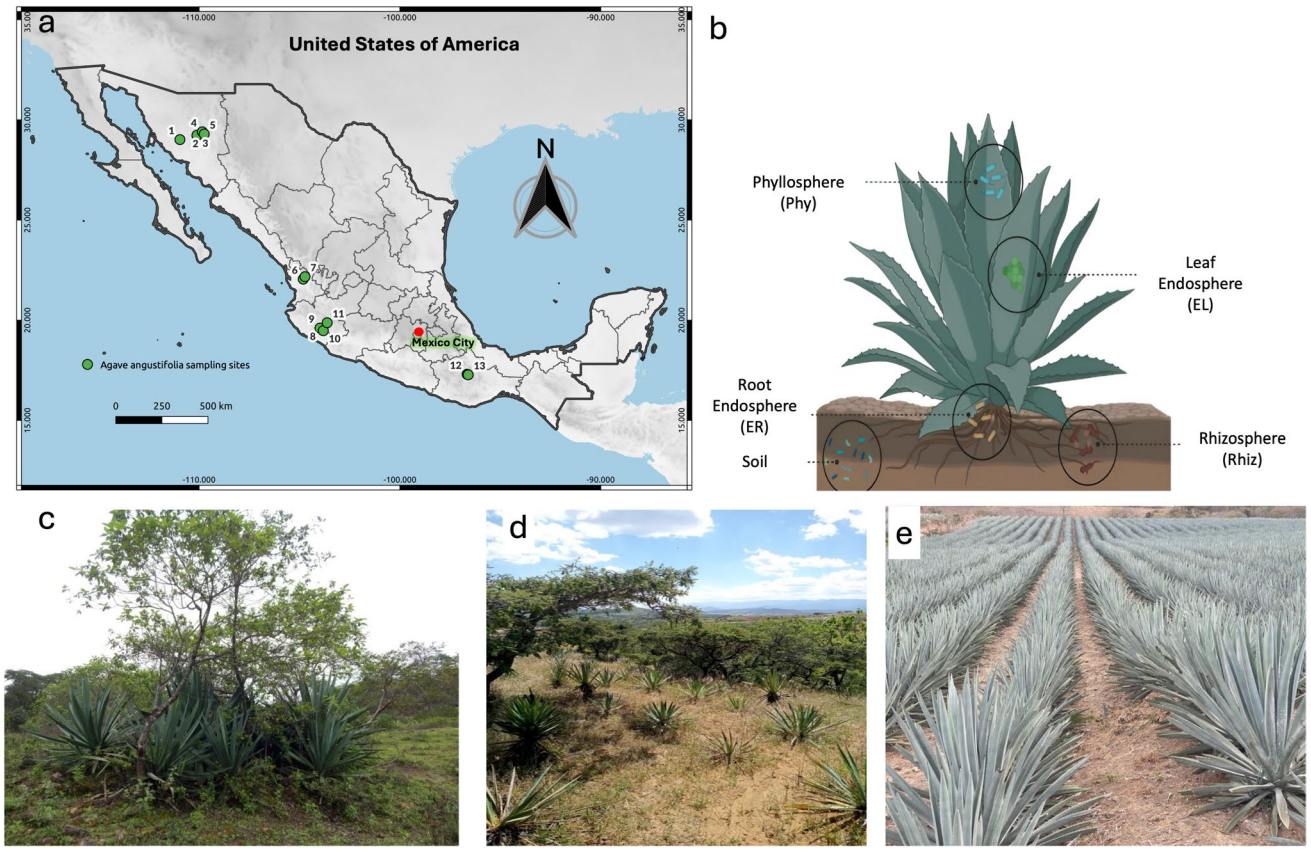

Fig. 1 Experimental design for *A. angustifolia* microbiome analysis: **a** *A. angustifolia* sampling sites from across Mexico. Code numbers correspond to Table 1. **b** Samples analyzed for each plant individual: The images represent the different management statuses of *A. angustifolia* **c** wild, **d** traditional, and **e** conventional

Table 1 Geographic location, status management, De Martonne Aridity Index (IAM) and bioclimatic variables of the collection sites of *Agave angustifolia* populations sampled across Mexico. Precipitation of wettest month (bio13), mean temperature of driest quarter (bio9), isothermality (bio3), mean diurnal range (bio2)

Population	State	Locality	Status	Latitude	Longitude	IAM	bio13	bio9	bio3	bio2
1	Sonora	Huepari	Wild	29.402	− 109.840	SAM	151.00	21.63	52.98	19.23
2	Sonora	San Pedro	Traditional	29.294	− 109.746	SAM	143.00	22.48	53.03	19.57
3	Sonora	Alamos	Conventional	29.233	− 110.107	SAM	168.00	19.70	56.55	19.79
4	Sonora	Mazatan	Wild	29.211	− 110.109	SAM	164.00	19.95	56.59	20.03
5	Sonora	Centro Ecológico	Wild	29.015	− 110.954	AR	84.00	21.55	55.49	18.70
6	Nayarit	Nayar3	Wild	22.172	− 104.727	HUM	278.00	21.45	64.93	16.17
7	Nayarit	Nayar2	Wild	22.048	− 104.816	HUM	318.00	26.18	65.26	15.47
8	Jalisco	Sayula	Wild	19.873	− 103.622	SH	200.00	18.35	63.74	15.81
9	Jalisco	Toliman	Wild	19.601	− 103.919	SH	133.00	23.85	70.12	18.25
10	Jalisco	Toliman	Traditional	19.597	− 103.917	SH	137.08	23.78	70.08	18.34
11	Jalisco	Tetepan	Traditional	19.47	− 103.815	HUM	165.00	22.85	69.74	16.46
12	Oaxaca	Huitzo	Conventional	17.300	− 96.652	SH	166.00	13.55	66.98	10.72
13	Oaxaca	Huitzo	Traditional	17.275	− 96.606	SH	164.00	14.42	66.04	11.43

X-100). For the root and leaf endosphere, sampled root and leaf tissues were surface sterilized with sterilization buffer (1:20 dilution of commercial bleach) for 15 min while mixing. After sterilization, root and leaf tissues were dissected into thin sections and placed into a 15% glycerol stock in 50 ml plastic tubes. Bulk soil was sampled by collecting topsoil (20 cm depth, ~ 50cm^3) approximately 1 m from the individual sampled; the soil subsamples were pooled per site (Fig. 1b). All processed samples were stored at − 80 °C for further DNA extraction. The final data set depended on the quality of the DNA samples for each plant compartment. A total of 254 samples were analyzed (137 for 16S prokaryotes and 137 for ITS2 fungi). The characteristics of the samples for both 16S and ITS2 markers, including the location, type of sample, status, and IAM, are summarized in Table S1 .

DNA Extraction and Sequencing

DNA extraction of root and leaf endosphere was performed using the Zymo Research Quick-DNA Plant/Seed Miniprep Kit; for phyllosphere and rhizosphere, we used the Zymo Research Microbiome Minikit; and for soil samples, the DNeasy PowerSoil Pro Kit (Qiagen). In all cases, the manufacturer's instructions were followed. High-quality DNA was sent to AIM—Advanced Identification Methods GmbH (Leipzig, Germany) for sequencing services as follows: for ITS2 and 16S region amplification, we used the ITS29F/ITS24R and 515F/816R primer pairs, respectively [2]. For 16S amplification, peptide nucleic acid (PNA) clamps were used as reported by Lundberg et al., [26], which significantly may reduce chloroplast and mitochondrial contamination and increased prokaryotic reads for root endosphere and leaf endosphere. Paired end 2 × 300 bp sequencing was performed, in two independent runs for each amplicon, on an Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA). The sequences associated with this project are available in SRA Bio Project **PRJNA1168991.**

Data Processing

Prokaryotic and fungal FASTQ reads were processed independently using the DADA2 pipeline [27], which characterizes microbiome composition through unique sequence variants, known as amplicon sequence variants (ASVs). In the R software, the 16S reads from each of two independent runs were first filtered and trimmed using DADA2 with the following parameters: Run_1: truncLen = c (220,180), maxN = 0, maxEE = c (2,6), truncQ = 2 and, Run_2: truncLen = c (250,180), maxN = 0, maxEE = c (2,6), truncQ = 2. For both ITS2 runs, we used with the following parameters: maxN = 0, maxEE = c (2,6), truncQ = 2. The filtered reads for each run for both 16S and ITS2 genes were then dereplicated and denoised using DADA2 default parameters. After removing chimeras, an ASV's table of each run was constructed and then merged for each gene (i.e., using the "*mergeSequenceTables*" function from "*phyloseq*" R). Taxonomic assignments for the merged non-chimeric ASV's table were performed using the Naïve Bayesian Classifier method [28], implemented in DADA2. For the 16S sequences, we used a training dataset formatted for DADA2 classifier based on the Silva v.138 reference database [29]. For the ITS2, the assignment was made against a classifier based on the UNITE ITS2 database (ver.10.0 [30]).

Statistical Analysis

Taxa Filtering and Alpha Diversity Analysis

Results for ASV's in 16S and ITS2 amplicon pipelines (DADA2) were imported into phyloseq objects ("*phyloseq*" package) for downstream bioinformatic analysis implemented in R (R project). For the 16S amplicons, the phyloseq object was trimmed of chloroplast and mitochondrial-like sequences, low prevalence, and non-taxonomic phyla.

Total sum scaling (TSS) normalization was used for alpha diversity measurements in both 16S and ITS2 markers. TSS uses the total read counts for each sample as size factors to scale the matrix counts. To normalize the count data, the ASV read counts are divided by the total number of reads in each sample to convert the counts to proportions, and the total number of ASVs (binned fragments) in the sample is used to adjust the abundance of each ASV [31]. Alpha diversity per sample (i.e., Shannon, Inverse Simpson, Richness, and Chao-1) was calculated using the "*microbiome*" package and compared among sample type/compartment, status, and IAM. Shared and unique ITS2 and 16S ASVs among sample type, IAM levels, and status were calculated and visualized with a Venn diagram using the "*ps_venn*" function of the "*MicEco*" package.

Correlation of Alpha Diversity with Bioclimatic Variation

To test the relationships between alpha diversity (i.e., Shannon diversity index) for both the whole microbiome and each compartment (i.e., leaf endosphere, root endosphere, phyllosphere, and rhizosphere) and the climatic variation, data for 19 bioclimatic raster layers available in WorldClim 2.1 [32] for current conditions (1970–2000) with 30 arcsecond resolution (available at: https://www.worldclim.org) were obtained for each collection site. A variance inflation factor, VIF [33], was applied to select the minimum redundant bioclimatic layers (variables with VIF < 10 were included). The resulting variables (i.e., bio13, bio9, bio3, bio2) after the VIF analysis were used to construct additive linear regression models against the Shannon diversity index using the entire dataset and for each compartment through the "*stats*"

package in R. In addition, we constructed a fully reduced model using AIC-based stepwise regression ("*stepAIC*," "*MASS*" packages) to determine the most parsimonious model explaining the relationships between alpha diversity (i.e., Shannon diversity) and bioclimatic variation. Microbial community composition analyses for key microbial players were plotted using the average relative abundance of each ASV within each taxonomic level for plant compartment, Status, and IAM communities using the "*MicrobiotaProcess*" library R software.

Beta Diversity Analysis

Phylogenetically based unweighted *UniFrac* distances were calculated using the "*vegdist*" function of the "*vegan*" package for beta diversity analysis. Two-dimensional nonmetric multidimensional scaling (NMDS) was performed using the "*phyloseq*," "*vegan*," and "*ggplot2*" packages, including sample type, status, and IAM. Statistical significance of differences in beta diversity between plant compartment, status, and IAM was tested using permutational multivariate analysis of variance (PERMANOVA). PERMANOVA's were also performed per compartment to test for differences in status and IAM levels ($p < 0.05$). PERMANOVA's were performed using the "*adonis*" function of the R "*vegan*" package. Additionally, the correlation between geographic distances and bioclimatic variables (i.e., bio13, bio9, bio3, bio2) and prokaryotic and fungal community composition (i.e., Unweighted UniFrac distances sample/site means) was analyzed by Mantel tests (permutations = 9999), for the whole community and for each compartment.

Differential Analysis of Microbiome Compositions with Bias Correction

Differential abundance (DA) analysis was performed using microbiome composition analysis with bias correction ("*ANCOM-BC*" package) at the genus level for each compartment (p and false discovery rate (FDR) < 0.05). Significance comparison between management statuses was performed using log fold changes (LFC); only leaf endosphere were excluded due the low number of ASV's to compare among statuses (Table S3).

Results

For the 16S gene, a total of 3,654,741 high quality reads were retained, clustering into 8,214 ASVs, after filtering and removal of mitochondrial and chloroplast-like sequences. On the other hand, the ITS2 data yielded a total of 2,073,457 high quality reads retained after filtering, clustered into 7,459 ASVs. The Venn diagram showed a core of 100 ASVs shared among the type compartments for 16S data, whereas the endosphere and episphere harbored 1,291 and 4,837 ASVs, respectively (Fig. S1), while the soil harbored 1,132 exclusive ASVs. By Status, a core of 457 ASVs was found, while wild and traditional Status harbored 3,973 and 2,013 ASVs, respectively (Fig. S1), while conventional Status harbored 964 exclusive ASVs. The IAM classification showed a core of 164 ASVs, while Semiarid Mediterranean (SAM) and Humid (HUM) harbored the highest number of exclusive ASVs (2,630 and 190, respectively; Fig. S1). For ITS2, no ASVs were shared among compartments (i.e., endosphere, episphere, and soil, Fig. S2), the highest number of exclusive ASVs were found in the endosphere (3,585) and episphere (3,256), which share less than 1% of ASVs, and soil (580), which shares ASVs only with episphere samples. By status, a core of only 6 ASVs was found, while wild and traditional status harbored 3,657and 2,458 ASVs, respectively (Fig. S2), and conventional status harbored 1,240 exclusive ASVs. The IAM classification showed no core ASVs and less than 1% shared between IAM levels, while SH and SAM harbored the highest number of exclusive ASVs (2,730 and 2,374 ASVs, respectively, Fig. S2).

Alpha Diversity

A marked increase in alpha diversity for the 16S gene (i.e., Shannon, observed, and Chao1 indices) was found from the endosphere to the rhizosphere and soil (Fig. 2a; Table S2). For ITS2, similar alpha diversity values were found among compartments, with the leaf endosphere exhibiting the lowest alpha diversity values, while the episphere compartments showed the highest values (Fig. 2b). Overall, alpha diversity showed no significant differences between Status and IAM for the entire dataset for both 16S and ITS2 markers (Fig. 3). However, at the compartment level for the 16S gene, significant differences between Status and IAM were found for phyllosphere Shannon diversity ($X^2 = 6.55$, $p = 0.03$; Fig. 3a and b). For ITS2 markers, significant differences were observed at IAM for the root endosphere ($X^2 = 10.81$, $p = 0.01$; Fig. 3d) and the rhizosphere ($X^2 = 9.25$, $p = 0.02$; Fig. 4d).

Relative Abundance Taxa

Among the different samples, 32 distinct prokaryotic phyla were identified in *Agave angustifolia* samples. Proteobacteria, Actinobacteria, and Firmicutes dominated, comprising up to 80% of the microbial community across different Status and IAM (Fig. S3 and S4). These phyla were especially prevalent in endosphere samples. In contrast, episphere and soil samples also exhibited high abundances of Bacteroidota, Crenarchaeota, and Chloroflexi (Fig. S10 and S11). At the order level, Burkholderiales, Enterobacterales,

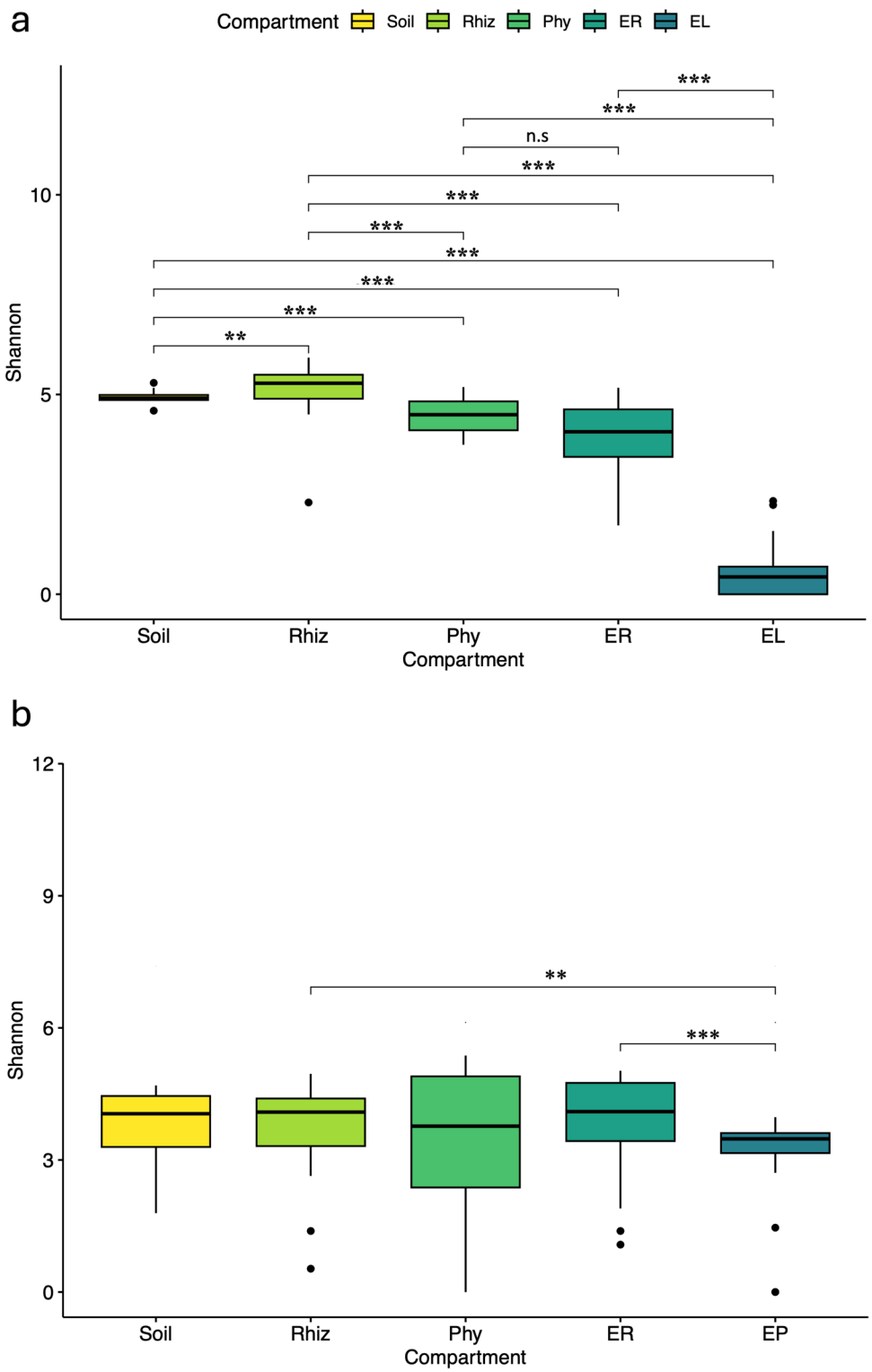

Fig. 2 Shannon diversity index among different plant compartments and soil for **a** prokaryotic and **b** fungal communities (**b** and **c**) associated with *A. angustifolia*. Significant differences (X^2 *Kruskal–Wallis*): ***$p = 0$; **$p < 0.001$. Only significant differences are shown

Rhizobiales and Pseudonocardiales dominated with up to 50% abundance between Status and IAM, while among compartments, Burkholderiales showed high prevalence in leaf compartments (leaf endosphere and phyllosphere), while Enterobacterales, Burkholderiales, Rhizobiales, Pseudonocardiales, Micrococcales, Pseudomonadales dominated in high relative abundance for root compartments (root endosphere and rhizosphere) and soil (Fig. S5 and S6). At the phyllosphere, a marked reduction at conventional status was found for Rhizobiales ($X^2 = 5.46$, $p = 0.04$) with a

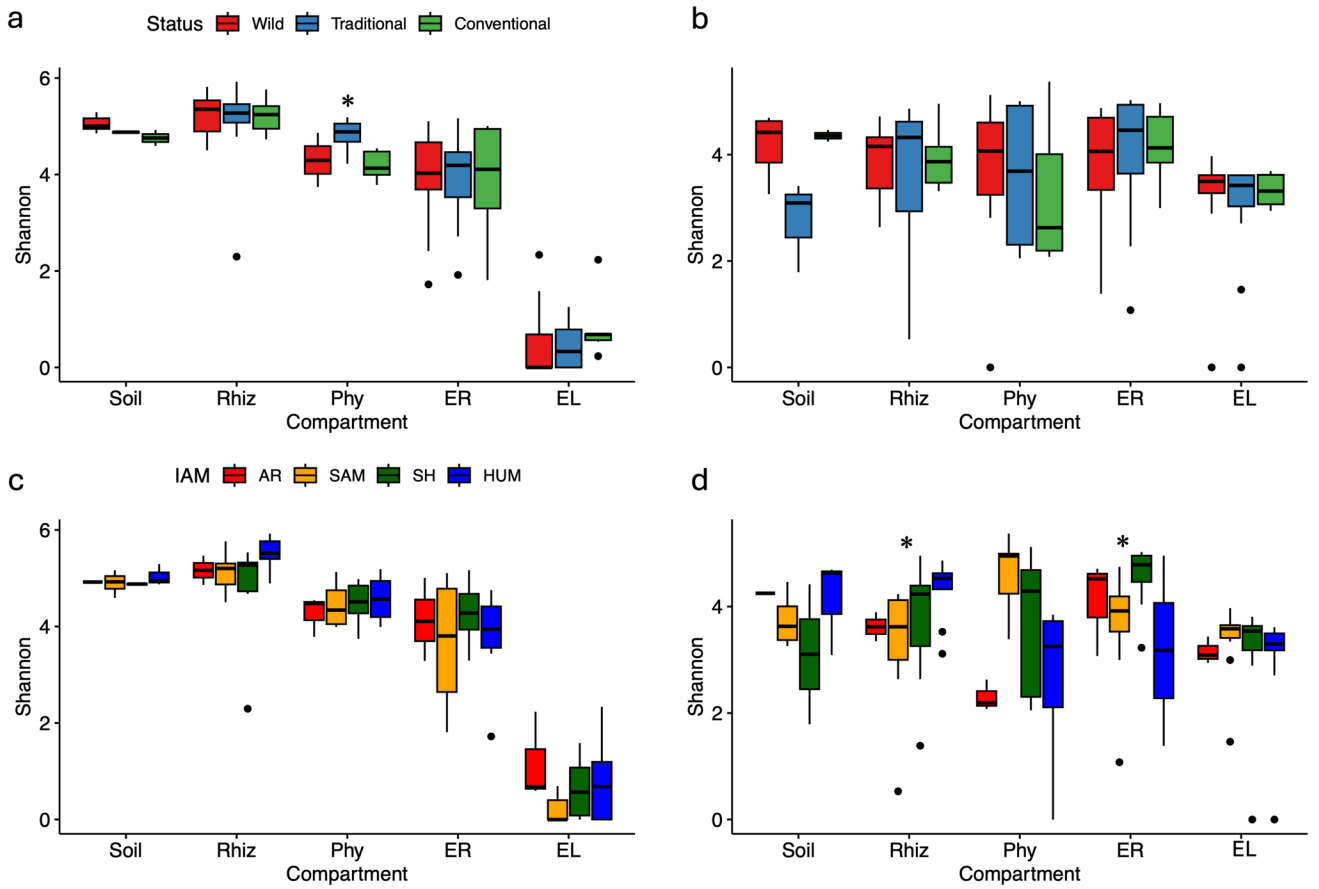

Fig. 3 Shannon diversity index in the prokaryotic (**a** and **c**) and fungal communities (**b** and **d**) associated with the management status and De Martonne aridity index (IAM) for *Agave angustifolia* populations from across Mexico, respectively. *Significant differences (X^2 *Kruskal–Wallis*) at compartment level for each status and IAM level are shown. AR, arid; SAM, semiarid Mediterranean; SH, subhumid; HUM, humid

marked increase in Lactobacillales ($X^2 = 6.28$, $p = 0.04$); for the rhizosphere, an increase in cultivated status was found for Rhizobiales ($X^2 = 7.152$, $p = 0.02$) while Bacillales ($X^2 = 5.49$, $p = 0.04$; Fig. S5), showed a substantial reduction. For IAM, at rhizosphere an increase in humid sites for Micrococcales ($X^2 = 15.99$, $p = 0.001$), Enterobacterales ($X^2 = 14.88$, $p = 0.001$), Bacillales ($X^2 = 9.70$, $p = 0.02$) was observed. For root endosphere, Enterobacterales ($X^2 = 10.70$, $p = 0.01$) increased in aridity, while Pseudomonales increase slightly in humid and semi-arid sites ($X^2 = 9.71$, $p = 0.02$; Fig. S6).

For ITS2 data, 11 different fungal phyla were found across *A. angustifolia* samples, with Ascomycota and Basidiomycota dominating with up to 90% relative abundance across Status and IAM (Fig. S11). Among the Status and IAM samples, the order Ascomycota Incertae sedis dominated, comprising up to 50%, followed by Pleosporales, Capnodiales, Eurotiales, and Hypocreales, which together accounted for up to 30% (Fig. S12). Across different compartments, from leaf endosphere to soil, the relative abundance of Ascomycota Incertae sedis decreased, while Pleosporales, Capnodiales, Eurotiales, Hypocreales, and Agaricales increased in relative abundance (Fig. S12).

At compartment level, only for soil the orders Pleosporales ($X^2 = 6.23$, $p = 0.04$) and Agaricales ($X^2 = 5.87$, $p = 0.04$) showed an increase in cultivated populations (Fig. S8). Otherwise, for IAM, Pleosporales ($X^2 = 9.41$, $p = 0.02$) and Xylariales ($X^2 = 10.31$, $p = 0.01$), displayed higher prevalence in semiarid zones while decreasing in humid and sub-humid sites. For root endosphere, an increase in prevalence for Pleosporales ($X^2 = 12.54$, $p = 0.005$) and Hypocreales was found in subhumid sites (Fig. S9).

Correlation of Alpha Diversity and Bioclimatic Variables

The results of the linear model including the bioclimatic variables resulting from the VIF and the Shannon diversity index for the ITS2, showed negative correlations among the root endosphere Shannon diversity index and the bio13

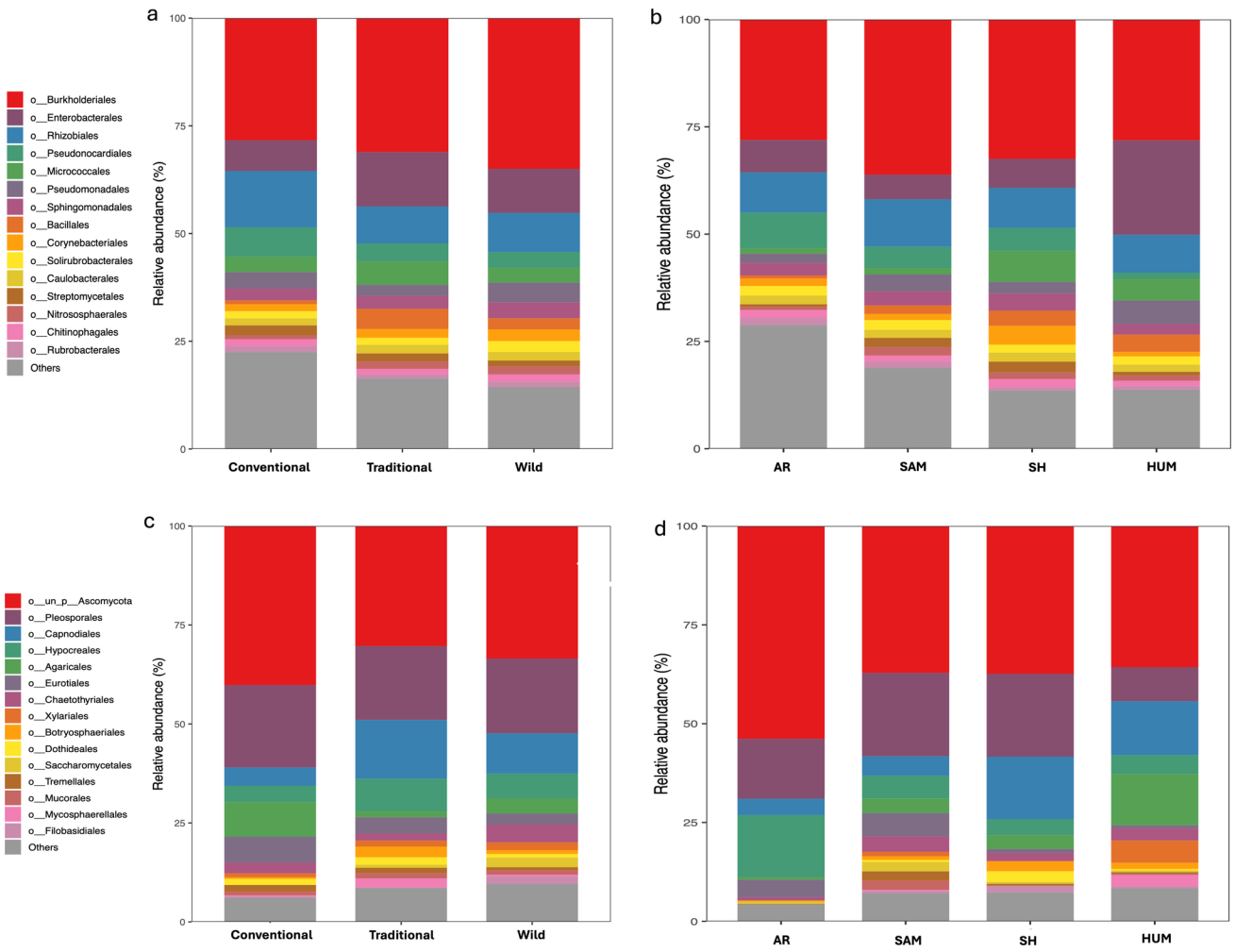

Fig. 4 Order-level relative abundance plots of global microbiome for prokaryotic taxa for Status (**a**) and IAM (**b**) in samples of *A. angustifolia* from across Mexico. Order-level relative abundance plots of fungal taxa for status (**c**) and IAM (**d**) in samples of *A. angustifolia* from across Mexico

(Precipitation of Wettest Month; AIC = −5.27; $R = -0.48$, $p = 0.003$; Fig. 5a), as well as among the rhizosphere Shannon diversity index and the bio2 (mean diurnal range; AIC = −6.08; $R = -0.46$, $p = 0.008$; Fig. 5b). Otherwise, 16S gene showed only a positive correlation between the rhizosphere Shannon diversity index and the bio13 (Precipitation of Wettest Month; AIC = −72.13; $R = 0.38$, $p = 0.031$, Fig. 5c).

Beta Diversity Patterns Among Management Statuses and IAM

In agreement with the PERMANOVA results (Table 2), NMDS analysis showed a clear separation between the microbial communities of the plant compartments for both 16S and ITS2 markers (Fig. 6a and b). However, PERMANOVA analysis revealed no significant differences when evaluating the effect of status on beta diversity patterns for the global microbiome using the 16S marker ($R^2 = 0.013$, $p = 0.38$; see Fig. 6a) and ITS2 ($R^2 = 0.015$, $p = 0.08$; see Fig. 6b). Nevertheless, the differences in major taxa orders between management statuses (i.e., Fig. 3a and c) and the total number of exclusive ASVs found for each management status reveal underlying differences between microbiomes from different management statuses (Fig. S1 and S2). For example, significant effects of management status were found for sample type (i.e., episphere; $R^2 = 0.044$, $p = 0.04$; Table 2), for prokaryotic communities. In line, at the compartment level, the effect of management status on prokaryotic community composition showed significant differences for phyllosphere ($R^2 = 0.144$; $p = 0.01$) and rhizosphere ($R^2 = 1.188$; $p = 0.04$), consistent with the NMDS grouping by compartment (Fig. 7a, b). On the other hand, significant differences for the ITS2 marker were found at the compartment level (Table 2), where the effect of management status was significant for soil, demonstrating that agricultural

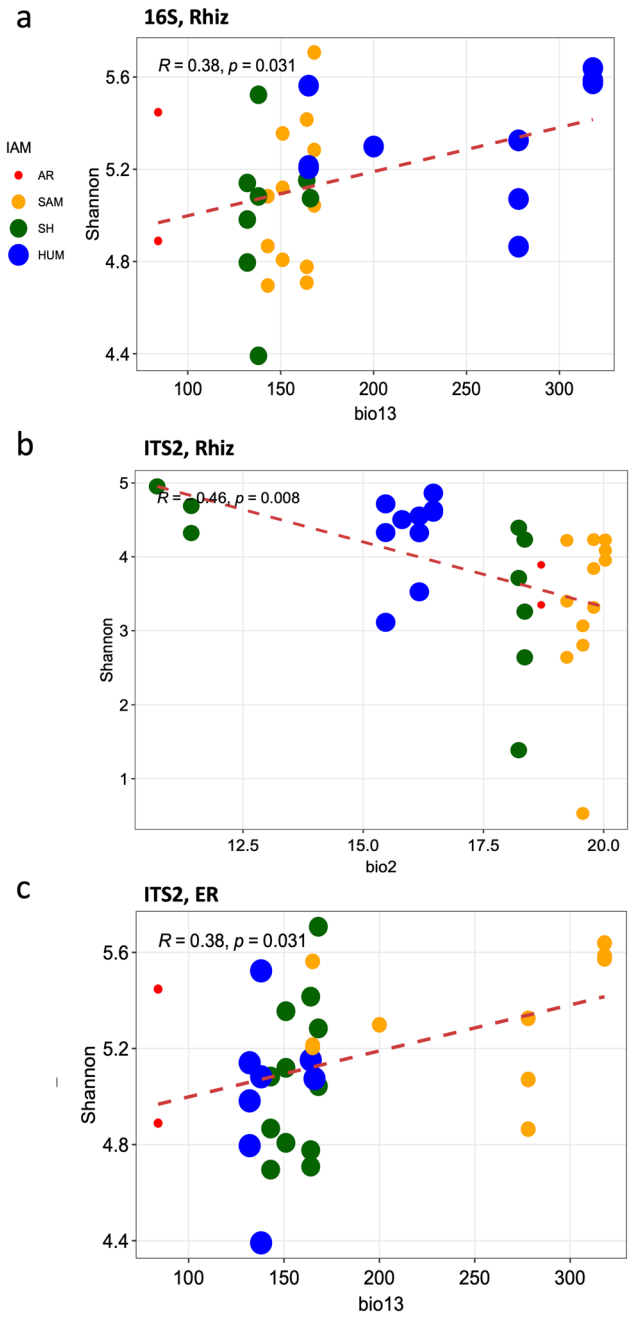

Fig. 5 Linear regression among compartment prokaryotic and fungal Shannon diversity and bioclimatic variables of the collection sites. **a** Prokaryotic rhizosphere Shannon diversity index and the Precipitation of Wettest Month (bio13), **b** fungal rhizosphere Shannon diversity and mean diurnal range (bio2), **c** fungal root endosphere Shannon diversity and Precipitation of Wettest Month (bio13)

practices significantly shape the microbiome at this scale, as shown by NMDS analyses for each compartment (Fig. 7c; Table S3).

In turn, IAM induced significant differences in prokaryotic communities for the episphere ($R^2 = 0.066$, $p = 0.002$) and endosphere compartments ($R^2 = 0.054$, $p = 0.02$; Table 2). At root compartments, IAM significantly influenced differences in prokaryotic communities (i.e., rhizosphere and root endosphere) and soil along the sampling sites of *A. angustifolia* (Table S3; Fig. S2 and S13). In addition, for the ITS2 marker, significant differences were found between IAM levels ($R^2 = 0.031$, $p = 0.007$) for global microbiome composition. Also, PERMANOVA revealed significant differences for episphere (IAM: $R^2 = 0.084$, $p = 0.0001$; IAM: compartment: $R^2 = 0.073$, $p = 0.002$; Table 2) and endosphere samples ($R^2 = 0.052$, $p = 0.02$). Interestingly, IAM differences among sampling sites showed that significant differences were found in all plant compartments (i.e., leaf endosphere, phyllosphere, root endosphere, rhizosphere), demonstrating that environmental variation is a determinant shaping both fungal and prokaryotic at compartment level microbial communities associated with *A. angustifolia* (Table S4; Fig. S14).

Correlation of Beta Diversity with Geographic and Bioclimatic Variation

The mantel test among communities' differences and geographic and bioclimatic variables, showed no differences for prokaryotic communities among unweighted UniFrac distances and geographic and bioclimatic variables. However, for ITS2 marker, positive correlations were found among unweighted UniFrac distances and geographic distances ($R = 0.395$, $p = 0.001$), and mean diurnal range (bio2) ($R = 0.133$; $p = 0.002$).

Differential Analysis of Microbiome Compositions with Bias Correction

The ANCOM-BC analysis identified bacterial and fungal taxa whose abundance was associated with different Status across various compartments. A total of 123 prokaryotic taxa were found for all interactions evaluated (Fig. 8a). For phyllosphere and soil in cultivated agaves, differential abundance for *Pantoea* (Proteobacteria) and *Pseudomonas* (Proteobacteria) (Fig. 6; Table S4) were observed. It also highlighted the differential abundance of Bacteroidetes genera in the root endosphere and rhizosphere of wild populations such as *Ohtaekwangia*, *Adhaeribacter*, and *Parasegetibacter*, *Dyadobacter*, and *Flavisolibacter* (Fig. 6; Table 3). In the cultivated rhizosphere and soil, the differential abundance of Firmicutes genera *Paenibacillus*, *Gaiella*, *Bacillus*, and *Tumebacillus* (Fig. 6; Table 3) was observed.

For fungi, an increase in differential abundance for the Ascomycota *Fusarium* and *Aspergillus*, in soils and root endosphere from cultivated populations compared to wild and traditional management (Fig. 8b). *Bisifusarium* was

Table 2 PERMANOVA analysis of the microbial communities associated with *A. angustifolia*, for the global microbiome, episphere (Rhiz, Phy) and endosphere compartments (RE, LE) considering compartment, management status and IAM. *AR*, arid; *SAM*, semiarid Mediterranean; *SH*, subhumid; *HUM*, humid

16S					ITS2				
Global		*F*	R^2	*p*	Global		*F*	R^2	*p*
Status	$Compartment_{2,122}$	13.576	0.175	**0.0001**	Status	$Compartment_{2, 125}$	8.174	0.200	**0.0001**
	$Status_{2,122}$	1.014	0.013	0.3877		$Status_{2,125}$	1.301	0.015	0.0805
	$Compartment:Status_{4, 122}$	0.986	0.025	0.4620		$Compartment:Status_{4, 125}$	1.139	0.055	0.0900
IAM	$Compartment_{2, 119}$	13.747	0.175	**0.0001**	IAM	$Compartment_{2, 119}$	7.085	0.096	**0.0001**
	$IAM_{3, 119}$	1.383	0.026	0.0617		$IAM_{3, 119}$	1.560	0.032	**0.0079**
	$IAM:Compartment_{6, 119}$	1.146	0.044	0.1528		$IAM:Compartment_{6, 119}$	1.189	0.048	0.0629
Episphere					**Episphere**				
Status	$Status_{2, 54}$	1.196	0.039	0.137	Status	$Status_{2, 47}$	1.177	0.043	0.1141
	$Compartment_{2, 54}$	9.166	0.149	**0.0001**		$Compartment_{1, 47}$	2.562	0.047	**0.0002**
	$Status:Compartment_{4, 54}$	1.367	0.044	0.049		$Status:Compartment_{2,47}$	1.141	0.042	0.1382
IAM					IAM				
	$IAM_{3,51}$	1.652	0.066	**0.0022**		$IAM_{3, 45}$	1.597	0.085	**0.0001**
	$Compartment_{2, 51}$	5.810	0.156	**0.0001**		$Compartment_{1, 45}$	2.634	0.047	**0.0001**
	$IAM:Compartment_{6, 51}$	1.165	0.094	0.0674		$IAM:Compartment_{3, 45}$	1.386	0.073	**0.0020**
Endosphere					**Endosphere**				
Status	$Status_{2, 62}$	1.101	0.022	0.298	Status	$Status_{2, 62}$	1.142	0.026	0.2302
	$Compartment_{1,62}$	29.972	0.312	**0.0001**		$Compartment_{1,62}$	18.25	0.207	**0.0001**
	$Status:Compartment_{2, 62}$	0.830	0.0173	0.617		$Status:Compartment_{2, 62}$	1.120	0.025	0.2577
IAM					IAM				
	$IAM_{3, 60}$	1.801	0.054	**0.0216**		$IAM_{3, 60}$	1.430	0.047	0.0500
	$Compartment_{1, 60}$	29.496	0.297	**0.0001**		$Compartment_{1, 60}$	18.956	0.082	**0.0001**
	$IAM:Compartment_{3, 60}$	1.448	0.044	0.0824		$IAM:Compartment_{3, 60}$	1.595	0.053	**0.0200**

found in high prevalence on root endophytes and rhizosphere in both wild and traditional samples, and Glomeromycota taxa, such as *Orientoglomus* and *Kamienskia*, showed differential abundance in soils and root compartments from wild populations compared to cultivated ones (Fig. 8b; Table S5).

Discussion

Sample Type Is the Main Factor Shaping the Diversity and Composition of Prokaryotic and Fungal Microbiomes in *A. angustifolia*

In our results, compartment is the primary factor shaping plant–microbe interactions, impacting diversity and composition more significantly than other factors such as status and IAM, in line with previous reports on agaves and cacti from arid and semiarid areas [2–4].This appear to strongly stabilize host-symbiont cooperation by isolating symbionts to reward beneficial interactions and prevent uncooperative ones [2–4, 34]. For prokaryotes, in leaf compartments a dominance of Burkholderiales, Rhizobiales, and Lactobacillales indicates diazotrophic functions, CO oxidation, nutrient availability and tolerance to drought, salinity and cold [10–12], as well as light sensing in the phyllosphere (i.e., Lactobacillales in *A. tequilana)* [13]. In contrast, for *Agaves* and cacti from warm deserts, *Cyanobacteria* dominates in phyllosphere, associated with autotrophy, biofilm formation, and water and nutrient retention [2, 13, 35, 36]. Similar alpha diversity levels were observed for the phyllosphere and rhizosphere, but rhizosphere showed a higher diversity than the surrounding soil, suggesting that root interactions improve conditions by increasing microbial richness, shaping community composition and diversity [37, 38].

Environmental Conditions Directly Affect the Diversity and Composition of Prokaryotic and Fungal Communities Along the Distribution of *A. angustifolia*

Consistent with this, we found an increase in alpha diversity in the rhizosphere that was directly associated with higher precipitation at the sampled sites (Fig. 5a). At wetter sites, the rhizosphere and root endosphere showed an increase in Micrococcales (e.g., *Pseudarthrobacter* spp.), Enterobacterales (e.g., *Enterobacter*, *Serratia*), and Bacillales (e.g., *Bacillus*,

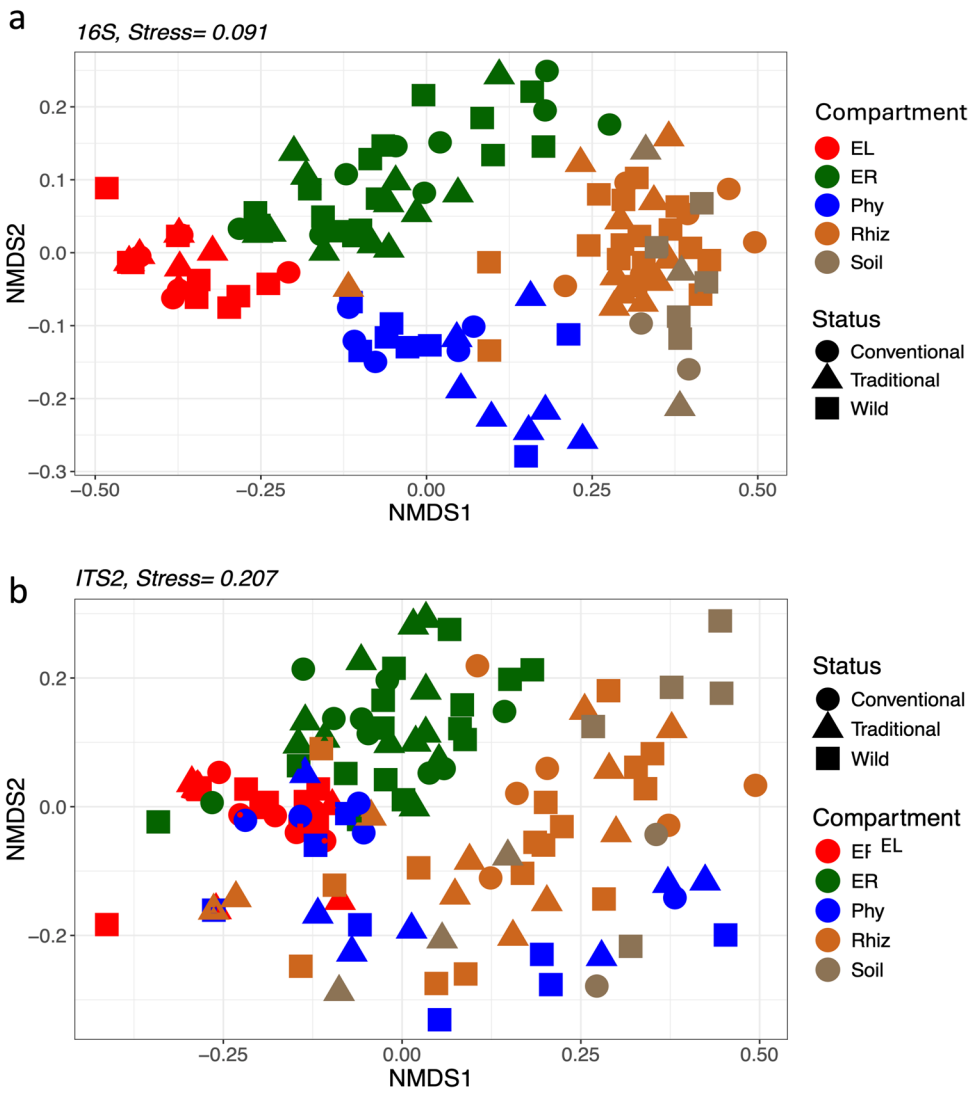

Fig. 6 Nonmetric multidimensional scaling (NMDS) plots for UniFrac distances of prokaryotic and fungal communities from *A. angustifolia* populations under different management status from across Mexico. **a** Prokaryotic communities from the different compartments according to the management status, **b** fungal communities from the different compartments according to the management status

Paenibacillus), associated with plant growth promotion, antibacterial activity, nitrogen fixation, phosphorus solubilization, antibiotic and chitinase production, and biological control [39–41]. Pseudomonadales (i.e., *Pseudomonas*) are known as plant growth enhancers and tolerance to heatwaves, floods, and salinity [42], and were enriched in the root endosphere. Water availability and microhabitat conditions directly affect the microbiome in *A. angustifolia* [38], while geographic distance does not impact the prokaryotic communities.

Otherwise, variations in bioclimate were the key factors shaping diversity and composition in the fungal microbiome or mycobiome, consistent with previous studies suggesting that biogeography influence fungal communities more than other microbial communities [43]. An increase in alpha diversity was found at root compartments in drier and hotter sites (Fig. 5b and c), particularly dominated by Pleosporales and Eurotiales. Pleosporales have protective spores against UV and dryness, enhancing plant host resilience, while Eurotiales in *O. ficus-indica* aid in phosphate solubilization as saprotrophs [14]. These traits could assist plant hosts in coping with drought and desiccation under arid conditions.

Fungal endemism may result from specific environmental conditions in different habitats, with dispersal limitation likely being the main cause of differences in fungal communities [44]. Previous studies on plants such as grasses, *Opuntia* and *Agave* have also found correlations between fungal community differences and geographic distance along environmental gradients [2, 3, 45]. Our results from the Mantel test demonstrated a clear influence of geographic distance and temperature patterns on global fungal communities, like findings in *O. ficus-indica*, where these factors significantly affected composition but not alpha diversity levels [3]. This suggests that spatial distance and environmental differences play a significant role in shaping fungal microbial communities, as seen in studies on *A. tequilana* and wild agaves from North American arid zones [2].

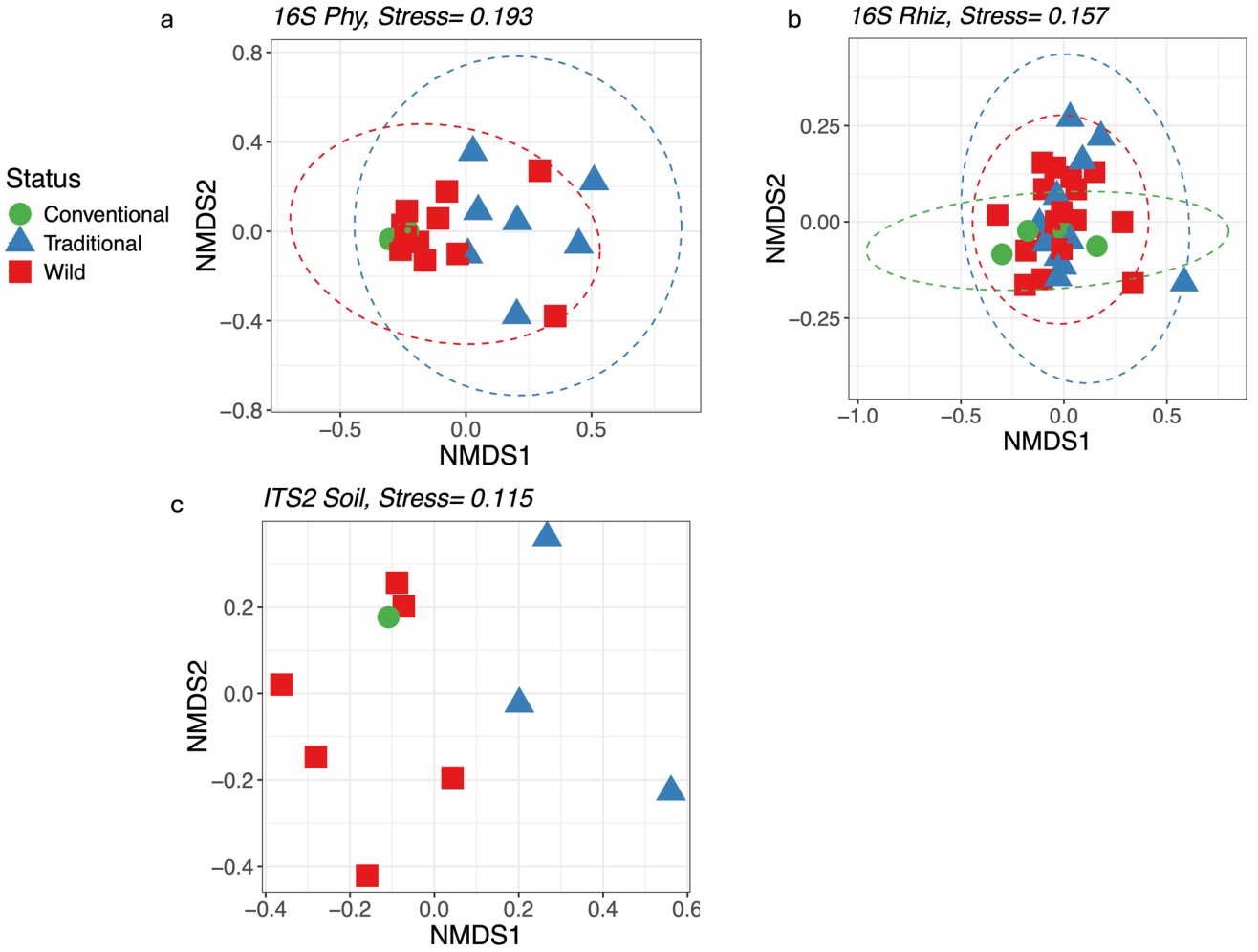

Fig. 7 Nonmetric multidimensional scaling (NMDS) plots for UniFrac distances of microbial communities at compartment level from *A. angustifolia* populations under different management status: prokaryotic communities: **a** phyllosphere; **b** rhizosphere; fungal communities: **c** soil

Management Status Influences the Diversity and Composition of the Microbiome at the Compartment Level in *A. angustifolia*

Although a decrease in microbial richness is not often found in cultivated plants compared to their wild relatives [46], a slight increase in prokaryotic alpha diversity was found for the phyllosphere under traditional management (Fig. 3). In managed crops, changes in microbiome diversity and composition are often associated with morphological and physiological changes in managed genotypes, but most reported for root compartments [6, 7, 9, 47]. In *A. tequilana*, a drastic reduction in phyllosphere alpha diversity compared to wild species was observed due to the dominance of a few prokaryotic families [2]. In our results, a slight reduction in conventional management was only found for Rhizobiales and Lactobacillales (Fig. S5). In *A. tequilana* monocultures, only the *Azul* variety is utilized for tequila production and is promoted via clones, disregarding the presence of 10 other varieties [20, 48]. This leads to lower genetic diversity compared to *A. angustifolia* [22, 48]. Instead, traditional management of *A. angustifolia* involves obtaining seedlings from vegetative propagules and seeds from different sources [22], as shown for populations used for Bacanora spirit production in Sonora, with an incipient history of cultivation and management, for which low genetic structure and high genetic diversity have been found [49]. Overall, these conditions can alter morphological and physiological variation and consequently affect microbial diversity. Similar findings were observed in the rhizosphere of maize [50, 51], potatoes [52], and barley [53], where alpha and beta diversity levels were directly associated with the analyzed genotypes. Moreover, in *Agave*, clonal propagation may influence microbial communities when passed down to offshoots, affecting microbial richness based on management practices [2].

While no differences were found in the global microbiome composition among management, differences at phyllosphere and rhizosphere for prokaryotes soil for fungi

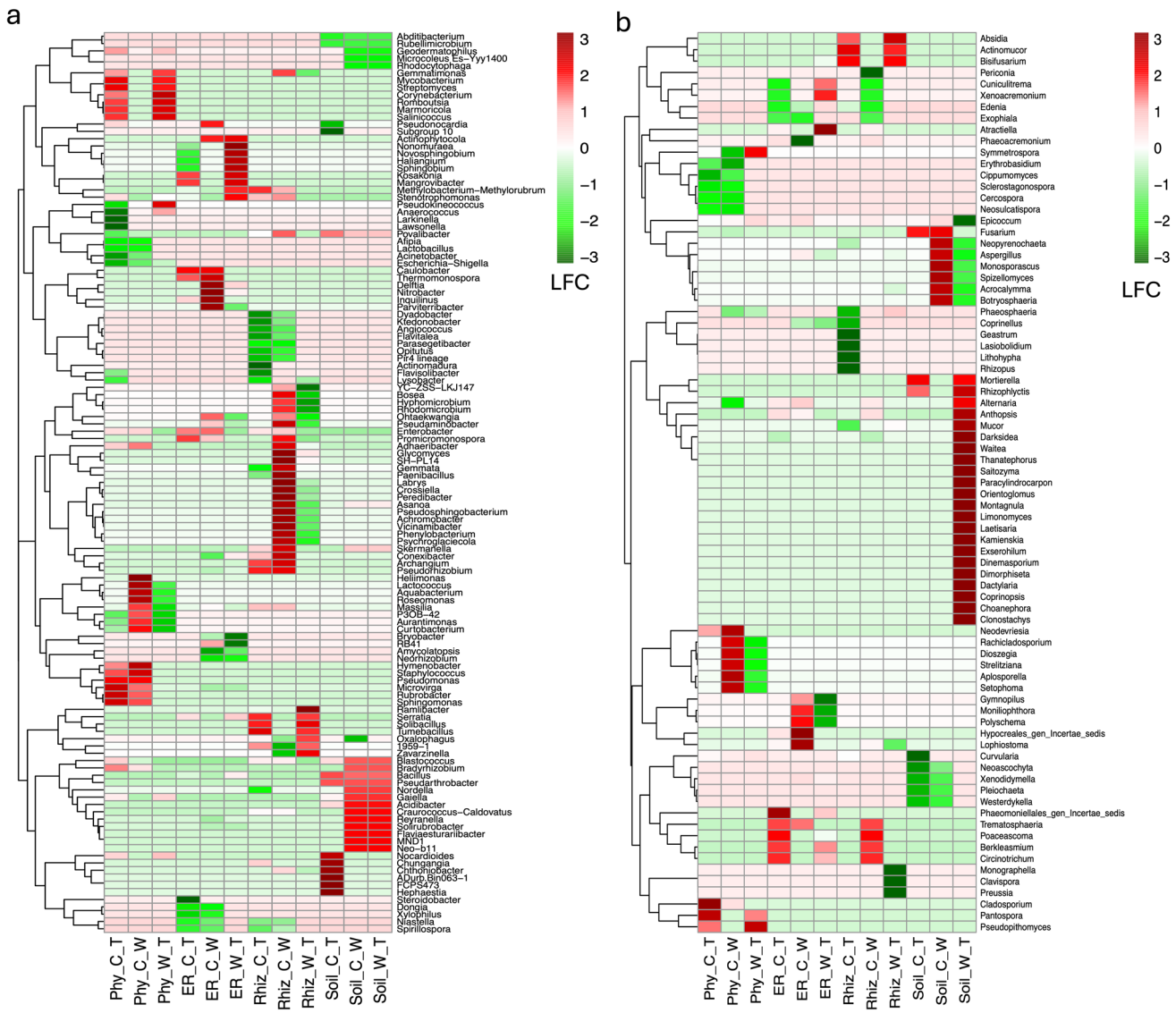

Fig. 8 ANCOM-BC differential abundance analysis to determine significant log fold changes (LFC) differences (FDR < 0.05) for **a** prokaryotic and **b** fungal genus at compartment level comparing different management statuses: Phy, phyllosphere; ER, root endosphere, Rhiz, rhizosphere; soil, soil. C, conventional; T, traditional; W, wild. The letter for each code indicates the statuses being compared. The first letter of each code represents the positive LFC

were found (Fig. 7), exhibiting the effect of management practices at the compartment level. Plant roots in species under selection showed an increase in Actinobacteria and Proteobacteria phyla, while Bacteroidetes decreased compared to their wild relatives [7, 47, 54, 55]. A decrease in Bacteroidetes prevalence was observed in the phyllosphere of cultivated populations. However, there were differential abundances of Bacteroidetes genera in the rhizosphere of wild populations, including *Ohtaekwangia*, *Adhaeribacter*, and *Parasegetibacter*, which are associated with plant growth promotion, phosphomonoesterase activities, phosphate and potassium dissolution, and nitrogen fixation [56–58]. Also, functions as synthesis of polysaccharide modifying enzymes, plant cell wall degrading enzymes, nitrogen fixation and soil pathogen protection as *Chitinophaga* and *Dyadobacter* [15–17] and *Flavisolibacter* which colonizes roots effectively, can contribute to biofertilization, biostimulation, and biocontrol activities, helping to limit the growth of *Fusarium oxysporum*. Interestingly, in the episphere of *A. tequilana*, a depletion of genes involved in carbon metabolism, secondary metabolite synthesis, and xenobiotic degradation was found, associated with the loss of microbes involved in these functions [13]. The functional impacts of changes in bacterial community composition due to domestication are not well understood and could be linked to compartment architecture.

Bacteroidetes, known for digesting complex polysaccharides in wild plants and promoting plant growth in harsh environments, might play a role in this context [6, 47]. Also, in the human and mice gut microbiome, abundance of Bacteroidetes has been associated with low carbohydrate diets [18]. This association may lead to healthier evolution for plants, as shown for animals, and whether its increase in abundance on the roots of wild relatives is a signature of coevolution remains speculative.

Otherwise, at the cultivated rhizosphere differential abundance of bacterial general related with nitrogen metabolism as *Paenibacillus* and *Gaiella*, production of IAA and L-tryptophan and growth promoters as *Nocardioides*, *Bacillus*, and *Tumebacillus*, previously reported to be recruited at root cucumber some help with N fixation [41]. Also, markedly differential abundance of *Pseudomonas,* was found in cultivated phyllosphere which previously was isolated from *A. palmeri* and has been recognized as growth promoter and widely used as bio stimulant [59]. A differential prevalence of N-fixing bacteria in cultivated agaves could be due to the regular application of nitrogen fertilizer inputs, which are identified to promote intense bacterial competencies as well as to increase the complexity of interaction networks and the depletion of ammonia-oxidizing archaea [60]. On the other hand, in the phyllosphere and soil in cultivated agaves differential abundance for *Pantoea*, frequently associated with the "*soft rot disease*," and in *A. tequilana* for which represents substantial annual losses [2] and reported for *A. angustifolia* [61, 62]. In terms of fungi, surprisingly, an increase in differential abundance for the Ascomycota *Fusarium* and *Aspergillus*, in soils from cultivated populations in comparison to wild and traditional managements was found (Fig. 8b). In *A. tequilana*, the soil-borne pathogens *F. xysporum* and *F. solani* have been identified as principal causing agent of the "*agave vascular wilt*" [63]. Otherwise, *Aspergillus* a saprotroph related with phosphate solubilizing functions in *O. ficus- indica* [64], but in *A. sisalana* has been identified as the main causal agent of "*bole rot*" [65]. Instead, *Bisifusarium* was found in high prevalence as root endophyte in both wild and traditional samples and previously reported as the causal agent of cladode "*soft rot*" in *Opuntia ficus-indica* [64] and *Nopalea cochenillifera* [66]. The differential abundance of specific fungal and prokaryotic pathogens in compartments and cultivated soils could be a response to conventional management practices in monoculture agaves, which promote high genetic homogeneity, allowing pathogens to develop strategies to evade plant defenses, as reported for prokaryotic communities in *A. tequilana* [2]. Surprisingly, even some Glomeromycota taxa, such as *Orientoglomus* and *Kamienskia*, showed differential abundance in the soils of wild populations compared to cultivated populations, a trend similar to that reported for *A. tequilana*, for which mycorrhizal taxa were absent from all samples, including soils [2]. The lack of mycorrhizal association has been linked to anthropogenic disturbances due to the introduction of non-native flora, such as monoculture agricultural practices [44], manifesting the effects of conventional agriculture on agave fungal communities.

Management Practices and Microbial Communities in *A. angustifolia* Cultivation

Our findings highlight the profound impact of agricultural management practices on the diversity and composition of microbial communities in *A. angustifolia*. Traditional management practices, characterized by the use of seeds and vegetative propagules from diverse sources, sustain higher genetic and microbial diversity compared to conventional monoculture systems reliant on clonal propagation. These practices not only preserve microbial richness but also support beneficial functional traits within the microbiome, crucial for nutrient cycling, plant growth promotion, and pathogen resistance. In contrast, conventional systems show a marked reduction in microbial diversity, particularly in compartments such as the phyllosphere and rhizosphere, with potential functional implications. For instance, the depletion of Bacteroidetes, a group linked to polysaccharide digestion and plant growth under harsh conditions, may compromise plant resilience. Similarly, the prevalence of pathogens such as *Pantoea*, *Fusarium*, and *Aspergillus* in conventional systems underscores the vulnerabilities introduced by genetic homogeneity and management intensification.

The application of nitrogen fertilizers in conventional systems further alters microbial interactions, favoring nitrogen-fixing while disrupting ammonia-oxidizing archaea and promoting competitive microbial dynamics [60]. In contrast, traditional practices appear to recruit beneficial taxa, associated with biofertilization and pathogen suppression.

Management practices must prioritize strategies that foster microbial diversity and functionality across compartments. Since it has been demonstrated that the conversion of native vegetation to cropland promotes both changes in soil physicochemical properties (i.e., reductions in carbon storage, nutrient cycling, organic matter decomposition) and the depletion of beneficial fungal communities, while pathogenic increase in dominance as conventional agricultural practices are accentuated [67]. For example, reduced nitrogen inputs and the integration of biofertilizers could mitigate the negative impacts of conventional practices. Indeed, application of synthetic communities or SynComs designed based on the observed microbe-microbe interactions from wild agaves can increase microbial diversity and plant health and productivity in the cultivated *A. tequilana*, confirming that the microbiome of wild plants can be used for the benefit of the cultivated species. Additionally,

promoting crop diversity through the cultivation of multiple agave varieties or intercropping can reduce pathogen pressure and enhance overall system resilience. For example, the maintenance of positive interactions among nurse and facilitator species in Agave mescal-producing systems are crucial to maintain phylogenetic diversity in these ecosystems, [68, 69] which, in the long-term, could maintain the "*eubiosis*" of the ecosystem (*i.e.,* by preserving the beneficial microbial communities). Therefore, the restoration of mycorrhizal associations, disrupted by monoculture practices, is critical for plant and soil health [23]. Mycorrhizal taxa, such as *Orientoglomus* and *Kamienskia*, play essential roles in nutrient acquisition and ecosystem stability. Thus, agroecological interventions, including organic amendments and reduced tillage, can help restore these beneficial relationships.

Concluding Remarks

Overall, we found substantial evidence that the microbiome of *A. angustifolia* is shaped by environmental and management factors. At the environmental level, we found patterns previously described in agave species and plants from arid and semi-arid zones: prokaryotic communities are determined by humidity and temperature patterns, while fungal communities respond to biogeographic features that shape composition, possibly due to their lower dispersion. On the other hand, at the compartment level, there is evidence of genotype/phenotype selection in agaves, evidenced at the prokaryote level by a significant decrease of taxa of the phylum Bacteroidota, associated with holobiont health. At the fungal community level, Glomeromycota taxa are absent in soils from conventionally managed sites, manifesting the negative effect of pesticide use. Despite their incipient level of domestication, variations in the abundance and composition of the microbiome in the different plant compartments evidences a trend comparable to that described in species that have been under long periods of selection. The transition from intensive monoculture systems to diversified, traditional or agroecological approaches offers a pathway to enhance microbial diversity and functionality, ensuring more sustainable and resilient agave cultivation systems. Future studies should further explore the functional implications of microbial shifts and identify scalable practices for integrating microbiome management into agricultural systems.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-025-02496-2.

Acknowledgements We are deeply grateful to the traditional mezcal producers who generously shared their knowledge and work with us; this research would not have been possible without their support. We also thank Jimena Jazmín Hurtado Olvera for her assistance with laboratory and bioinformatic analyses, and Piero Foncerrada Elizondo for his support with bioinformatic analyses. Gonzalo Contreras acknowledges CONAHCYT for the postdoctoral fellowship Estancias Posdoctorales por México 2022(1), CV549242.

Author Contributions GCN: Conceptualisation, Data acquisition and curation, Investigation, Writing – original draft, Writing – review & editing, Visualisation. AVB: Formal analysis, Resources, Writing – review & editing, Funding acquisition. FMF: Data acquisition and curation, Resources, Writing – review & editing. LPPM: Formal analysis, Resources, Writing – review & editing, Funding acquisition. AHL: Conceptualisation, Data curation, Investigation, Resources, Writing – original draft, Writing – review & editing, Funding acquisition, Visualisation.

Funding This work was funded by the CONAHCYT—PRONACES grant 319061 awarded to AVB, AHL, and LPPM.

Data Availability The raw sequencing data generated during this study are available in the NCBI Sequence Read Archive (SRA) under BioProject ID PRJNA1168991. All relevant data, including metadata and analysis scripts, will be made available upon reasonable request.

Declarations

Competing Interest The authors declare no competing interests.

References

1. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663. https://doi.org/10.1111/1574-6976.12028
2. Coleman-Derr D, Desgarennes D, Fonseca-Garcia C et al (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. New Phytol 209:798–811. https://doi.org/10.1111/nph.13697
3. Gargouri M, Karray F, Chebaane A et al (2021) Increasing aridity shapes beta diversity and the network dynamics of the belowground fungal microbiome associated with *Opuntia ficus-indica*. Sci Total Environ 773:145008. https://doi.org/10.1016/j.scitotenv.2021.145008
4. Karray F, Gargouri M, Chebaane A et al (2020) Climatic aridity gradient modulates the diversity of the rhizosphere and endosphere bacterial microbiomes of *Opuntia ficus-indica*. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.01622
5. Wei G, Ning K, Zhang G et al (2021) Compartment niche shapes the assembly and network of *Cannabis sativa*-associated microbiome. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.714993

6. Martínez-Romero E, Aguirre-Noyola JL, Taco-Taype N et al (2020) Plant microbiota modified by plant domestication. Syst Appl Microbiol 43. https://doi.org/10.1016/j.syapm.2020.126106
7. Pérez-Jaramillo JE, Mendes R, Raaijmakers JM (2016) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol 90:635–644. https://doi.org/10.1007/s11103-015-0337-7
8. Soldan R, Fusi M, Cardinale M et al (2021) The effect of plant domestication on host control of the microbiota. Commun Biol 4:1–9. https://doi.org/10.1038/s42003-021-02467-6
9. Yue H, Yue W, Jiao S et al (2023) Plant domestication shapes rhizosphere microbiome assembly and metabolic functions. Microbiome 11:1–19. https://doi.org/10.1186/s40168-023-01513-1
10. Pal G, Saxena S, Kumar K et al (2022) Endophytic *Burkholderia*: multifunctional roles in plant growth promotion and stress tolerance. Microbiol Res 265:127201. https://doi.org/10.1016/j.micres.2022.127201
11. Bolívar-Anillo HJ, Contreras-Zentella ML, Teherán-Sierra LG (2016) *Burkholderia tropica* una bacteria con gran potencial para su uso en la agricultura. TIP 19:102–108. https://doi.org/10.1016/j.recqb.2016.06.003
12. Palmer JL, Hilton S, Picot E et al (2021) Tree phyllospheres are a habitat for diverse populations of CO-oxidizing bacteria. Environ Microbiol 23:6309–6327. https://doi.org/10.1111/1462-2920.15770
13. Flores-Núñez VM, Fonseca-García C, Desgarennes D et al (2020) Functional signatures of the epiphytic prokaryotic microbiome of agaves and cacti. Front Microbiol 10:1–13. https://doi.org/10.3389/fmicb.2019.03044
14. Sterflinger K, Tesei D, Zakharova K (2012) Fungi in hot and cold deserts with particular reference to microcolonial fungi. Fungal Ecol 5:453–462. https://doi.org/10.1016/j.funeco.2011.12.007
15. Astafyeva Y, Gurschke M, Qi M et al (2022) Microalgae and bacteria interaction—evidence for division of diligence in the alga microbiota. Microbiol Spectr 10. https://doi.org/10.1128/spectrum.00633-22
16. DiLegge MJ, Manter DK, Vivanco JM (2022) Soil microbiome disruption reveals specific and general plant-bacterial relationships in three agroecosystem soils. PLoS One 17:1–24. https://doi.org/10.1371/journal.pone.0277529
17. Funnicelli MIG, Pinheiro DG, Gomes-Pepe ES et al (2021) Metagenome-assembled genome of a *Chitinophaga* sp. and its potential in plant biomass degradation, as well of affiliated *Pandoraea* and *Labrys* species. World J Microbiol Biotechnol 37:1–17. https://doi.org/10.1007/s11274-021-03128-w
18. Brown K, DeCoffe D, Molcan E, Gibson DL (2012) Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 4:1095–1119. https://doi.org/10.3390/NU4081095
19. García Mendoza A, Colunga-Garcia Marin P, Bye BR (1993) Los usos del Agave angustifolia Haw., ancestro silvestre del henequén en su área de distribución geográfica. Bol Soc Bot Méx 62:109–128. https://doi.org/10.13140/RG.2.1.4602.3200
20. Colunga-GarcíaMarín P, Zizumbo-Villarreal D (2007) El tequila y otros mezcales del centro-occidente de México : domesticación, diversidad y conservación de germoplasma. In: Colunga-García Marín P, Larqué Saavedra LE y DZ-VA (eds) En lo ancestral hay futuro: del tequila, los mezcales y otros agaves. Centro de Investigación Científica de Yucatán, Merida, México, Centro de Investigación Científica de Yucatán, A.C.,Mérida, Yucatán, Mexico, pp 113–131
21. Colunga-GarcíaMarín P, Zizumbo-Villarreal D, Torres I et al (2017) Los agaves y las prácticas mesoamericanas de aprovechamiento, manejo y domesticación. In: Domesticación en el Continente Americano vol. 2, Investigación para el manejo sustentable de recursos genéticos en el Nuevo. Mundo Universidad Nacional Autónoma de México, Mexico City, Mexico, pp 273–309
22. Cabrera-Toledo D, Mendoza-Galindo E, Larranaga N et al (2022) Genomic and morphological differentiation of spirit producing *Agave angustifolia* traditional landraces cultivated in Jalisco, Mexico. Plants 11. https://doi.org/10.3390/plants11172274
23. Chávez-González JD, Flores-Núñez VM, Merino-Espinoza IU, Partida-Martínez LP (2024) Desert plants, arbuscular mycorrhizal fungi and associated bacteria: exploring the diversity and role of symbiosis under drought. Environmental Microbiology Reports 16. https://doi.org/10.1111/1758-2229.13300
24. Flores-Núñez VM, Camarena-Pozos DA, Chávez-González JD et al (2023) Synthetic communities increase microbial diversity and productivity of *Agave tequilana* plants in the field. Phytobiomes J 7:435–448. https://doi.org/10.1094/PBIOMES-01-23-0001-R
25. Rahimi J, Ebrahimpour M, Khalili A (2013) Spatial changes of Extended De Martonne climatic zones affected by climate change in Iran. Theor Appl Climatol 112:409–418. https://doi.org/10.1007/s00704-012-0741-8
26. Lundberg DS, Yourstone S, Mieczkowski P et al (2013) Practical innovations for high-throughput amplicon sequencing. Nat Methods 10:999–1002. https://doi.org/10.1038/nmeth.2634
27. Callahan BJ, McMurdie PJ, Rosen MJ et al (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869
28. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM.00062-07
29. Quast C, Pruesse E, Yilmaz P et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:590–596. https://doi.org/10.1093/nar/gks1219
30. Abarenkov K, Nilsson RH, Larsson KH et al (2024) The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. Nucleic Acids Res 52:D791–D797. https://doi.org/10.1093/nar/gkad1039
31. Dillies M-A, Consortium on behalf of TFS, Rau A et al (2013) A comprehensive evaluation of normalization methods for Illumina high-throughput RNA sequencing data analysis. Brief Bioinform 14:671–683. https://doi.org/10.1093/BIB/BBS046
32. Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int J Climatol 37:4302–4315. https://doi.org/10.1002/joc.5086
33. Brauner N, Shacham M (1998) Role of range and precision of the independent variable in regression of data. AIChE J 44:603–611. https://doi.org/10.1002/aic.690440311
34. Chomicki G, Werner GDA, West SA, Kiers ET (2020) Compartmentalization drives the evolution of symbiotic cooperation. Philos Trans R Soc B: Biol Sci 375. https://doi.org/10.1098/rstb.2019.0602
35. Fonseca-García C, Coleman-Derr D, Garrido E et al (2016) The cacti microbiome: interplay between habitat-filtering and host-specificity. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.00150
36. Marasco R, Mosqueira MJ, Cherif A, Daffonchio D (2022) Diversity and plant growth-promoting properties of microbiomes associated with plants in desert soils. In: Ramond JB., Cowan DA (eds) Microbiology of hot deserts. ecological studies, vol 244. Springer, Cham. https://doi.org/10.1007/978-3-030-98415-1_8
37. Desgarennes D, Garrido E, Torres-Gomez MJ et al (2014) Diazotrophic potential among bacterial communities associated with wild and cultivated *Agave* species. FEMS Microbiol Ecol 90:844–857. https://doi.org/10.1111/1574-6941.12438

38. López-Lozano NE, Echeverría Molinar A, Ortiz Durán EA et al (2020) Bacterial diversity and interaction networks of *Agave lechuguilla* rhizosphere differ significantly from bulk soil in the oligotrophic basin of Cuatro Cienegas. Front Plant Sci 11:1–14. https://doi.org/10.3389/fpls.2020.01028
39. Ham SH, Yoon AR, Oh HE, Park YG (2022) Plant growth-promoting microorganism *Pseudarthrobacter sp*. NIBRBAC000502770 enhances the growth and flavonoid content of *Geum aleppicum*. Microorganisms 10. https://doi.org/10.3390/microorganisms10061241
40. Jha CK, Aeron A, Patel BV et al (2011) Enterobacter: role in plant growth promotion. In: Maheshwari DK (ed) Bacteria in Agrobiology: Plant Growth Responses. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 159–182
41. Li Z, Zheng Y, Li Y et al (2022) Genotype-specific recruitment of rhizosphere bacteria from sandy loam soil for growth promotion of *Cucumis sativus* var. *hardwickii*. Front Microbiol 13:1–12. https://doi.org/10.3389/fmicb.2022.910644
42. Zboralski A, Filion M (2023) *Pseudomonas spp*. can help plants face climate change. Front Microbiol 14:1–13. https://doi.org/10.3389/fmicb.2023.1198131
43. Jumpponen A, Herrera J, Porras-Alfaro A, Rudgers J (2017) Biogeography of root-associated fungal endophytes. In: Tedersoo L (ed) Biogeography of Mycorrhizal Symbiosis. Springer International Publishing, Cham, pp 195–222
44. Delavaux CS, Weigelt P, Dawson W et al (2019) Mycorrhizal fungi influence global plant biogeography. Nat Ecol Evol 3:424–429. https://doi.org/10.1038/s41559-019-0823-4
45. Rudgers JA, Fox S, Porras-Alfaro A et al (2022) Biogeography of root-associated fungi in foundation grasses of North American plains. J Biogeogr 49:22–37. https://doi.org/10.1111/jbi.14260
46. Gutierrez A, Grillo MA (2022) Effects of domestication on plant-microbiome interactions. Plant Cell Physiol 63:1654–1666. https://doi.org/10.1093/pcp/pcac108
47. Pérez-Jaramillo JE, Carrión VJ, de Hollander M, Raaijmakers JM (2018) The wild side of plant microbiomes. Microbiome 6:4–9. https://doi.org/10.1186/s40168-018-0519-z
48. Mondragon KYR, Aguirre-Planter E, Gasca-Pineda J et al (2022) Conservation genomics of *Agave tequilana* Weber var. *azul*: low genetic differentiation and heterozygote excess in the tequila agave from Jalisco. Mexico PeerJ 10:1–24. https://doi.org/10.7717/peerj.14398
49. Klimova A, Mondragón KYR, Freaner FM et al (2022) Genomic analyses of wild and cultivated Bacanora *Agave* (*Agave angustifolia* var. *pacifica*) Reveal inbreeding, few signs of cultivation history and shallow population structure. Plants 11. https://doi.org/10.3390/plants11111426
50. Walters WA, Jin Z, Youngblut N et al (2018) Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proc Natl Acad Sci U S A 115:7368–7373. https://doi.org/10.1073/pnas.1800918115
51. Peiffer JA, Spor A, Koren O et al (2013) Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci U S A 110:6548–6553. https://doi.org/10.1073/pnas.1302837110
52. Inceoğlu Ö, Al-Soud WA, Salles JF et al (2011) Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. PLoS One 6. https://doi.org/10.1371/journal.pone.0023321
53. Bulgarelli D, Garrido-Oter R, Münch PC et al (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe 17:392–403. https://doi.org/10.1016/j.chom.2015.01.011
54. Pan X, Raaijmakers JM, Carrión VJ (2023) Importance of Bacteroidetes in host–microbe interactions and ecosystem functioning. Trends Microbiol 31:959–971. https://doi.org/10.1016/j.tim.2023.03.018
55. Pérez-Jaramillo JE, Carrión VJ, Bosse M et al (2017) Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. ISME J 11:2244–2257. https://doi.org/10.1038/ismej.2017.85
56. Lin H, Liu C, Li B, Dong Y (2021) *Trifolium repens* L. regulated phytoremediation of heavy metal contaminated soil by promoting soil enzyme activities and beneficial rhizosphere associated microorganisms. J Hazard Mater 402. https://doi.org/10.1016/j.jhazmat.2020.123829
57. Palla M, Turrini A, Cristani C et al (2022) Impact of sheep wool residues as soil amendments on olive beneficial symbionts and bacterial diversity. Bioresour Bioprocess 9. https://doi.org/10.1186/s40643-022-00534-2
58. Yuan Q, Wang P, Wang X et al (2022) Phytoremediation of cadmium-contaminated sediment using *Hydrilla verticillata* and *Elodea canadensis* harbor two same keystone rhizobacteria Pedosphaeraceae and Parasegetibacter. Chemosphere 286:131648. https://doi.org/10.1016/j.chemosphere.2021.131648
59. Zhang Q, Kingsley KL, White JF (2022) Endophytic *Pseudomonas sp.* from *Agave palmeri* participate in the rhizophagy cycle and act as biostimulants in crop plants. Biology (Basel) 11. https://doi.org/10.3390/biology11121790
60. Chen Y, Li Y, Qiu T et al (2024) High nitrogen fertilizer input enhanced the microbial network complexity in the paddy soil. Soil Ecol Lett 6. https://doi.org/10.1007/s42832-023-0205-3
61. Palemón-Alberto F, Ortega-Acosta SÁ, Domínguez-Monge S et al (2021) First report of bud soft rot on *Agave angustifolia* caused by *Pantoea dispersa* in México. Plant Dis 105:3286. https://doi.org/10.1094/PDIS-02-21-0316-PDN
62. Rodríguez Velázquez ND, Pérez Pérez GO, Vergara Arellano G et al (2024) *Pantoea vagans* causing soft rot disease in *Agave angustifolia*, in Mexico. J Phytopathol 172:1–9. https://doi.org/10.1111/jph.13280
63. de Jesus Ramírez-Ramírez M, Mancilla-Margalli NA, Meza-Álvarez L et al (2017) Epidemiology of Fusarium agave wilt in Agave tequilana Weber var. Azul. Plant Prot Sci 53:144–152. https://doi.org/10.17221/142/2016-PPS
64. Gryzenhout M, Fouche HJ, Swart WJ (2017) First report of a serious cladode disease of Opuntia ficus-indica (prickly pear) in South Africa caused by *Bisifusarium lunatum*. Plant Dis 101:2148. https://doi.org/10.1094/PDIS-03-17-0377-PDN
65. De Souza JT, Silva ACM, de Jesus Santos AF et al (2021) Endophytic bacteria isolated from both healthy and diseased *Agave sisalana* plants are able to control the bole rot disease. Biol Control 157. https://doi.org/10.1016/j.biocontrol.2021.104575
66. da Silva Xavier LM, de Farias OR, Barbosa PRR et al (2024) *Bisifusarium lunatum* causing cladode soft rot in pear cactus (*Nopalea cochenillifera* L. Salm-Dyck) in Brazil. Plant Dis 1–5. https://doi.org/10.1094/PDIS-02-24-0456-PDN
67. Qu X, Li X, Bardgett RD et al (2024) Deforestation impacts soil biodiversity and ecosystem services worldwide. Proc Natl Acad Sci 121. https://doi.org/10.1073/pnas.2318475121
68. Sortibrán L, Verdú M, Valiente-Banuet A (2019) A nurse plant benefits from facilitative interactions through mycorrhizae. Plant Biol 21:670–676. https://doi.org/10.1111/plb.12948
69. Montesinos-Navarro A, Verdú M, Querejeta JI, Valiente-Banuet A (2017) Nurse plants transfer more nitrogen to distantly related species. Ecology 98:1300–1310. https://doi.org/10.1002/ecy.1771

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All co-authors have approved the manuscript and agree with its submission to Microbial Ecology.