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Bacterial community characterization of the rhizobiome of plants belonging to *Solanaceae* family cultivated in desert soils

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Abstract

Purpose: The plant *Solanaceae* family is one of the most important for global agriculture and nutrition. Within this plant family, two plant species stand out for their economic importance and for human consumption, which are tomato (*Solanum lycopersicum*) and chili pepper (*Capsicum annuum*). Moreover, those plants support diverse and characteristic microbial communities that are uniquely suited to the plant habitat and intimately connected to plant health. The main objective of this work is the bacterial community characterization in the rhizobiome of tomato and chili pepper, cultivated in arid environments.

Methods: Five crop fields located in the south of the peninsula of Baja California, Mexico, were sampled. Total DNA was extracted from rhizosphere, rhizoplane, and endophytic root compartment and sequenced by Illumina MiniSeq platform technology applied to 16S rRNA gene V3 region.

Results: We were able to obtain 1,195,426 total reads and 1,725,258 total reads for tomato and chili pepper samples, respectively. The analysis of the bacterial community structures confirmed that the two plant species showed differences in their microbial community structures. Nonetheless, the microbial community structures were directly and equally influenced by the crop field localization of each plant species. Interestingly, we determined that in both plant species, the *Proteobacteria* was the main phylum.

Conclusion: In conclusion, we found that several bacterial families are part of the core rhizobiome (28 OTUs) for both tomato and chili pepper, but the most abundant were the *Pseudomonadaceae* family and the *Pseudomonas* genus, which most probably play a pivotal role in the microbial ecology to benefit both crop plants.

Keywords: *Capsicum annuum*, *Solanum lycopersicum*, Rhizobiome, Rhizosphere, Rhizoplane, Endophytic root bacteria

Introduction

Plants are capable to support diverse microbial communities. Those are specific to the plant habitat and are intimately coupled with plant requirements. Those microbial communities are species, and sometimes, genotype-specific (Allard et al. 2016). The plant bacterial communities contribute to crop productivity by plant growth promotion and biocontrol of phytopathogens, therefore into ecofriendly agriculture (Posada et al. 2018). Microbiota encompasses several functional contexts: stimulation of seed germination, plant growth, and resistance promotion to abiotic and biotic stresses (Kalam et al. 2017; Larousse et al. 2017). Previous reports have been shown that the microbial communities associated with diverse plant hosts, tissue, geographic location, and season are all able to influence directly onto bacterial community structure composition. Albeit, the specific extent to which each factor has played an effect has varied from study to study (Coleman-Derr et al.

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2016). The microbial community associated with roots known as "rhizobiome" was proposed to be assembled in two steps: (i) the rhizosphere is colonized by a subset of the bulk soil community and (ii) the rhizoplane and the endosphere are colonized by a subset of the rhizosphere community. All those plant-associated microorganisms together establish a core microbiome in the rhizobiome. The core microbiome consisting of bacterial groups is common to the species (Olanrewaju et al. 2018; Sasse et al. 2018; Ávila et al. 2019). The rhizosphere (defined as microbes living in the zone around root (Sasse et al. 2018)) is rich in nutrients due to the accumulation of plant exudates, and these contain amino acids and sugars that provide a nitrogen and carbon source required for colonizing bacteria. This induces the bacterial community to colonize the rhizoplane and rhizosphere (Qiao et al. 2017; Posada et al. 2018). The rhizoplane microorganisms (defined as root surface adhered microbes (Sasse et al. 2018)) can metabolize essential inorganic nutrients (P, S, Mg, Mn, and Cu) directly from minerals, fixing atmospheric nitrogen, and then promoting plant growth (Lopez et al. 2011). Finally, some bacteria from the rhizoplane can colonize intercellular spaces in several internal plant tissues without causing any harm or disease, and some bacteria enhance plant growth and increase plant resistance to pathogens; those bacteria are collectively known as endophytic bacteria (defined as microbe living within plant tissue in the endosphere (Sasse et al. 2018)) (Lopez et al. 2011; Passari et al. 2016).

Solanaceae is one of the most important plant families for global agriculture. Among the different solanaceous species, tomato (*Solanum lycopersicum*) and chili pepper (*Capsicum annuum*) are two major crops of outstanding importance worldwide (Haak et al. 2014; Seguí-Simarro 2016). Chili peppers can be used as a spice, vegetable, and food coloring, in addition to pharmaceutical applications. In Mexico, which is the center of origin, its production was estimated in more than two million tons with a market value of more than one billion dollars (SIAP 2016; Barchenger et al. 2018). Otherwise, tomato is also an important crop since its production is more than three million tons with a market value of approximately one billion dollars (SIAP 2016). The importance of tomato lies in its content of vitamin C, potassium, folic acid, lycopene and other carotenoids, and several phytochemicals with pharmaceutical potential (Perveen et al. 2015).

Up to today, the global human population is projected to reach up to 9 billion people by 2050, with an increase in food demand by 1.7-fold. Furthermore, the increase in food production must be achieved by a reduction of supply cost for agriculture, since up to 50% of crop production arises from N-based fertilizers (Camilios-Neto et al. 2014). Otherwise, arid and semi-arid regions represent one third of the Earth's land surface area. These ecosystems are characterized by their low water availability which restrains biological activity. Plants in these habitats are subjected to many types of abiotic stress including extreme temperature fluctuations, high ultraviolet radiation, low nutrient content soils, and drought (Fonseca-García et al. 2016). Arid, semi-arid, and Mediterranean-type regions currently support agricultural production for close to a third of the world's population (Mickan et al. 2019). Soil microorganisms in semi-arid regions are commonly subject to seasonal water stress; some groups of organisms are better able to adapt to soil water deficits than others by activating strategies such as dormancy, among others (Manzoni et al. 2012). Thus, the whole shared microbiome ("core") and those microorganisms that are shared by some individuals in the same growth conditions ("specific") of plants microbiome members are compared. That is the species/genotypes of microorganisms that are grown in the same environment in several plants or the species/genotypes of microorganisms grown in many environments for a specific plant (Lebeis 2014; Tian et al. 2015). It is of utmost importance to improve agricultural production processes sustainably by developing better biofertilizers that help crops adapt to the increasingly extreme conditions that occur in different parts of the world.

Metagenomic analysis and comparison of the plant-associated microbiome have successfully led to a novel phylogenetic and functional insight in the plant microbiome and their interactions with host plants (Tian et al. 2015). The objective of this work is to study the core microbiome of the rhizobiome (rhizosphere, rhizoplane, and endophytic root compartment) of plant solanaceous species of chili pepper (*C. annuum*) and tomato (*S. lycopersicum*), grown in arid environments for a later comparative analysis between both rhizobiome. The above would allow finding which microorganism group is conforming to the *Solanaceae* family core rhizobiome, which group is specific for chili or tomato, and which are location specific. Altogether, it will allow a deeper understanding of the plant-microbe interaction to achieve an adaptation to their environment and that benefiting agricultural practices to develop specific and better biofertilizers for arid and semi-arid regions.

Materials and methods

Plant material sampling

Tomato (*S. lycopersicum* var. *cerasiforme*) and chili pepper (*C. annuum* var. *annuum*) samples (roots, soil attached to roots, and soil) were collected from five crop fields located in the southern region of the Baja California peninsula where the arid environment predominates: (i) La Matanza: 23.65.52 N, − 110.26.72 W;

(ii) Todos Santos: 23.46.68 N, – 110.21.59 W; (iii) Comitán: 24.06.10 N, – 110.21.05 W; (iv) Pescadero: 23.36.67 N, – 110.19.04 W; and (v) Los Planes: 23.95.57 N, – 109.93.75 W. All samples were collected in the production period of 2017–2018. Roots were excised from the plant stem and placed in plastic bags with soil attached. Three crop fields for tomato (La Matanza, Pescadero, Los Planes) and three crop fields for chili pepper (Comitán, La Matanza, Todos Santos) were sampled. Three samples per crop field were collected for both tomato and chili pepper. Fifty-four samples were obtained (27 for tomato and 27 for chili pepper) and designated as rhizoplane (9 samples), rhizosphere (9 samples), and root endophytic compartment (9 samples) for each plant. Those samples were pooled in 3 samples of rhizoplane, 3 samples of rhizosphere, and 3 samples of root endophytic compartment, for each plant, totaling 18 samples for sequencing (9 for tomato and 9 for chili pepper) (Fonseca-García et al. 2016). All samples were processed immediately for total DNA extraction.

Sample preparation and DNA extraction

We collected soil around the root (~ 15 cm, rhizosphere) in 50 mL sterile tubes, and the soil attached to root samples (rhizoplane) were washed with sterile distilled water several times and vortexed for 5 min to obtain all the bacteria (including bacterial biofilms) (Kalam et al. 2017). After, to obtain the endophytic root sample, root tissue samples were sterilized (with 70% ethanol solution for 2 min, then washed with sterile and distilled water for 5 min, then washed with 5% (v/v) sodium hypochlorite for 10 min, and finally twice with sterile and distilled water) (Desgarennes et al. 2014). From rhizosphere and rhizoplane solutions, the microbial pellet was obtained by centrifugation at 3000 rpm for 5 min. Root sterilized samples were powdered with mortar and pestle using liquid nitrogen and stored at – 80 °C until DNA extraction.

Total DNA was extracted as follows: starting from ~ 500 mg of soil (rhizoplane and rhizosphere) samples and root tissue (root endophytic compartment) with 1 mL of pre-warm extraction buffer (2% SDS, 1.4 M NaCl, 100 mM Tris, and 20 mM EDTA). The samples were incubated for 1 h at 55 °C and then allowed to incubate for up to 72 h at room temperature. Then, 1 mL of the supernatant was taken and transferred into a 1.5-mL new tube, and 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1) pH 8.0 (Sigma-Aldrich, Saint Louis, MO, USA) was added, mixed, and centrifuged at 12,000×*g*'s for 15 min. The resulting aqueous phase was transferred into 1.5 mL new tubes, and total DNA was precipitated with 500 μL of isopropanol for 24 h at – 20 °C. The samples were centrifuged at 12,000×*g*'s for 10 min, and the supernatant was discarded. The samples were washed with 70% (v/v) ethanol. The total DNA pellet was air-dried and resuspended in 50 μl of buffer TE (10:1). Total DNA was visualized on an agarose gel to ensure the integrity and measured in a NanoDrop 2000 system (Thermo Fisher Scientific, Waltham, MA, USA) to determine the quality (ratio of λ 260 nm/280 nm) for all samples. Finally, total DNA samples were stored at – 20 °C until sequencing.

16S rDNA V3 region PCR amplification and sequencing

Total DNA was processed as follows: PCR amplification of 16S rDNA V3-V4 region was carried out using V3-338f and V4-806r universal primers. The resulting PCR amplification product was used as a template to amplify only the V3 region using V3-338f and V3-533r primers with Illumina adapters, and indices were also added following the manufacturer's recommendations (Illumina, San Diego, CA, USA). The resulting V3 region-amplified products were quantified in a Qubit (Thermo Fisher Scientific, Waltham, MA, USA) to obtain an equimolar pool for each sample. Sequencing reads were generated using the 2 × 150 (300 cycles) base-read length chemistry of the Illumina MiniSeq platform.

Bioinformatics and statistical analysis

Sequencing reads generated were filtered for quality control ($Q \geq 33$) using the modified Mott algorithm, paired-end, and merged with BBmerge to obtain sequence reads of tomato and chili pepper samples. Those sequence reads were then assembled with a minimum identity of 98%, and both contigs and unassembled reads were compared against the GenBank non-redundant database using Megablast. Megablast results were used to create a curated database specific for each sample. Sequence reads per sample were clustered against its corresponding created database using Sequence Classifier to obtain operational taxonomic unit (OTU) frequencies or OTU tables using Geneious Prime (www.geneious.com). These OTU tables were then processed by "R" programming language using a variety of packages and custom scripts (www.r-project.org). We estimated Chao1, Shannon, and Simpson α-biodiversity indices. Venn's diagrams were plotted using the package "VennDiagram." Bray-Curtis distance estimations were calculated using the "vegdist" function with the package "vegan," as well as principal component analysis using "prcomp" function with the package "ggfortify" (Oksanen et al. 2012; Coleman-Derr et al. 2016; Castañeda and Barbosa 2017). PERMANOVA statistical analysis was performed with "adonis" function with the package "vegan." To identify the OTUs that were enriched or depleted as a function of the microbial environment (soil, rhizosphere, and root endophytic compartment), differentially abundant OTUs (DAOTUs) were evaluated by fitting a generalized linear

model (GLM) with a negative binomial distribution (Poudel et al. 2019). Likelihood ratio tests and contrast analyses were performed on the fitted GLM to identify DAOTUs. The OTU counts from soil were used as a control and compared with root endophytic compartment and rhizosphere samples in a contrast analysis. All tests were adjusted to soil (control) for the false-discovery rate (FDR) ($P < 0.05$) using the Benjamini-Hochberg method. General community profiles were constructed using OTUs labeled at the phylum level and visualized in a bar plot graph.

Finally, sequencing data were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under BioProject accession number PRJNA562381.

Results

Sequencing run metrics

The total DNA samples (root endophytic compartment, rhizosphere, and rhizoplane) of *C. annuum* and *S. lycopersicum* were sequenced by the 16S rDNA V3 region with the Illumina MiniSeq (2 × 150, 300 cycles) platform. The NGS sequencing yielded ~ 2.9 million raw reads for the 18 samples analyzed. For tomato samples, 1,195,426 raw reads (on average 123,333 sequences per sample) were acquired (Table 1). These data were further processed for control quality analysis (trimming, filtering, pairing-end, and merging) to obtain 554,999 sequences and classified into 6529 OTUs, for which 82.41% were classified and 17.59% were not classified into any known OTU reported in the "*nt-nr*" database (GenBank). In chili pepper, 1,725,258 raw reads (on average 166,003 sequences per sample) were acquired (Table 1). After sequence processing, we obtained 758, 362 sequences classified into 6244 OTUs, from which 89.89% of the processed sequences were assigned into a known OTU and 10.11% were not classified into any known OTU reported in the "*nt-nr*" database (GenBank). For both totaling 12,773 OTUs, the average paired-end sequence length was ~ 185 bp after merging.

Table 1 Sequencing reads generated, filtered for the quality control ($Q \geq 33$), and assembled with a minimum identity of 98%, for comparison against the GenBank non-redundant database using Megablast

Samples	Reads	QC 33	Classified (%)	Unclassified (%)
C. annuum				
CCR	476624	416578	88.67	11.33
CCSR	94578	81808	91.76	8.24
CCS	1246008	129392	74.98	25.02
CMR	171288	150112	96.81	3.19
CMSR	91774	80626	96.13	3.87
CMS	116478	103222	98.73	1.27
CTR	223200	196626	79.6	20.4
CTSR	192752	169662	98.64	1.36
CTS	212736	188698	90.63	9.37
S. lycopersicum				
TMR	192064	167744	82.48	17.52
TMSR	76236	69698	87.57	2.43
TMS	159230	150390	92.9	7.1
TPR	131974	123956	48	52
TPSR	67318	64358	99.45	0.55
TPS	174164	163012	92.16	7.84
TLR	84630	78984	40.94	59.06
TLSR	82800	78432	99.25	0.75
TLS	227010	213424	86.63	13.37

CCR chili pepper, *Comitán* endophytic root; *CCSR* chili pepper, Comitán, rhizosphere; *CCS* chili pepper, Comitán, rhizoplane; *CMR* chili pepper, La Matanza, endophytic root; *CMSR* chili pepper, La Matanza, rhizosphere; *CMS* chili pepper, La Matanza, rhizoplane; *CTR* chili pepper, Todos Santos, endophytic root; *CTSR* chili pepper, Todos Santos, rhizosphere; *CTS* chili pepper, Todos Santos, rhizoplane; *TMR* tomato, La Matanza, endophytic root; *TMSR* tomato, La Matanza, rhizosphere; *TMS* tomato, La Matanza, rhizoplane; *TPR* tomato, Pescaderos, endophytic root; *TPSR* tomato, Pescaderos, rhizosphere; *TPS* tomato, Pescaderos, rhizoplane; *TLR* tomato, Los Planes, endophytic root; *TLSR* tomato, Los Planes, rhizosphere; *TLS* tomato, Los Planes, rhizoplane

Diversity of communities associated with *C. annuum* and *S. lycopersicum*

The microbial community diversity for rhizosphere, rhizoplane, and root endophytic compartment samples from three crop fields sampled for each crop plant *Solanaceae* species (*C. annuum* and *S. lycopersicum*) was assessed through alpha indices estimations. Interestingly, we were able to determine that for both *Solanaceae* species, the alpha-diversity (observed OTUs, Chao1 index, and Shannon index) indices were greater for both the root endophytic compartment and the rhizoplane samples than those determined for the rhizosphere samples (Fig. 1, Tables 1 and 2). Moreover, when these alpha-diversity indices were examined by crop field localization, we also determine that alpha-diversity followed a peculiar trend of greater indices for crop fields located near the sea shores (Comitán and La Matanza) than those crop fields afar the coast (Pescadero, Todos Santos, Los Planes) (Fig. S1, Table S1).

The bacterial diversity, richness, and evenness analyses (observed OTUs and Chao1 index, Shannon index, and Simpson index, respectively) were carried out to establish bacterial community structures based on several approaches such as plant *Solanaceae* species (*C. annuum* and *S. lycopersicum*), plant species and type of samples (rhizoplane, rhizosphere, and root endophytic compartment), type of samples, and crop field locations. In the first approach, no significant differences were found between the species for any diversity, richness, and evenness indices (Fig. S3). We analyzed the differences

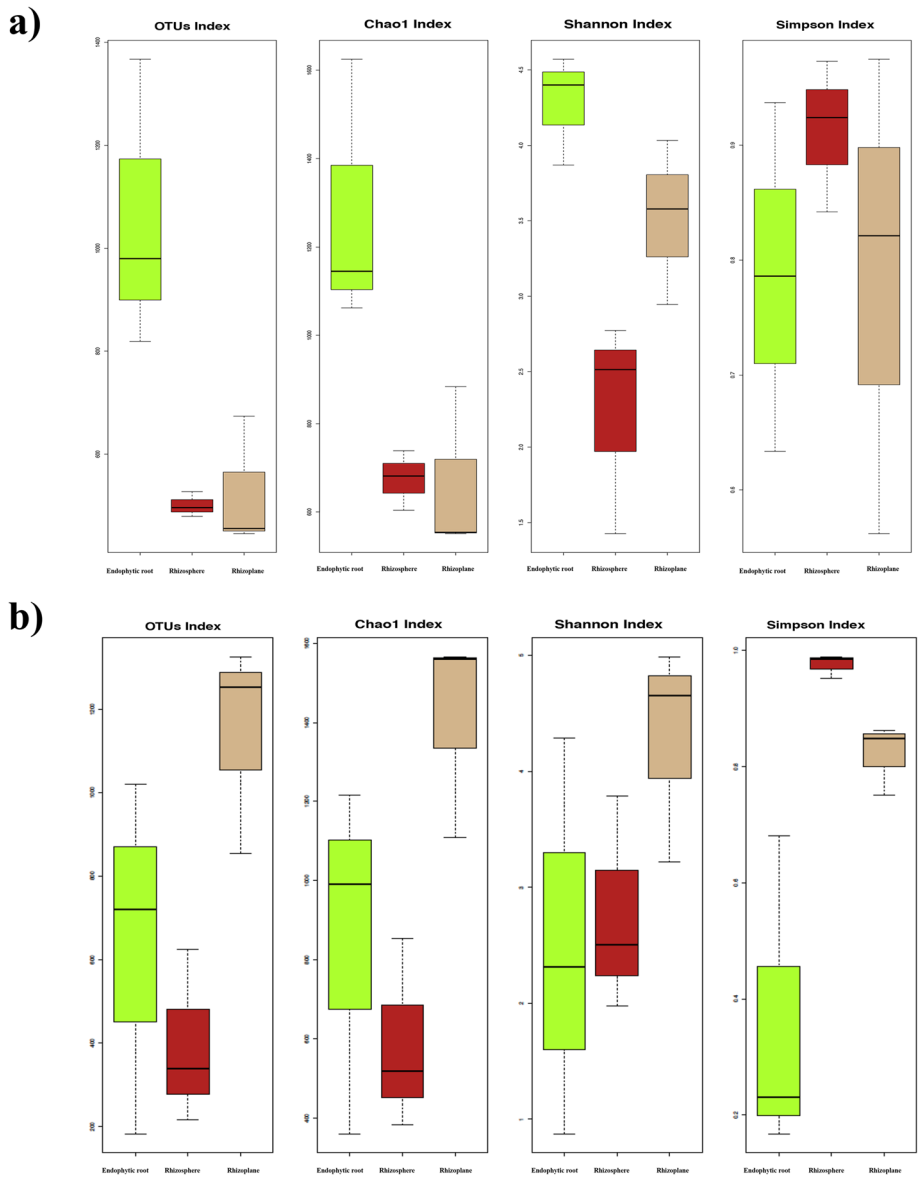

Fig. 1 Alpha-diversity index of bacterial communities across the different samples of **a** *C. annuum* and **b** *S. lycopersicum*

Table 2 Alpha-diversity indices of bacterial communities across the different samples

Biodiversity indices	OTUs	Chao-1	Shannon	Simpson
C. annuum				
Root endophytic compartment	1055	1277.34	4.28	0.786
Rhizosphere	500	674.67	2.24	0.913
Rhizoplane	525	663.46	3.52	0.786
S. lycopersicum				
Root endophytic compartment	640	1277.34	2.49	0.359
Rhizosphere	391	674.67	2.76	0.975
Rhizoplane	1144	663.46	4.29	0.821

between both plants and by sample type (rhizoplane, rhizosphere, and root endophytic compartment); we found significant differences in the diversity indices (observed OTUs and Chao1 index) only for rhizoplane samples between *C. annuum* and *S. lycopersicum* ($P < 0.05$) (Fig. S4a, b). In the analysis by sample type and both plant species treated as a single plant family, we were able to determine that root endophytic compartment samples showed the highest estimations of diversity (observed OTUs and Chao1 index) and richness (Shannon index). The rhizosphere samples showed the lowest diversity and richness estimations, except for the evenness estimation which was the highest (Fig. S5). Finally, when we analyzed the five crop field locations for diversity, richness, and evenness estimations, we did not determine any differences, in spite of the geographical location differences and the agronomical practices applied to each crop field (Fig. S6).

We proceeded to determine the differences between root endophytic compartment samples, rhizosphere samples, and rhizoplane samples from *C. annuum* crop fields through beta-diversity analysis. We were able to determine that the microbial community structures for the root endophytic compartment samples and in the rhizoplane were quite similar, regardless of the location where each sample came from by a hierarchical clustering analysis based on a Bray-Curtis distance estimation matrix (Fig. 2a). Also, when we carry out an unsupervised hierarchical biclustering analysis of the most abundant OTUs (> 100 reads/sample) for *C. annuum* samples, we found that samples from La Matanza (CMS, CMSR, and CMR) were grouped in the same clade (hierarchical clustering for samples) sharing this clade with rhizoplane and rhizosphere samples from Comitán (CCS and CCSR) (Fig. 3a). On the other hand, samples from the rhizosphere and rhizoplane from Todos Santos were grouped in a well-defined clade (CTS and CTSR) (Fig. 3a). And also, the root endophytic compartment samples from Comitán and Todos Santos (CCR and CTR) were grouped in another clade (Fig. 3a). These last clustering results followed the same trend applying principal components (PCA) and principal coordinates (PCoA) analyses (Fig. 4a, b).

The root endophytic compartment samples, rhizosphere samples, and rhizoplane samples from *S. lycopersicum* crop fields were assessed through beta-diversity analysis to determine differences between samples. All samples from *S. lycopersicum* crop fields were analyzed by a hierarchical clustering analysis based on a Bray-Curtis distance estimation matrix. This analysis showed that microbial community structure for rhizosphere samples and root endophytic compartment samples were similar, in spite of the different crop field location they came from, except in the case of root endophytic compartment sample from La Matanza (TMR), which was grouped with the rhizoplane samples (TPS, TLS, TMS) (Fig. 2b). Similar results were obtained after an unsupervised hierarchical biclustering analysis of the most abundant OTUs (> 100 reads/sample) (Fig. 3b). When PCA and PCoA analyses were applied, we were able to determine, as well as hierarchical clustering based on Bray-Curtis distance estimation matrix and unsupervised hierarchical biclustering analyses, that the microbial community structures were independent of the tomato crop field location (Fig. 4c, d). Besides, we carried out a permutational analysis of variance (PERMANOVA) to determine whether the type of sample (rhizoplane, rhizosphere, and root endophytic compartment) and crop

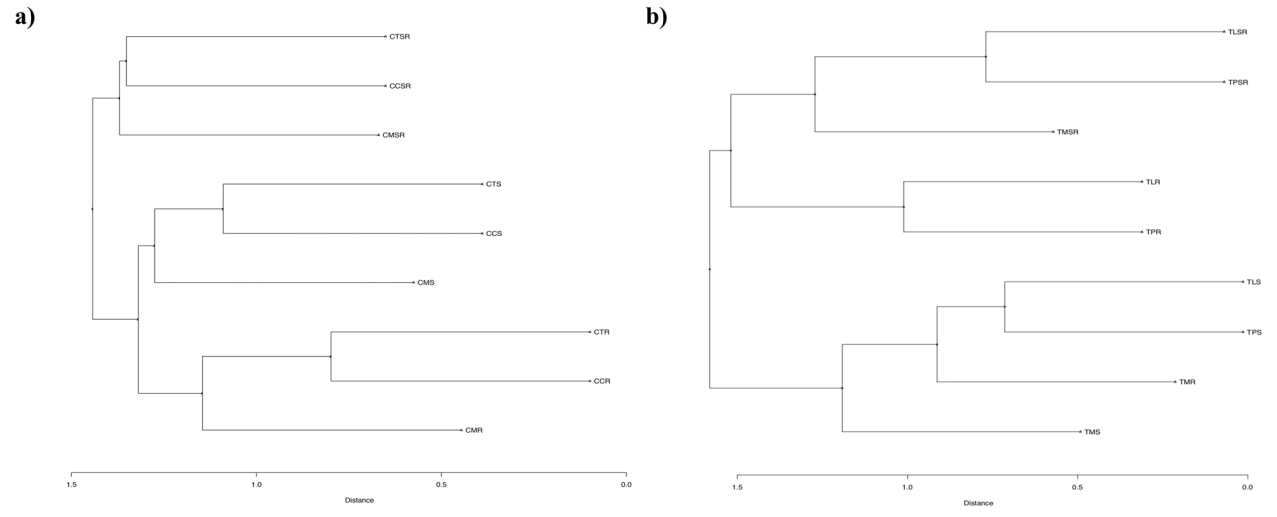

Fig. 2 Analysis of the Bray-Curtis beta-diversity index in the rhizosphere, rhizoplane, and root endophytic compartment of **a** *C. annuum* and **b** *S. lycopersicum*

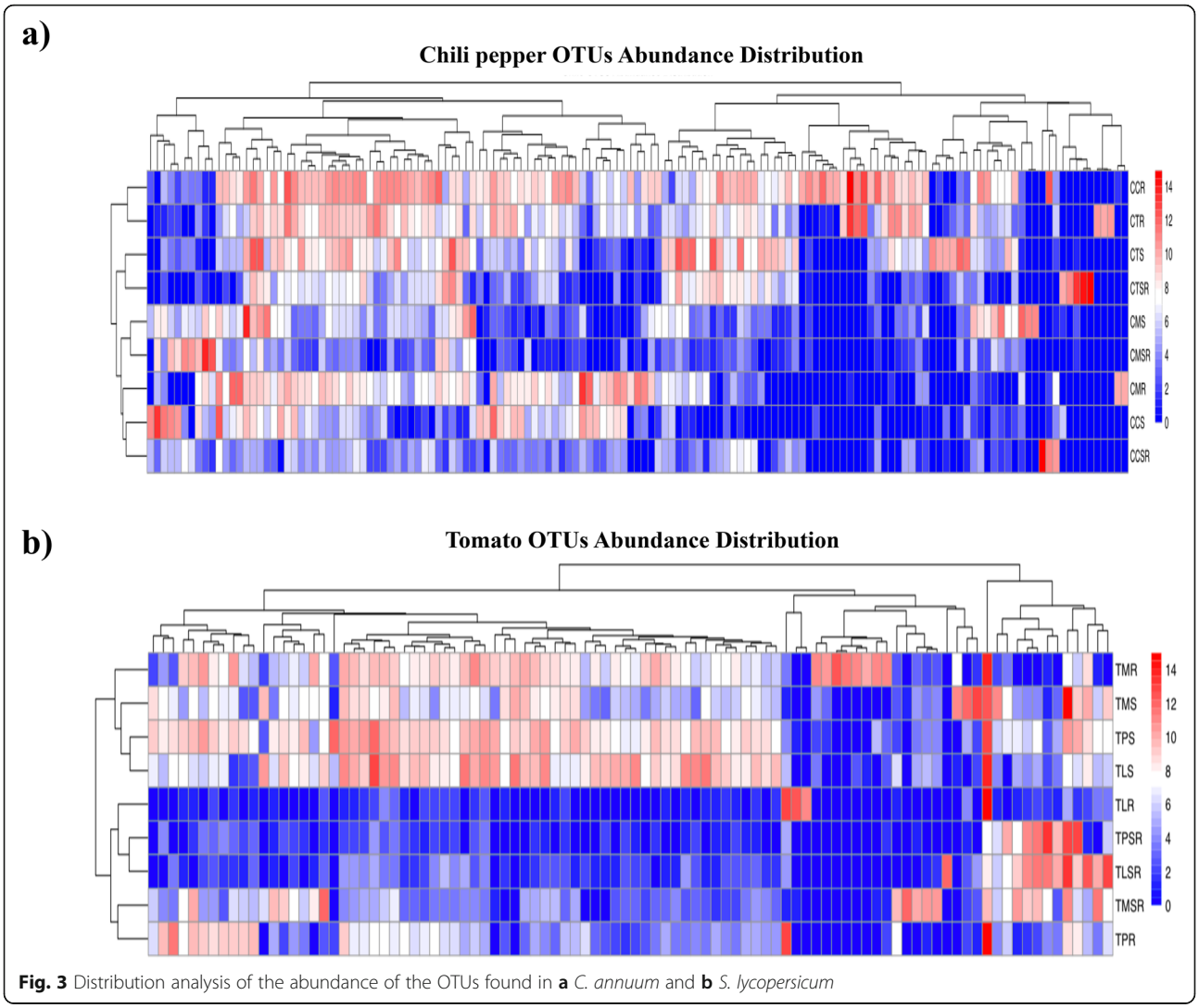

Fig. 3 Distribution analysis of the abundance of the OTUs found in **a** *C. annuum* and **b** *S. lycopersicum*

plant species affected the bacterial community structures. PERMANOVA confirmed that the type of sample was the main factor (crop plant species was not statistically significant) that influenced bacterial community structures (R^2 = 0.18245, P = 0.003).

Composition of microbial communities associated with *C. annuum* and *S. lycopersicum*

The microbial communities associated with *C. annuum* and *S. lycopersicum* in the rhizosphere, rhizoplane, and root endophytic compartment were analyzed. The chili pepper microbial community structures for the rhizoplane from the three crop field locations (CTS, CCS, and CMS) were mainly composed of the bacterial phyla of *Proteobacteria* (up to 64.45%), *Firmicutes* (up to 15.55%), and *Actinobacteria* (up to 10.87%) (Fig. 5a). The most abundant classes were *Gammaproteobacteria* (up to 29.14%), *Betaproteobacteria* (up to 20.50%), *Alphaproteobacteria* (up to 12.46%), and *Bacilli* (up to 12.75%) (Fig. 5b). The most abundant families were *Pseudomonadaceae* (up to 12.63%), *Enterobacteriaceae* (up to 11.70%), *Burkholderiaceae* (up to 11.35%), and *Bacillaceae* (up to 3.85%) (Fig. 5c), and from the *Pseudomonadaceae* family, the genus *Pseudomonas* (up to 10.34%) was the most abundant (Fig. S2b). The rhizosphere microbial community structures were mainly composed of the bacterial phyla of *Firmicutes* (up to 77.79%), *Cyanobacteria* (up to 61.22%), and *Proteobacteria* (up to 22.65%) (CCSR, CMSR, and CTSR) (Fig. 5a). The most abundant classes were *Bacilli* (up to 26.39%), *Clostridia* (up to 22.66%), *Alphaproteobacteria* (up to 5.26%), *Betaproteobacteria* (up to 8.14%), and *Gammaproteobacteria* (up to 8.61%) (Fig. 5b). The main families were *Aerococcaceae* (up to 25.74%), *Clostridiaceae* (up to 21.29%), *Enterobacteriaceae* (up to 5.45%), *Chromobacteriaceae* (up to 4.57%), and *Rhizobiaceae* (up to 1.45%) (Fig. 5c). The root endophytic compartment microbial community structures were mainly composed of the bacterial phyla

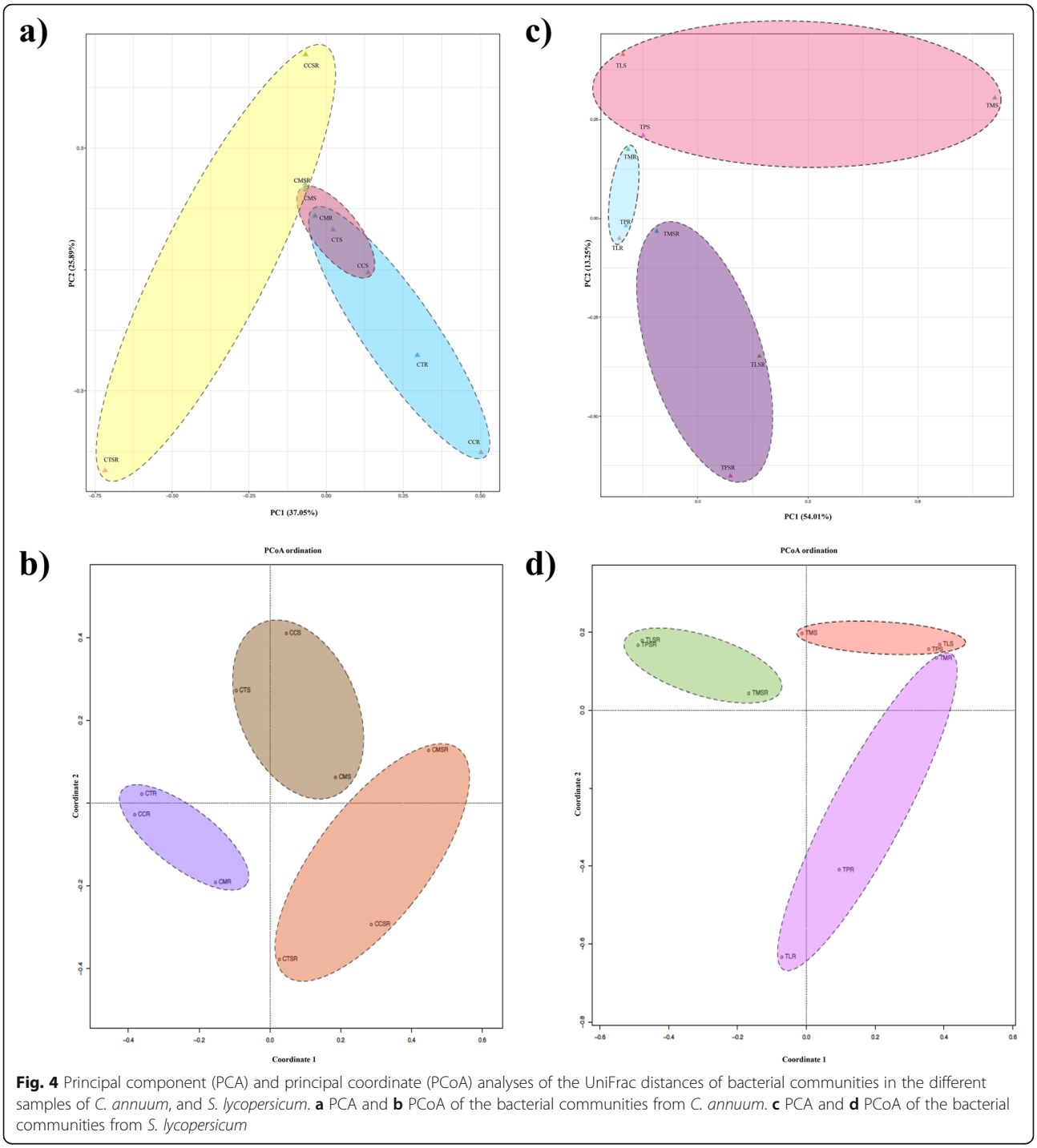

Fig. 4 Principal component (PCA) and principal coordinate (PCoA) analyses of the UniFrac distances of bacterial communities in the different samples of *C. annuum*, and *S. lycopersicum*. **a** PCA and **b** PCoA of the bacterial communities from *C. annuum*. **c** PCA and **d** PCoA of the bacterial communities from *S. lycopersicum*

Proteobacteria (up to 45.23%), *Firmicutes* (up to 17.85%), *Bacteroidetes* (up to 12.46%), *Actinobacteria* (up to 9.82%), and *Acidobacteria* (up to 4.06%) (CTR, CCR, and CMR) (Fig. 5a). The most abundant classes were *Alphaproteobacteria* (up to 23.06%), *Betaproteobacteria* (up to 12.15%), *Clostridia* (up to 12.47%), *Gammaproteobacteria* (up to 8.33%), *Bacilli* (up to 4.30%), and *Chitinophagia* (up to 5.42%) (Fig. 5b). The main families were *Thermoanaerobacteraceae* (up to 11.65%), *Chitinophagaceae* (up to 5.42%), and *Rhizobiaceae* (up to 4.29%) (Fig. 5c).

The microbial communities associated with *S. lycopersicum* in the rhizosphere, rhizoplane, and root endophytic compartment were analyzed. The microbial community structures from rhizosphere were dominated almost exclusively by *Proteobacteria* (up to 99.33%) and

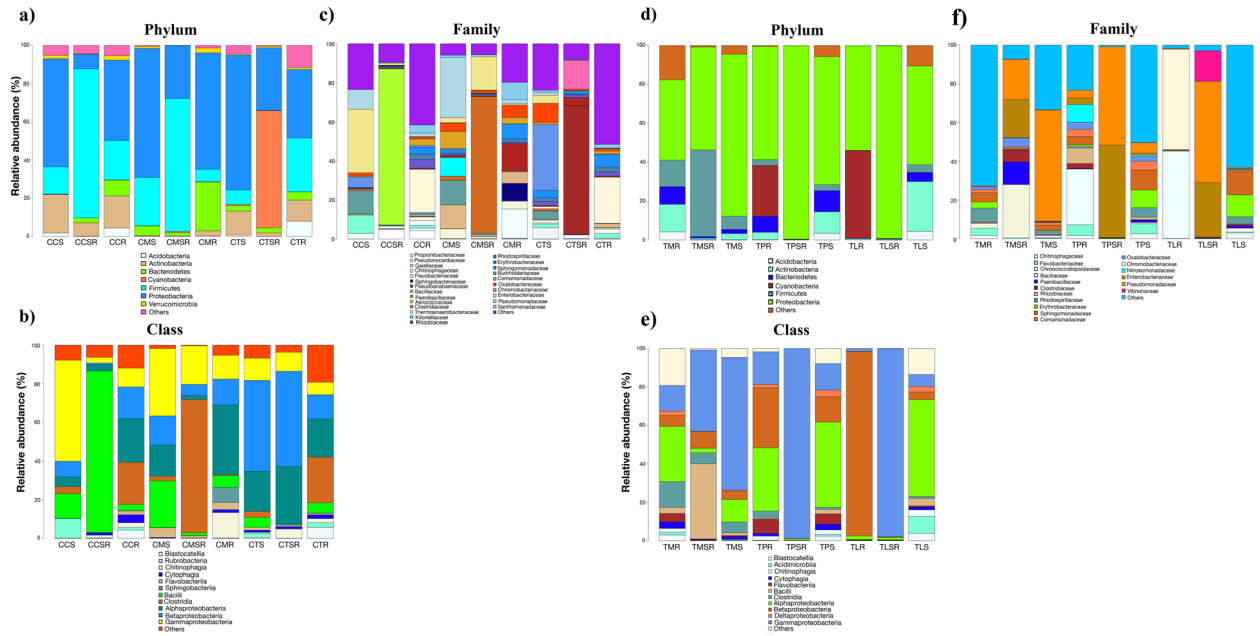

Fig. 5 Profile of bacterial communities from *C. annuum* and *S. lycopersicum*. Dominant phylum, classes, and families in *C. annuum* (**a**–**c**) and *S. lycopersicum* (**d**–**f**).

Firmicutes (up to 44.40%) (Fig. 5d); two classes, from these phyla, were the main components of the rhizosphere: *Gammaproteobacteria* (up to 79.42%) and *Bacilli* (up to 12.91%) (Fig. 5e). Moreover, the most abundant families were *Pseudomonadaceae* (up to 38.81%), *Enterobacteriaceae* (up to 30.37%), and *Bacillaceae* (up to 8.84%) (Fig. 5f), and the most represented genera were *Pseudomonas* and *Bacillus* (TPSR, TLSR, and TMSR). The rhizoplane microbial community structures were mainly composed of *Proteobacteria* (up to 66.11%), *Actinobacteria* (up to 13.17%), *Bacteroidetes* (up to 5.74%), and *Firmicutes* (up to 4.57%) (Fig. 5d). The main classes and families that we found were those belonging to the *Proteobacteria* phylum: *Alphaproteobacteria* (up to 29.76%), *Gammaproteobacteria* (up to 27.69%), and *Betaproteobacteria* (up to 6.25%) (Fig. 5e), and *Pseudomonadaceae* (up to 19.33%), *Sphingomonadaceae* (up to 7.24%), and *Erythrobacteraceae* (up to 5.92%), respectively (Fig. 5f), and the most abundant genus was *Pseudomonas* (TLS, TPS, and TMS). The microbial community structures from root endophytic compartment samples were dominated by *Proteobacteria* (up to 56.07%), *Firmicutes* (up to 13.51%), *Actinobacteria* (up to 6.15%), and *Bacteroidetes* (up to 5.70%) (Fig. 5d). The most abundant classes were *Betaproteobacteria* (up to 26.09%), *Alphaproteobacteria* (up to 15.73%), and *Clostridia* (up to 4.50%) (Fig. 5e). And the most abundant families we found were *Chrooccidiopsidaceae* (up to 23.72%), *Chromobacteriaceae* (up to 17.25%), *Rhodospirillaceae* (up to 2.38%), and *Sphingomonadaceae* (up to 2.82%) (Fig. 5f).

Comparison of bacterial communities associated with the *Solanaceae* family

To determine which OTUs were shared and exclusive for rhizoplane, rhizosphere, and root endophytic compartment between the two plant *Solanaceae* species (*C. annuum* and *S. lycopersicum*), we performed several comparative analyses. We proceeded to carry out the 2628 OTU comparison between plant *Solanaceae* species, for which 573 OTUs were exclusive in *S. lycopersicum*, 521 OTUs were exclusive for *C. annuum*, and 1534 OTUs were shared for both species (Fig. 6a). Next, the comparison was carried out from 2032 OTUs found between the rhizoplane of *C. annuum* and the rhizoplane of *S. lycopersicum*, for which 1032 OTUs were found exclusively in *S. lycopersicum* rhizoplane, 204 OTUs were exclusive for *C. annuum* rhizoplane, and 796 OTUs were shared for the rhizoplane of both plant *Solanaceae* species (Fig. 6b). The comparison between the 1243 OTUs found for *C. annuum* rhizosphere and *S. lycopersicum* rhizosphere was performed, for which 457 OTUs were exclusively in *C. annuum* rhizosphere, 292 OTUs were found exclusively in *S. lycopersicum* rhizosphere, and 494 OTUs were shared for the rhizosphere of both plant *Solanaceae* species (Fig. 6c). Finally, the comparison between the 1978 OTUs found for *C. annuum* root endophytic compartment and *S. lycopersicum* root endophytic compartment was performed, for which we found that 705 OTUs were exclusive for *C. annuum* root endophytic compartment, 294 OTUs were exclusive for *S. lycopersicum* root endophytic compartment, and 979

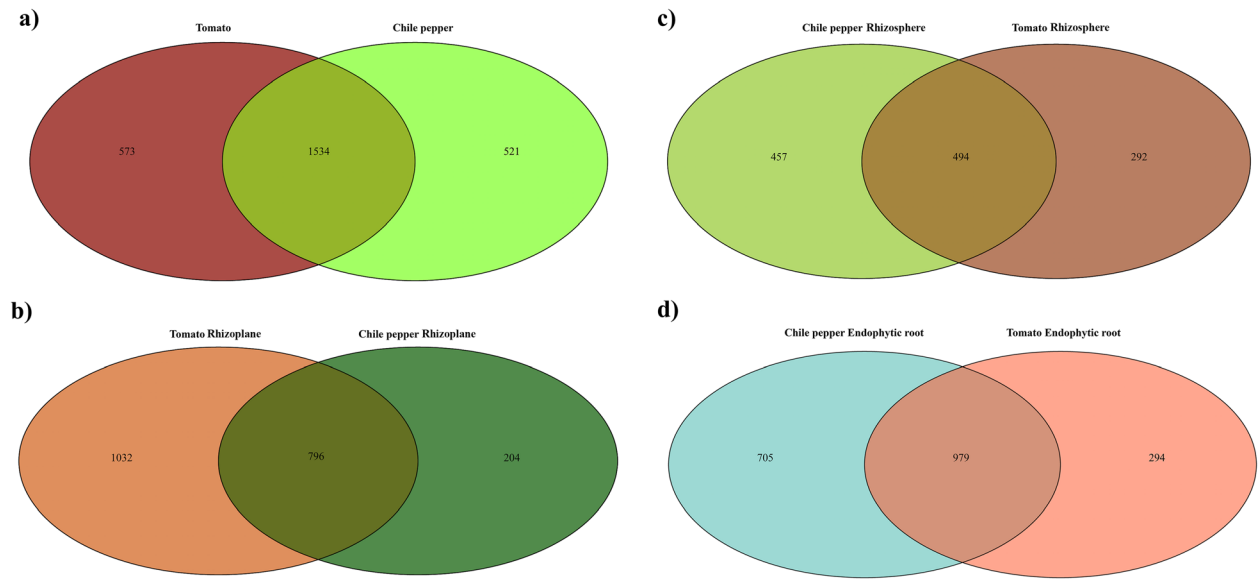

Fig. 6 Analysis of the Bray-Curtis beta-diversity index in the *Solanaceae* family in **a** both species, **b** rhizoplane, **c** rhizosphere, and **d** endophytic root samples

OTUs were shared for the root endophytic compartment of both plant *Solanaceae* species (Fig. 6d).

Furthermore, when we analyzed the core microbiome (OTUs present in the range from 50 to 75% of the samples) for both plant *Solanaceae* species, we found that 462 OTUs were present in at least 50% of the samples, while 199 were present in at least 75% of the samples. Finally, 28 OTUs were present in 100% of the samples. It is worth noting that the main bacterial class was the *Gammaproteobacteria*, which comprises the *Pseudomonadaceae* family, and the genus *Pseudomonas*, followed by *Rhizobiales*, *Rhodospirillales*, and *Sphingomonadales* (Table S3).

Differentially abundant OTU analysis in the *Solanaceae* family

To identify the OTUs that were depleted or enriched in the samples of rhizoplane, rhizosphere, and root endophytic compartment in *C. annuum* and *S. lycopersicum*, the total number of differentially abundant OTU (DAOTU) analysis was made together as *Solanaceae* family. This test permits to determine the changes that occurred in the bacterial community composition for the *Solanaceae* family, between the different types of samples analyzed. DAOTU analysis was made comparing OTU proportion in the rhizoplane as a control against root endophytic compartment or rhizosphere samples. The total number of DAOTUs (enriched or depleted) was greater in the root endophytic compartment (33 OTUs) than in the rhizosphere samples (20 OTUs) (Fig. 7). In the rhizosphere, the enriched OTUs included taxa from *Proteobacteria* (16 OTUs) and *Bacteroidetes* (1 OTU) (Fig. 7a). Enriched *Proteobacteria* and *Bacteroidetes* OTUs were belonging to *Chitinophagaceae* (1 OTUs), *Pseudomonadaceae* (3 OTUs), *Erwiniaceae* (1 OTUs), *Enterobacteriaceae* (5 OTUs), *Burkholderiales* (2 OTUs), *Rhizobiaceae* (2 OTUs), *Hyphomicrobiaceae* (2 OTUs), and *Caulobacteraceae* (1 OTUs). In contrast, 3 OTUs were depleted in the rhizosphere. The depleted OTUs included taxa from *Proteobacteria* (*Oxalobacteraceae*, 1 OTU), *Firmicutes* (*Thermoanaerobacteraceae*, 1 OTU), and *Tenericutes* (*Mycoplasmataceae*, 1 OTU) (Fig. 7a). Otherwise, in the case of root endophytic compartment, 18 OTUs were significantly enriched (Fig. 7b). OTUs included taxa from *Proteobacteria* (6 OTUs), *Actinobacteria* (4 OTUs), *Firmicutes* (3 OTUs), *Bacteroidetes* (2 OTUs), *Caldiserica* (2 OTUs), and *Planctomycetes* (1 OTU). The enriched OTUs were belonging to families *Streptomycetaceae* (1 OTU), *Pseudonocardiaceae* (1 OTU), *Microbacteriaceae* (2 OTUs), *Cytophagaceae* (1 OTU), *Chitinophagaceae* (1 OTUs), *Caldisericaceae* (2 OTUs), *Thermoanaerobacteraceae* (2 OTUs), *Gracilibacteraceae* (1 OTU), *Gemmataceae* (1 OTU), *Enterobacteriaceae* (2 OTUs), *Burkholderiales* (2 OTUs), and *Comamonadaceae* (2 OTUs). Finally, 15 OTUs were found depleted in the root endophytic samples and included taxa from *Proteobacteria* (13 OTUs) and *Firmicutes* (2 OTUs) (Fig. 7b). Those OTUs belong to families *Pseudomonadaceae* (2 OTUs), *Legionellaceae* (1 OTU), *Enterobacteriaceae* (3 OTUs), *Burkholderiales* (6 OTUs), *Phyllobacteriaceae* (1 OTU), and *Clostridiaceae* (2 OTUs).

Design in silico of biofertilizer

The study of the core microbiome of the rhizobiome of plant species of *Solanaceae* family *C. annuum* and *S.*

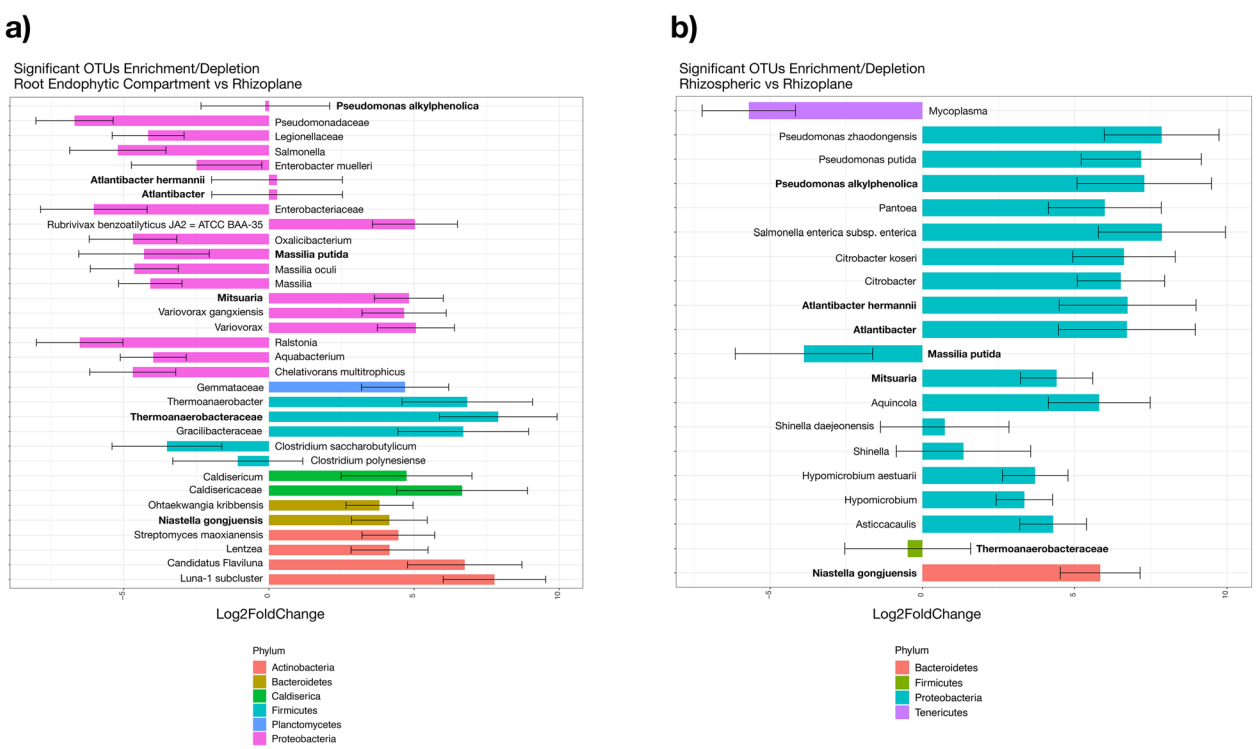

Fig. 7 Comparisons of differentially abundant OTUs. Differentially abundant OTUs (DAOTUs) were evaluated from *C. annuum* and *S. lycopersicum* for the **a** root endophytic compartment and **b** rhizosphere

lycopersicum was the main objective of this work. Likewise, which bacterial group is specific for chili or tomato and which locations are specific were also objectives of this study. All of the above were for the in silico design of better biofertilizers for *Solanaceae* family and for species and specific crop area. At this point, with the data, we can say that a *Solanaceae* family-specific biofertilizer should include mainly *Pseudomonas*, but also *Rhizobiales*, *Rhodospirillales*, and *Sphingomonadales*. In the case of chili pepper, it should be composed of bacteria from families *Pseudomonadaceae* (in average 12.63% OTUs), *Bacillaceae* (on average 2.16% OTUs), *Paenibacillaceae* (on average 3.08% OTUs), or *Rhodospirillaceae* (in average 1.26% OTUs). For the development of biofertilizers for chili pepper specific to each area, these could be composed of bacteria from the following families: (a) Comitán: *Aerococcaceae* (77.23% OTUs) and/or *Thermoanaerobacteraceae* (16.07% OTUs); (b) La Matanza: *Clostridiaceae* (63.8% OTUs), *Rhizobiaceae* (11.97% OTUs), *Chitinophagaceae* (12.73% OTUs), and/or *Bacillaceae* (10.83% OTUs); and c) Todos Santos: *Burkholderiaceae* (28.7% OTUs), *Thermoanaerobacteraceae* (18.9% OTUs), and/or *Chromobacteriaceae* (13.61% OTUs). In the case of tomato, it should be composed of bacteria from families *Pseudomonadaceae* (on average 19.86% OTUs) and/or *Sphingomonadaceae* (on average 7.24% OTUs). For the development of biofertilizers for tomato specific to each area, these could be composed of bacteria from the following families: (a) La Matanza: *Bacillaceae* (26.46% OTUs), *Rhodospirillaceae* (6.23% OTUs), and/or *Sphingomonadaceae* (4.17% OTUs); (b) Pescadero: *Chroococcidiopsidaceae* (26.04% OTUs); and (c) Los Planes: *Chroococcidiopsidaceae* (45.10% OTUs) and/or *Chromobacteriaceae* (51.76% OTUs).

Discussion

In this study, next-generation sequencing 16S rDNA gene amplicons-based metagenomic analysis was used to characterize the core microbiome of the rhizosphere, rhizoplane, and root endophytic compartment for plant solanaceous species in arid zones, such as in the Baja California peninsula south region. This approach has the full potential to find the bacterial groups shared among the plant *Solanaceae* species, as well as to determine which groups are exclusive for each plant species and even to determine which bacteria might be related to plant survival and adaptation to arid environments.

By NGS sequencing, we were able to determine a greater diversity (observed OTUs and Chao1 index) in root endophytic compartment samples than in rhizosphere samples for both tomato and chili pepper. These results reflected a more homogeneous composition of the bacterial communities in the rhizoplane and the rhizosphere. Bacteria with a scarce abundance in the

rhizoplane and the rhizosphere that did not influence in the bacterial community structure probably were under the detection limit of the NGS technique, while in the root, this limitation was surpassed by abundance of nutrients that favored the enrichment (proliferation) of those bacteria in this organ. Interestingly rhizoplane samples from tomato and chili pepper crop fields showed a contrasting and specific behavior for each crop. It is worth noting that a limited (or a few) number of studies have been addressed to explore bacterial communities associated with these two plants, despite their agricultural and economic importance. Tian et al. (2015) determined a greater bacterial diversity and richness (observed OTUs and Chao1, and Shannon index) in rhizoplane microbial communities than those for root endophytic compartments. Endophytic bacteria have been considered a subset of rhizospheric bacteria, with the advantage to be in close contact with the plant host for a constant supply of nutrients (Afzal et al. 2019). The results between both studies in tomato-related microbial communities are quite remarkable, since both studies were conducted independently and in the most contrasting conditions (greenhouse conditions for Tian et al. (2015) and crop fields in arid zones for our study). Those results suggest a tightly and broad regulation of the surrounding microbiota exerted by tomato plants, in spite of the environmental conditions, which enhance the plant survival.

When we analyzed the differences/similitudes between the rhizosphere, rhizoplane, and root endophytic compartment samples for both plant *Solanaceae* species through beta-diversity indices (Bray-Curtis distance matrix, hierarchical clustering analysis, PCoA, and PCA analysis), the grouping of the samples followed a trend. That can be clustered by the type of sample (rhizosphere, rhizoplane, and root endophytic compartment). Besides, the alpha indices showed the influence of the location on the diversity (observed OTUs and Chao1 index), since the samples that were collected from the crop fields nearer to the shores showed the greatest diversity indices. These results were quite similar for previous reports that the crop field location exerted a significant effect on crop-associated bacterial assemblages (Allard et al. 2016; Rashid et al. 2012).

The bacterial community structures for all types of samples analyzed and for chili pepper were dominated by the *Proteobacteria* and *Firmicutes* phyla, and the main classes were *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Bacilli*. Interestingly, the family relative abundances showed specific differences for each type of sample: *Xanthomonadaceae*, *Bacillaceae*, and *Burkholderiaceae* were among the most abundant families for rhizoplane samples; *Aerococcaceae*, *Clostridiaceae*, and *Chromobacteriaceae* for rhizosphere samples; and *Thermoanaerobacteraceae* for root endophytic compartment samples. However, several abundant families were shared between the type of samples, such as *Enterobacteriaceae*, *Pseudomonadaceae*, and *Rhizobiaceae*. Other previous reports have found that the most abundant bacterial phyla for rhizoplane, rhizosphere, and root endophytic compartment samples were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* (Rasche et al. 2006; Kolton et al. 2011; Marasco et al. 2012; Asaff-Torres et al. 2017; Ros et al. 2017). Finally, in the case of root endophytic compartment samples, this is the first report for chili pepper describing the microbial community structures applying next-generation sequencing techniques.

The tomato samples for rhizoplane, rhizosphere, and root endophytic compartment were composed mainly by the *Proteobacteria* phylum and, to a lesser extent, by *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*. The dominant classes for all samples were *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*, and the most abundant families were *Pseudomonadaceae* and *Sphingomonadaceae*, which also belong to the *Proteobacteria* phylum. Other previous reports have been shown that the *Proteobacteria* phylum and the *Pseudomonadaceae* family were the most representative OTUs for all tomato-related samples (Ottese et al. 2013; Ottese et al. 2015; Tian et al. 2015; Kalam et al. 2016; Pii et al. 2016; Larousse et al. 2017; Qiao et al. 2017).

The comparative analysis of the bacterial communities related to both plant *Solanaceae* species unveiled the remarkable feature of the high proportion of shared microbiome among these plants. Interestingly, the DAOTU analysis showed a contrasting behavior among rhizosphere and root endophytic compartment samples for the enrichment and depletion of OTUs belonging to *Pseudomonadaceae*, *Enterobacteriaceae*, and *Burkholderiales*, respectively. This reflects the selectivity exerted by the plant *Solanaceae* species and translated into a high proportion of the shared microbiome. This feature reflects that both plant *Solanaceae* species have similar ecological, and even physiological, requirements leading to select specific bacteria, or *Solanaceae*-related bacteria, to address their needs. Furthermore, from the core microbiome analysis for both plant *Solanaceae* species and related samples (rhizoplane and rhizosphere) taking the OTU presence in at least 50%, 75%, and 100% (absolute) of the samples, we found that the *Pseudomonadaceae* family and the genus *Pseudomonas* were present at 100% of the samples, and this genus was also the most abundant in the core microbiome at 100% criteria. Previous reports that analyzed the microbiome associated to plant *Solanaceae* also found that the genus *Pseudomonas* was among the most abundant genera, and the presence of this genus has been related directly with an incidence

reduction of certain diseases caused by phytopathogenic fungi (Ottese et al. 2013; Hu et al. 2016).

Finally, improved formulations of the biofertilizers are necessary for creating and commercializing new biofertilizers that are more effective, stable, and of better quality (Bashan et al. 2014; Mahanty et al. 2017). Initially, studies to characterize plant-associated communities relied on cultivation-based methods. Massive parallel sequencing has dramatically improved our ability to identify and quantify community members (Lebeis 2014). At this point, using the next-generation sequencing technologies for the in silico design of biofertilizers that can be developed from the bacterial communities of each species of crop and area to be cultivated, such as those proposed in this work, could accelerate the obtaining of better eco-friendly biofertilizers that help to increase agricultural production without impacting the environment.

Conclusion

In our study, we were able to define by NGS coupled with metagenomic analysis of the core microbiome in the rhizobiome (rhizosphere, rhizoplane, and root endophytic compartment) of plant *Solanaceae* species in several crop fields from arid zones. We found that the *Proteobacteria* was the main phylum in the rhizobiome for both plant *Solanaceae* species. The crop field location (near the shores) had a direct and positive impact on bacterial diversity. Finally, the *Proteobacteria* phylum was represented mainly by the *Pseudomonadaceae* family and the *Pseudomonas* genus, which also were present for all the samples, and in turn, these integrate the core microbiome in the rhizobiome of these two plants belonging to the *Solanaceae* family.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13213-020-01572-x.

Additional file 1: Figure S1. Alpha-diversity indices of bacterial communities across the different localities of a) *C. annuum*, and b) *S. lycopersicum*.

Additional file 2: Figure S2. Profile of bacterial communities from *C. annuum* and *S. lycopersicum*. Dominant order and genus in *C. annuum* (a and b), and *S. lycopersicum* (c and d).

Additional file 3: Figure S3. Alfa index between *C. annuum*, and *S. lycopersicum*.

Additional file 4: Figure S4. Alfa index between *C. annuum*, and *S. lycopersicum* by type of sample.

Additional file 5: Figure S5. Alfa index in the *Solanaceae* family by type of sample.

Additional file 6: Figure S6. Alfa index in the *Solanaceae* family by sampling site.

Additional file 7: Table S1. Alpha-diversity indices of bacterial communities across the different crop field locations. **Table S2.** Alpha-diversity indices of bacterial communities across the different localities by type of sample. **Table S3.** OTUs present in the 100% of the samples.

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Authors' contributions

Aarón Barraza: Investigation, Methodology, Formal analysis, Writing -original draft. Goretty Caamal-Chan: Investigation, Writing - review & editing. Thelma Castellanos: Funding acquisition, Writing - review & editing. Abraham Loera-Muro Conceptualization, Formal analysis, Writing - original draft, review & editing, Project administration. The author(s) read and approved the final manuscript.

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Ethics approval and consent to participate

N/A

Consent for publication

N/A

Competing interests

The authors declare that there are no competing interest.

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