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CROP PROTECTION

Rhizosphere bacterial and fungal communities of healthy and wilted pepper (*Capsicum annuum* L.) in an organic farming system

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ABSTRACT: Rhizosphere microorganisms play an important role in the growth and health of plants. Around the world, diverse soil-borne pathogens attack *Capsicum annuum* causing significant damage and economic losses. This study determined whether the diversity and composition of microbial communities in the rhizosphere soil of *C. annuum* plants is significantly changed by wilt disease. We used the 16S rRNA gene for bacteria and the internal transcribed spacer region for fungi to characterize the rhizosphere microbiomes of healthy and wilted plants. The most abundant bacterial phyla were Proteobacteria and Gemmatimonadetes, while the most abundant fungal phyla were Ascomycota and Mucoromycota. The bacterial α-diversity did not show significant differences in richness and diversity, but did show a significant difference in evenness and dominance of species. Rare taxa were present in both healthy and wilted conditions with relative abundances < 1%. In the fungi, all evaluated estimators showed a significant reduction in the wilted condition. The β-diversity showed significant differences in the structure of bacterial and fungal communities, which were segregated according to plant health conditions. The same occurred when comparing the alpha and beta diversity of this study based on organic agriculture with that of other studies based on conventional agriculture. We observed a significant difference with estimators analyzed by segregating rhizosphere communities depending on the farming method used. Finally, the differential abundance analysis did not show significant results in the bacterial communities; however, in the fungal communities, *Fusarium, Thanatephorus*, *Rhizopus*, *Curvularia*, *Cladosporium*, and *Alternaria* were more abundant in the rhizosphere of wilted than healthy plants. Species from these genera have been previously reported as phytopathogens of several plants, including *C. annuum*.
Key words: bacteria, chili pepper, fungi, 16S rRNA, microbiome, rhizosphere.

Comunidades bacterianas e fúngicas da rizosfera de pimentão sadio e murcho (*Capsicum annuum* L.) em sistema de cultivo orgânico

RESUMO: Microrganismos na rizosfera desempenham um papel importante no crescimento e saúde das plantas. Em todo o mundo, vários patógenos do solo atacam o *Capsicum annuum* causando danos significativos e perdas econômicas. Este estudo teve como objetivo determinar se a diversidade e composição das comunidades microbianas no solo da rizosfera de plantas de *C. annuum* é alterada significativamente pela murcha. Usamos o gene 16S rRNA para bactérias e a região espaçadora transcrita interna para fungos para caracterizar os microbiomas da rizosfera de plantas saudáveis e plantas com murcha. Os filos bacterianos mais abundantes foram Proteobacteria e Gemmatimonadetes, enquanto os filos fúngicos foram Ascomycota e Mucoromycota. A diversidade alfa bacteriana não mostrou diferenças significativas na riqueza e diversidade, mas mostrou uma diferença significativa na uniformidade e dominância das espécies. Táxons raros estavam presentes em condições saudáveis e murchas com abundância relativa < 1%. Em fungos, todos os estimadores avaliados apresentaram redução significativa na condição de murcha. A diversidade beta apresentou diferenças significativas na estrutura das comunidades bacterianas e fúngicas, que foram segregadas de acordo com as condições fitossanitárias. O mesmo aconteceu ao comparar a diversidade alfa e beta deste estudo baseado na agricultura orgânica com a de outros estudos baseados na agricultura convencional. Uma diferença significativa foi observada com os estimadores analisados segregando as comunidades da rizosfera dependendo do método de cultivo utilizado. Por fim, a análise de abundância diferencial não apresentou resultados significativos nas comunidades bacterianas; entretanto, nas comunidades fúngicas, os gêneros *Fusarium, Thanatephorus, Rhizopus, Curvularia, Cladosporium* e *Alternaria* foram mais abundantes na rizosfera de plantas murchas do que saudáveis. Várias espécies desses gêneros foram previamente relatadas como fitopatógenos de várias plantas, incluindo *C. annuum*.
Palavras-chave: bactérias, pimenta, fungos, 16S rRNA, microbioma, rizosfera.

INTRODUCTION

The soil microbiota plays an essential role in decomposing organic matter, cycling nutrients, and fertilizing the soil, as well as in interaction with plants to provide protection against pathogens (BERENDSEN et al., 2012), water stress (MANZONI et al., 2012), and the assimilation of minerals such

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as phosphorus (CASTRILLO et al., 2017). The rhizosphere is a zone of high biological activity, with many interactions between plants and rhizobacteria that enhance plant growth and biological control activity (HASSAN et al., 2019). This relationship provides the plants with protection against pathogens, by altering their microbiome to a beneficial community (BERENDSEN et al., 2012). Beneficial organisms reported in the rhizosphere include plant growth-promoting rhizobacteria (PGPR), nitrogen-fixing bacteria, and mycorrhizal fungi (MENDES et al., 2013; MADRID-DELGADO et al., 2021), as well as *Trichoderma* spp., *Metarhizium* spp., *Beauveria* spp. (GUIGÓN-LÓPEZ et al., 2010; ORDÓÑEZ-BELTRÁN et al. 2020), and others. Many genera of PGPR have been reported to interact with plants, including *Agrobacterium*, *Azotobacter*, *Azospirillum, Bacillus*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Serratia*, as well as nitrogen-fixing endophytic rhizobacterial genera, such as *Bradyrhizobium*, *Allorhizobium*, *Mesorhizobium*, and *Azorhizobium* (HOSSAIN et al., 2015; DUY et al., 2016; HARMAN & UPHOFF, 2019; HASSAN et al., 2019).

Diverse pathogens such as *Phytophthora capsici* (ERWIN & RIBEIRO, 1996), *Verticillium dahliae* (VELÁSQUEZ-VALLE et al., 2001; SANOGO & CARPENTER, 2006), *Fusarium oxysporum* (VELARDE-FÉLIX et al., 2018), *Fusarium lateritium, Macrophomina* sp. (VÁSQUEZ-LÓPEZ et al., 2009), *Fusarium solani*, *Sclerotinia sclerotiorum, Sclerotium rolfsii* (THAKUR & PAUL, 2013), *Rhizoctonia solani*, and *Pythium* spp. (VELÁSQUEZ-VALLE et al., 2001) trigger root-rot as well as wilting, stem-, leaf-, and fruit-blight. All causing significant damage to chili pepper crops, and thus, economic losses in production in Mexico (GUIGÓN-LÓPEZ & GONZÁLEZ-GONZÁLEZ, 2001; SILVA-ROJAS et al., 2009; CASTRO-ROCHA et al., 2016; SÁNCHEZ-GURROLA et al., 2019). Previous studies have reported that beneficial microbes, such as PGPR and beneficial fungi (KANG & KIM, 2004; JIANG et al., 2016; LOMBARDI et al., 2018), can be recruited by host plants to counteract pathogen infection (DUDENHÖFFER et al., 2016).

The interactions between plants and pathogens have been studied under the concept of an individual plant–microorganism relationship, an approach that ignores the complexity of such interactions and the involvement of many other groups of microorganisms that affect the outcome of infection. Aside from these studies of disease-causing agents and biocontrol microorganisms, to our knowledge, no studies have been conducted using Next-Generation Sequencing to identify communities of microorganisms in the rhizosphere of *C. annuum* L. in an organic farming system, so it is interesting to know if the composition of the rhizosphere of this crop changes as a function of its health status. Culture-dependent and culture-independent methods have led to the successful identification of plant-associated microbiomes that are either beneficial or pathogenic to plants. This study characterized the diversity and composition of the rhizosphere microbiome in healthy and wilted *C. annuum* L. plants through the 16S rRNA gene and ITS region amplicon sequencing by Illumina MiSeq. In addition, we compared our results obtained from an organic system with other studies based on a conventional farming system in order to identify those variations in alpha and beta diversity in both farming systems, which will serve as a baseline in the study of microorganisms associated with the rhizosphere of *C. annuum*.

MATERIALS AND METHODS

Site description and samples collection

We collected soil samples from organic farm plots under *Capsicum annuum* cultivation, containing healthy and wilt-diseased plants, at a location in the municipality of Camargo, in Chihuahua State, Mexico (27°39’75’’N, 105°07’84’’W; 1240 masl) in June 2020. Three replicates of soil from plants in each health condition (healthy or wilted) were collected. Briefly, we selected rhizosphere soil samples from three plants at the fruiting stage (~40 cm tall) in each condition. First, we collected rhizosphere soils from diseased plants by *Fusarium* and *Thanatephorus* (wilted condition) 40 m apart from each other; then, we collected rhizosphere soil samples from healthy plants (healthy condition) at a distance of 100 m from the wilted plants, to complete a total of three replicates for each condition. Approximately 10 g of rhizosphere soil were collected per individual plant using a dry-sterile toothbrush to brushing the soil around the surface of the root, placed into sterile bags and stored them on ice for transport to the laboratory, where they were then stored in an ultralow temperature freezer at -80 ºC until processing. In addition, physical-chemical analysis of soil was done at the Facultad de Ciencias Agrícolas y Forestales’s Soils’ Laboratory (UACH) using standard methodologies for measuring: soil texture, pH, electrical conductivity (EC), $CaCO_3$, NO_3, P, K, Fe, Zn, Cu, and Mn. The physical and chemical properties of the soil in the experimental area are presented in table 1.

Table 1 - Physical-chemical characteristics of the rizospheric soil samples.

Plant soil condition	Texture	pH	†EC	% $CaCO_3$	‡NO_3	‡P	‡K	‡Fe	‡Zn	‡Cu	‡Mn
Healthy	Clay	8.05	3.64	45.02	8.21	5.20	854	2.02	0.32	0.23	2.03
Diseased	Clay	7.85	5.82	41.03	8.54	6.24	875	2.33	0.34	0.24	2.18

†Electrical conductivity (EC) is presented in dS m^{-1}. ‡NO_3, P, K, Fe, Zn, Cu, and Mn are presented in parts per million (ppm).

DNA extraction, library preparation, and sequencing

We extracted the total genomic DNA individually from each soil sample using a ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, Irvine, CA, U.S.A.) following the manufacturer's instructions. We quantified the quality and integrity of the DNA using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE) based on its A260/280 ratio, and observed it in a 1.0% agarose gel electrophoresis. The DNA samples were sent to Novogene (Beijing, China) for analysis using MiSeq sequencing platform through paired-end 2 × 250 bp strategy on an Illumina MiSeq sequencer (Illumina Inc., San Diego, CA, U.S.A.). For bacteria, a fragment of the 16S rRNA gene was amplified using the primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') flanking the V3 and V4 regions (YU et al., 2005). For fungi, the ITS1 region was amplified using the primer pair ITS5-1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3') (BELLEMAIN et al., 2010).

Bioinformatic analysis

Sequence data were obtained as FASTQ files in the CASAVA 1.8 paired-end demultiplexed format. Files from forward and reverse sequencing runs were merged using FLASH v1.2.11 with default settings (MAGOC & SALZBERG, 2011) to create a FASTQ file containing all the sequences for each sample. We quality-filtered, trimmed, dereplicated, and de-noised the merged sequences using DADA2 (CALLAHAN et al., 2016) in Quantitative Insights Into Microbial Ecology (QIIME2 v2020.2) (BOLYEN et al., 2019) to obtain representative amplicon sequence variants (ASVs). After completing the quality-filtering step, we did multiple sequence alignment and phylogenetic reconstruction using MAFFT (KATOH & STANDLEY, 2013) and FastTree (PRICE et al., 2010), respectively, to generate a rooted phylogenetic tree and conduct subsequent analyses. We extracted representative sequences and their abundances by feature-table, and did the taxonomy assignment with a pre-trained naïve Bayesian classifier using the Greengenes database (v.13_8) for bacteria, and the UNITE database (v.8_99) for fungi. Venn diagrams were plotted using the feature-table at the genus level with the R package *ggvenn*, based on the presence of bacterial and fungal genera regardless of their relative abundance.

In order to analyze the α- and β-diversity of bacterial and fungal communities and conduct related statistical tests, we rarefied the samples at the depth of the library with the lowest number of reads and calculated the metrics using the R package *vegan*. To explore α-diversity within these communities in both healthy and wilted conditions, we estimated species richness using Chao1, species diversity with the Shannon index, dominance with the Simpson index, and the species evenness index, using the Kruskal–Wallis test considering statistically significant difference at P values < 0.05. To investigate differences in bacterial and fungal communities' composition between healthy and wilted conditions, we performed a principal coordinate analysis (PCoA) based on Bray–Curtis distances and unweighted UniFrac distances, and we then used an analysis of similarities (ANOSIM) to test for significant differences in bacterial and fungal communities. In addition, we decided to include in the analysis a couple of studies whose sequences are deposited as bioprojects at the NCBI site in which the rhizospheric soil communities under a conventional farming system were explored for comparison with the alpha and beta diversity of the rhizospheric communities of this study based on an organic farming system. Briefly, 16S rRNA gene and ITS region libraries were included from a study of the rhizosphere of *C. annuum* conducted by ASAFF-TORRES et al. (2017) in Chihuahua City, Mexico under a conventional agriculture scheme, which also analyzed rhizosphere soil with treatments based on the inoculation of a synthetic microbial consortium and with

application of root exudate inductors. Also, libraries were included from the study by ZHANG et al. (2019) where the modification of the bacterial microbiome of the rhizosphere of jalapeño bell pepper under conventional agriculture and with the inoculation of growth-promoting bacteria was analyzed.

Finally, we did a differential abundance test using ALDEx2 with default settings (FERNANDES et al., 2013) to identify bacterial and fungal taxa that were significantly different across the rhizosphere samples from both healthy and wilted conditions at the genus level. ALDEx2 uses the centred log-ratio (clr) transformation, which ensures the data are scale invariant and compositionally coherent. All bacterial and fungal genera with an effect size higher than 1 and a P-value calculated by the Welch's test lower than 0.05 are considered as differentially abundant. After the differential test, the results were depicted as volcano plots using the R package *ggplot2*.

RESULTS

Sequencing results

We obtained a total of 1,218,821 and 1,154,540 raw sequences from bacteria and fungi, respectively. After applying the quality control criteria, we retained a total of 857,743 and 1,040,617 high-quality sequences from bacteria and fungi, respectively. To conduct subsequent analyses, we rarefied the samples to the lowest number of reads per library; in the case of bacteria, we homogenized all samples to 134,116 reads; and for fungi, we homogenized the samples to 162,089 reads. Rarefaction curves of bacterial and fungal samples tended to approach the saturation plateau, indicating that the sequencing effort was adequate for all samples (Figure 1).

Microbial community composition

A total of 43 distinct phyla, 241 families, and 458 bacterial genera were identified. At the phylum level, Proteobacteria was the most abundant with > 36% of relative abundance, followed by Gemmatimonadetes (20.34%), Actinobacteria (11.51%), Bacteroidetes (9.71%), Acidobacteria (7.72%), Firmicutes (4.24%), Chloroflexi (4.11%), Nitrospirae (1.36%), and Verrucomicrobia (1.16%); the remaining 34 phyla had relative abundance < 1.00%. At the family level, Cytophagaceae was the most abundant (4%), followed by Sphingomonadaceae (3.73%), Xanthomonadaceae (2.83%), Rhodospirillaceae (2.61%), Hyphomicrobiaceae (1.66%), Bacillaceae (1.32%), Chitinophagaceae (1.15%), and Bradyrhizobiaceae (1.13%); the remaining families represented < 1.00%. At the genus level, a high number of bacteria were reported (458 genera), the relative abundance distribution was very homogeneous. In fact, the most abundant genera were *Kaistobacter*, *Bacillus*, *Rubrobacter*, *Streptomyces*, *Balneimonas*, *Nitrospira*, and *Salinimicrobium*, with values ranging from 5.67% to 3.17% of relative abundance; 16 genera ranged from 2% to 1%, and the remaining 435 genera represented values < 1.00% of relative abundance (Figure 2a shows the 20 most abundant bacterial genera).

Among the fungi, 13 distinct phyla, 132 families, and 257 genera were identified.

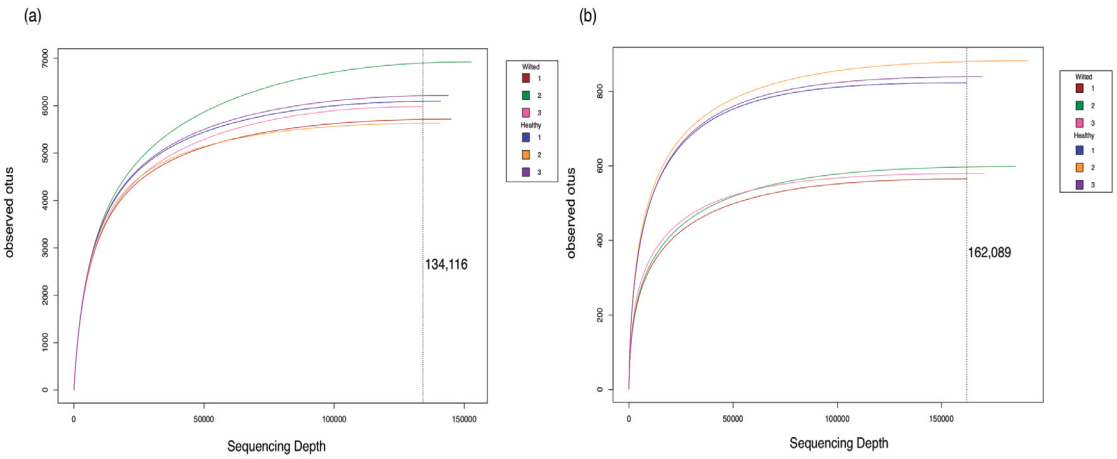

Figure 1 - Rarefaction curves of (a) bacterial and (b) fungal communities.

The phylum Ascomycota was the most abundant taxonomic group, representing > 75% of the relative abundance, followed by Mucoromycota (14.88%), Mortierellomycota (2.84%), and Basidiomycota (1.2%); and the remaining 9 phyla were present at abundances < 1.00%. At the family level, the Aspergillaceae family represented > 28% of relative abundance, followed by Nectriaceae (25.43%), Rhizopodaceae (14.83%), Chaetomiaceae (5.97%), Hypocreales fam Incertae sedis (3.2%), Mortierellaceae (2.83%), Sporormiaceae (1.29%), and Cladosporiaceae (1.25%). The remaining 124 families represented < 1.00%. At the genus level, the most abundant was *Aspergillus* with > 27% of relative abundance, followed by *Rhizopus* (14.83%), *Fusarium* (12.09%), *Acremonium* (3.09%), *Mortierella* (2.83%), *Chaetomium* (2.75%), *Cladosporium* (1.25%), *Acrophialophora* (1.19%), and *Preussia* (1.14%); the remaining 248 genera had relative abundances < 1.00% (Figure 2b shows the 20 most abundant fungal genera).

Microbial communities Venn diagrams displayed the bacterial and fungal genera shared by rhizosphere in both healthy and wilted conditions, and those exclusive to plants in either condition. In the case of bacteria, out of a total of 458 genera found, 312 genera were shared in both conditions; and 82 and 64 genera were exclusively reported in healthy and wilted conditions, respectively (Figure 3a). Conversely, of the 257 fungal genera identified, 149 were shared by both conditions, while 75 were exclusive to the healthy condition, and 33 were unique to the wilted condition (Figure 3b).

α- and β-diversity

The results of estimators of α-diversity did not show significant differences in the diversity and species richness of bacterial communities using the Shannon and Chao1 indexes ($P > 0.05$); however, the species equity and dominance measured with Evenness and Simpson index showed significant differences ($P < 0.05$) (Figure 4a). For fungi, the Shannon, Chao1, Evenness, and Simpson indexes were significantly different, evidenced by higher values in the rhizospheres of healthy plants than those of wilted ones ($P < 0.05$) (Figure 4b). In the case of the comparison of this study based on organic agriculture with those based on conventional agriculture, a statistically significant difference was shown for the case of bacteria using Shannon and Chao1 indices ($P < 0.05$), but not in evenness or Simpson ($P > 0.05$). In fungi, Shannon, Chao1, evenness and Simpson showed a significant difference ($P < 0.05$).

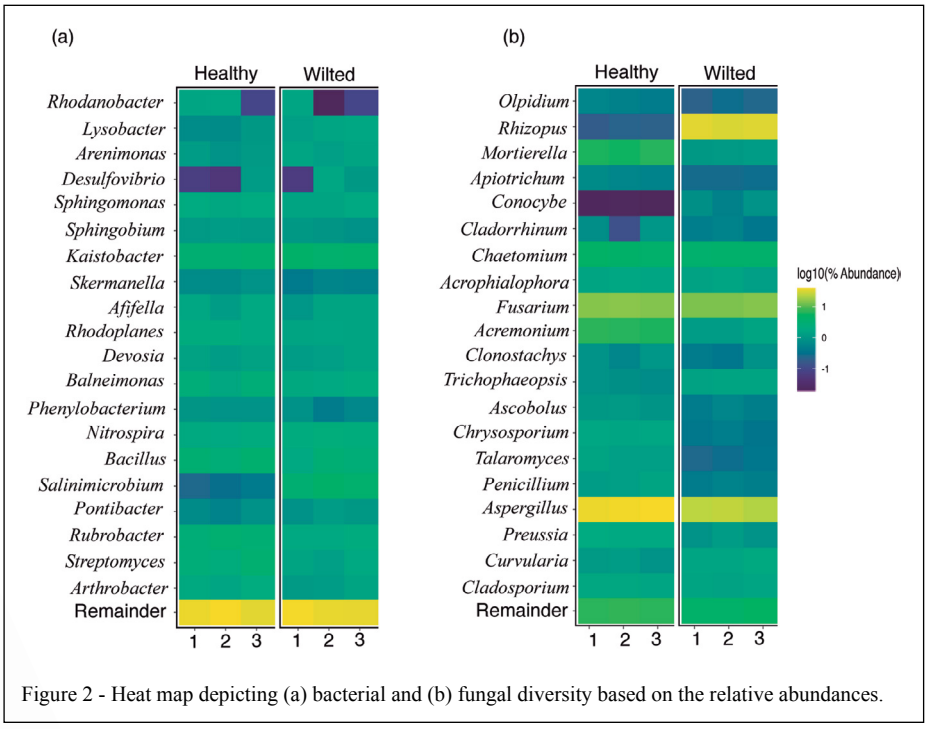

Figure 2 - Heat map depicting (a) bacterial and (b) fungal diversity based on the relative abundances.

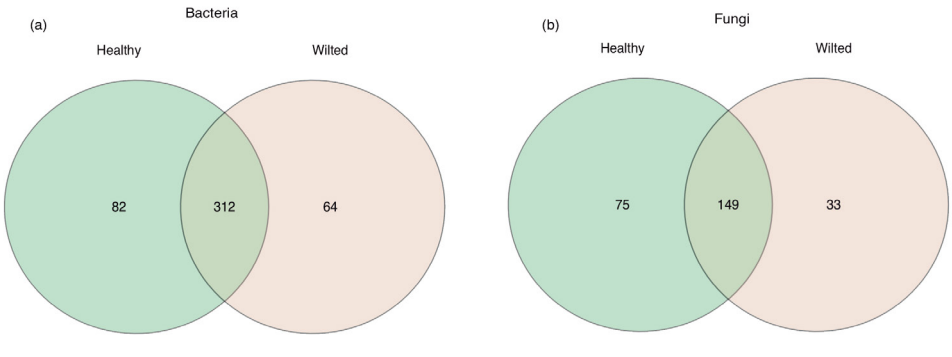

Figure 3 - Venn diagrams showing the distribution of (a) bacterial and (b) fungal genera shared by the rhizospheres of both healthy and wilted *Capsicum annuum* L. plants, and those exclusive to either condition.

Using principal-coordinate analysis (PCoA) we examined the variation of bacteria in healthy and wilted conditions (β-diversity) based on weighted UniFrac and Bray–Curtis distances, which explained 94.1% and 75.4% of the total observed variation, respectively, and revealed that rhizosphere bacterial communities were clustered by health conditions (ANOSIM; $P<0.05$) (Figure 5a). Similarly, in the fungal communities, PCoA showed clustering according to health conditions (ANOSIM; $P < 0.05$), which explained 99.6% (weighted UniFrac) and 99.1% (Bray–Curtis) of the total observed variation (Figure 5b). The comparison of the community structure of this study with that of studies based on conventional agriculture showed a significant difference in bacteria and fungi based on weighted UniFrac and Bray-Curtis distances (ANOSIM; $P < 0.05$). In the case of bacteria, despite the fact that both works included are from conventional agriculture, presented a different structure in their communities.

Differential analysis of microbial community abundances

Our analysis of the data with ALDEx2 helped us gain a better understanding of the significant changes in the abundance of certain members of microbial communities in both healthy and wilted conditions. In bacteria, significant differences in abundance were not observed ($P > 0.05$) (Figure 6a); however, in the fungi, a total of 17 genera had abundances that differed significantly between plant health conditions ($P < 0.05$) (Figure 6b). The genera with significant abundance in the healthy condition were *Setophaeosphaeria*, *Pseudogymnoascus*, and *Mortierella*. While *Rhizopus*, *Conocybe*, *Thanatephorus*, *Trichophaeopsis*, *Myceliophthora*, *Curvularia*, *Fusarium*, *Podospora*, *Cladosporium*, *Chaetomium*, *Alternaria*, *Acrophialophora*, *Coprinopsis*, and *Neurospora* showed significant abundance in the wilted condition.

DISCUSSION

The spread of *Capsicum annuum* wilt and the high incidence of damage, together with the few studies of the associated microorganisms that inhabit the rhizosphere, result in great concern for all personnel involved in the cultivation and management of this agriculturally important crop. In the present study, we analyzed the diversity and structure of bacterial and fungal communities through the 16S rRNA gene and ITS region amplicon sequencing in rhizosphere soil of healthy as well as wilt-diseased plants under organic farming conditions. From the results, we identified a diverse community of microorganisms, of which several bacterial and fungal members were either shared by, or exclusive to, the rhizospheres of *C. annuum* plants depending on their health condition.

The taxonomic assignment confirms that rhizospheric soil of *C. annuum* is composed mainly of the Proteobacteria, Gemmatimonadetes, Actinobacteria, Bacteroidetes, and Acidobacteria phyla, the first being the most abundant. The phylum Proteobacteria has been reported in many studies for its association with the rhizosphere of plants, particularly in some studies on soil growing *C. annuum* (LI et al., 2019; ZHANG et al., 2019; BARRAZA et al., 2020; SONG et al., 2020). In general, at the genus level, the microbial diversity was very homogeneous, independent of the health conditions of the plants, although genera such as *Kaistobacter*, *Bacillus*, *Rubrobacter*, *Streptomyces*,

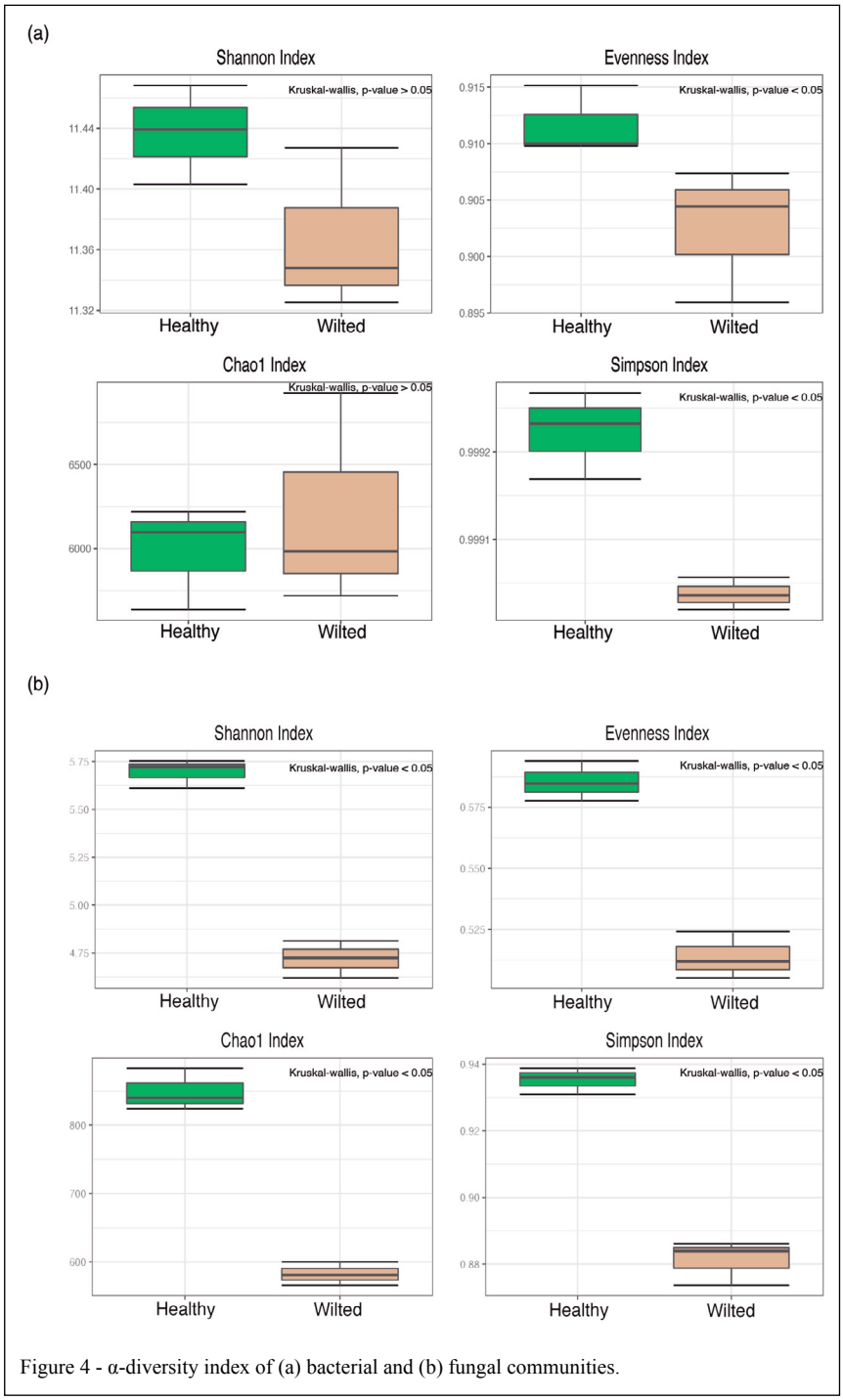

Figure 4 - α-diversity index of (a) bacterial and (b) fungal communities.

Balneimonas, *Nitrospira*, and *Salinimicrobium* were slightly more abundant than others in the rhizosphere. These genera have been reported as the most frequent in soil (GKARMIRI et al., 2017; WU et al., 2017). Little information is available about the genus *Kaistobacter*. However, some studies have reported species of this genus as being associated with active disease suppression in the rhizosphere of tobacco plants (LIU et al., 2016; GKARMIRI et al., 2017). Moreover, genera including *Bacillus*, *Rubrobacter*,

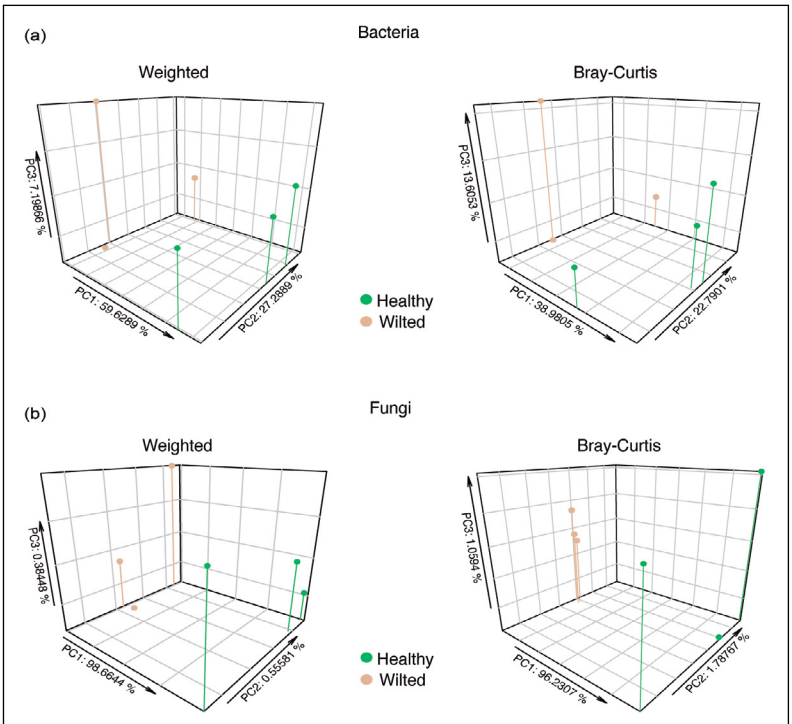

Figure 5 - PCoAs built with weighted UniFrac and pairwise Bray–Curtis dissimilarity matrices of (a) bacterial and (b) fungal communities.

and *Streptomyces* have been reported as suppressors of rhizosphere fungi that are pathogenic to other plant species, such as *Fusariumoxysporum*, *Rhizoctonia solani*, and *Verticillium dahliae* (CHAURASIA et al., 2005; ISLAM et al., 2012; CAO et al., 2016; SIEGEL-HERTZ et al., 2018; YAO et al. 2020). These species were also reportedin this study, so future analyses should be carried out to evaluate the biological control capabilities of these bacterial members.

The Ascomycota, Mucoromycota, Mortierellomycota, and Basidiomycota were the most abundant fungal phyla, of which the first had the highest abundance. Although, it is reported as a widely reported phylum in the rhizosphere, only a few studies have described the fungal communities associated with *C. annuum* as well as other plants belonging to the Solanaceae family (SINGH et al., 2014; NAZIYA et al., 2019). The genus *Aspergillus*, the most abundant in this study, has been reported for its antifungal activity against *Phytophthora capsici* (KANG & KIM, 2004); however, as that study was conducted on *in vitro* bioassays, caution must be exercised in drawing conclusions on biological interactions that occur under *in vivo* conditions.

Studies that have evaluated the α-diversity of rhizosphere communities in agricultural crops have reported variations in the diversity, presumably because of the different factors to be considered at the time of the study (e.g., temperature, plant age, sampling season, crop rotation). Similar to the results reported in this study on bacterial richness and diversity, in plants of the Solanaceae and Piperaceae families, a high bacterial diversity has been reported regardless of whether the plants were healthy or diseased (LI et al., 2016; HU et al., 2020). Although, this was not so for evenness, which significantly declined when the crop became diseased (SHE et al., 2017). In fungal communities, similar to what occurred in this study, a higher α-diversity has been reported in healthy plants than in diseased plants (LI et al., 2016; TAN et al., 2017; YANG et al., 2020). It is worth mentioning that the agronomic practices in the plots sampled in this study were organic, so soil microorganisms had not been exposed to agrochemicals. Indeed, a greater richness and diversity was shown in the organic farming samples than in the conventional farming samples, which has been reported in several studies and has been largely attributed to the effect

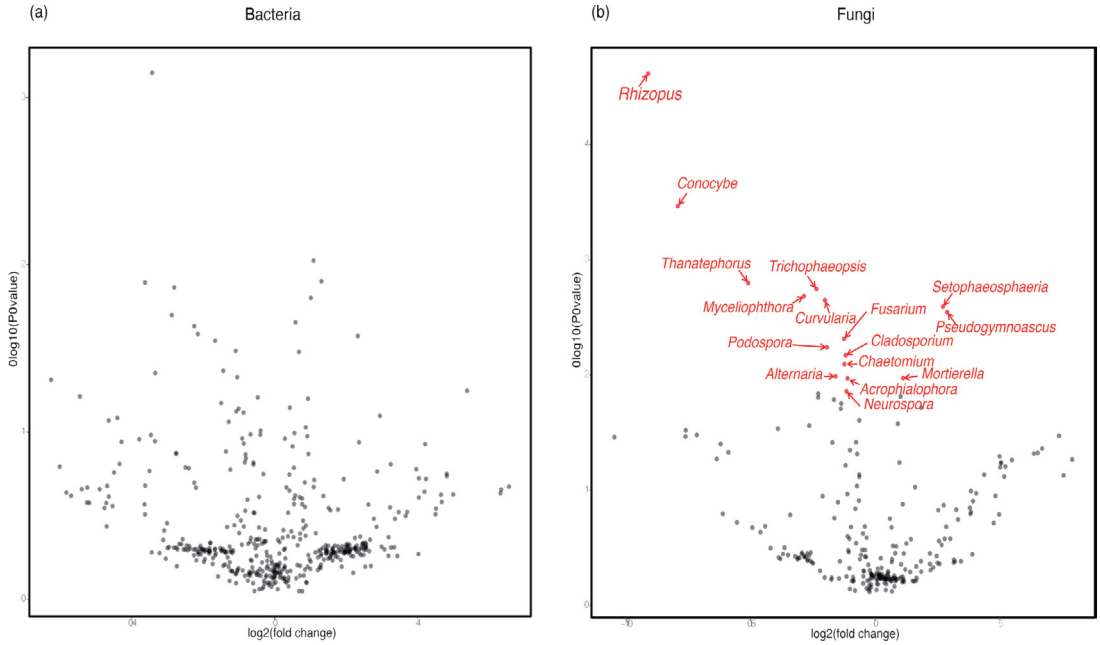

Figure 6 - Volcano plot of differential abundance analysis of (a) bacterial and (b) fungal communities. Dots in red represent genera with differentially significant abundance.

of the fertilizers applied (HARTMANN et al., 2015; PELTONIEMI et al., 2021). Moreover, organic practices trigger the activities of soil indigenous microorganisms non-specifically and some of the alterations of microbial communities are associated with disease suppression (LI et al., 2019).

In terms of community structure, several studies showed changes in the β-diversity of bacterial and fungal rhizosphere communities caused by plant pathogen infestation (TSANG et al., 2020). Our results also demonstrated that the β-diversity was segregated according to plant health conditions. Furthermore, these variations were also demonstrated according to the agricultural practices of the rhizosphere analyzed, which segregated the communities independently of whether the samples were from soil rhizosphere under conventional or organic farming conditions. These differences in microbial communities may have occurred for several reasons, such as modification of soil properties due to attack and colonization of *C. annuum*plants by pathogens (e.g., pH, EC, nutrient solubility, O_2, CO_2, moisture). Another reason is the agricultural practices applied, which triggered a modification of the ecological niche and resulted in the recruitment of microorganisms that exert either deleterious or beneficial effects on the plants (EL-SHATNAWI & MAKHADMEH, 2001).

The differential abundance test showed no significant difference in the bacterial rhizosphere regardless of plant health condition,some genera are visually separated from the rest. However, they were not significant, whereas the differential abundance in the fungal rhizosphere differed significantly in some genera between plant conditions. In the rhizosphere of healthy plants, a significant difference was observed in the genus*Mortierella*, members of which have been reported as plant growth promoters, as well as antibiotics and phytohormone producers, thereby improving resistance to phytopathogens in plants of agricultural importance (MARES-PONCE DE LEÓN et al., 2018; OZIMEK et al., 2018; ZHANG et al., 2020). In the rhizosphere of wilted plants, significant differences were shown in several fungal taxa, particularly in *Fusarium* and *Thanatephorus* genera. These genera have been reported to be the dominant fungal genera in rhizosphere soil chili pepper, and two of the main wilting agents (VELÁSQUEZ-VALLE et al., 2001; THAKUR & PAUL, 2013; PÉREZ-HERNÁNDEZ et al., 2014; BASHIR et al., 2018; FAJARDO-REBOLLAR et al., 2021). This increase

in the abundance of phytopathogens genera may be responsible for triggering the symptomatology that affects the plants, and which subsequently allows opportunistic pathogens (e.g., *Rhizopus*, *Curvularia*, *Cladosporium*, and *Alternaria*) to jointly infect the plant until its decay.

The genus *Rhizopus* was reported with a higher abundance in the rhizosphere of wilted plants, and this pathogen has been previously associated with the deterioration of crops in chili pepper (AJOKPANIOVO & OYEYIOLA, 2011; FATIMOH et al., 2018) and other plants (HANSON, 2010; BAI et al., 2015; SUN et al., 2017). Also, *Curvularia* is a plant pathogen reported as the causal agent of maize leaf spot (LIU et al., 2009), leaf spot disease of *Clerodendrum indicum* (MUKHERJEE et al., 2013), and root rot of strawberry (VERMA & GUPTA, 2010). Finally, *Cladosporium* species have been reported as pathogenic fungi of members of the Solanaceae family such as tomato (THOMMA et al., 2005) and chili pepper (HUANG et al., 2012), in which they cause foliar damage. Surprisingly, *Phytophthora* was not reported in the rhizosphere of wilted or healthy chili pepper plants, while other studies carried out even in the same region, but not under organic farming, have reported it as the causal organism of pepper wilt (SÁNCHEZ-CHÁVEZ et al., 2017). These results indicated the possibility that changes in the soil microbiome associated with organic farming contribute to soil suppressiveness to *P. capsici*, which seems to be the most susceptible to the activities of indigenous soil microorganisms (ROS et al., 2017).

Diseases caused by soil-borne plant pathogens can be difficult to control for a variety of reasons, as many soil-borne pathogens produce persistent resistance structures that can survive in the soil for many years. Even in the absence of a susceptible host, the agricultural practices focused on reducing pathogens are either unsuitable or insufficient, as well as the selective pressure from the other microorganisms in the rhizosphere, because of competition for nutrients and essential elements (PASCALE et al., 2020). However, as many other groups of microorganisms affect plant health, their pathogenic action could occur in conjunction. For example, it has been demonstrated that pathogens have the ability to secrete effector molecules that can affect the communication between plants and beneficial microorganisms, and that they can also recruit other microorganisms that compete against native microorganisms and help in the colonization of the host (BERENDSEN et al., 2012; SNELDERS et al., 2018).

CONCLUSION

Our findings provide evidence that wilt-disease in *C. annuum* has an impact on reducing diversity indexes and changes in the structure of bacterial and fungal rhizosphere communities. Several of the fungal genera we found have been reported as phytopathogens in chili pepper and other plants where a change in their individual abundance was observed, increasing significantly as the chili pepper plants wilted. Conversely, bacterial and fungal communities of systems based on organic and conventional agriculture harbor different microbiomes, influenced by the agricultural practices carried out. Finally, further experiments should be done that isolate potentially beneficial microorganisms from the rhizosphere soil, to study their importance and focus on the interactions within soil microbial communities to elucidate possible biocontrol strategies.

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DECLARATION OF CONFLICT OF INTEREST

The authors declares that there is no conflict of interest regarding the publication of this paper.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

AVAILABILITY OF SUPPORTING DATA

The datasets generated and analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) repository under the BioProject with accession code PRJNA728362.

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