



## LONG-TERM IMPACT OF MAIZE AGROECOLOGICAL MANAGEMENT ON BACTERIAL COMMUNITIES AND SOIL HEALTH IN THE ARID NORTH OF MEXICO †

[IMPACTO A LARGO PLAZO DEL MANEJO AGROECOLÓGICO DEL MAÍZ EN LAS COMUNIDADES BACTERIANAS Y LA SALUD DEL SUELO EN EL NORTE ÁRIDO DE MÉXICO]

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### SUMMARY

**Background.** Soil management practices modify the microbial communities and the carbon stocks (organic, inorganic, and total). The increase in microbiological communities' diversity improves the production of plants; thus, it is essential to understand the predominant bacterial taxa in the soil. **Objective.** The objective of the present study was to establish the bacterial communities' alteration by agroecological management in maize crops in arid northern Mexico. **Methodology.** Bacterial diversity and composition were determined in soils from Coahuila, Mexico, under three different scenarios: *i*) Agroecological management (AM), *ii*) Conventional management (CM), and *iii*) Control (T, with no vegetation). In addition, pH, electrical conductivity (EC), and soil organic matter (SOM) were analyzed using standard methods. Bacterial DNA was extracted from the soil, amplifying the V3-V4 region of the 16S rRNA gene and sequencing with Illumina. The gene sequences were analyzed in QIIME. **Results.** A total of 20 bacterial phyla and 631 genera were registered. For AM, CM, and T, respectively, the most abundant genera were *Tepidisphaera* (7.02, 9.29, and 9.93 %), *Sphingomonas* (6.55, 5.15, and 4.06 %), *Microvirga* (2.64, 2.39, and 3.63 %), and *Blastococcus* (2.91, 3.10, and 3.37 %). A significant difference was observed among groups ( $p = 0.004$ ), where AM was different, which suggests that the type of substrate determines the diversity and abundance of the bacterial community. Significant differences were found for pH and EC, with higher pH in CM (7.87) and T (7.86) soils. The EC was higher in AM ( $446 \mu \text{Scm}^{-1}$ ) and T ( $419 \mu \text{Scm}^{-1}$ ). On the other hand, AM showed the best result in SOM content ( $21.80 \pm 1.10 \text{ gC kg}^{-1}$ ). **Implication.** Therefore, AM in maize crops has the potential to conserve or

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restore C stock in degraded arid lands, increasing bacterial diversity, favoring the health of the soil. **Conclusion.** Agroecological management of maize crops soils in arid North of Mexico promotes greater bacterial diversity, which will favor the availability of nutrients for the future development of healthy plants.

**Keywords:** Bacterial diversity; Electrical conductivity; Microbiome; pH; 16S rRNA.

## RESUMEN

**Antecedentes.** Las prácticas de manejo del suelo modifican las comunidades microbianas y las reservas de carbono (orgánico, inorgánico y total). El aumento de la diversidad de comunidades microbiológicas mejora la producción de las plantas, por lo que es fundamental conocer los taxa bacterianos predominantes en el suelo. **Objetivo.** El objetivo del presente estudio fue establecer la alteración de comunidades bacterianas por manejo agroecológico en cultivos de maíz en el norte árido de México. **Metodología.** Se determinó la diversidad y composición bacteriana en suelos de Coahuila, México, bajo tres escenarios diferentes: *i*) Manejo agroecológico (MA), *ii*) Manejo convencional (MC) y *iii*) Control (T, sin vegetación). Además, se analizó el pH, la conductividad eléctrica (CE) y la materia orgánica del suelo (SOM) utilizando métodos estándar. Se extrajo DNA bacteriano del suelo, amplificando la región V3-V4 del gen 16S rRNA y se realizó secuenciación con Illumina. Las secuencias de genes se analizaron en QIIME. **Resultados.** Se registró un total de 20 filos bacterianos y 631 géneros. Para AM, CM y T, respectivamente, los géneros más abundantes fueron *Tepidisphaera* (7.02, 9.29 y 9.93 %), *Sphingomonas* (6.55, 5.15 y 4.06 %), *Microvirga* (2.64, 2.39 y 3.63 %) y *Blastococcus* (2.91, 3.10 y 3.37 %). Se observó diferencia significativa entre grupos ( $p = 0.004$ ), donde AM fue diferente, lo que sugiere que el tipo de sustrato determina la diversidad y abundancia de la comunidad bacteriana. Se encontraron diferencias significativas para pH y EC, con mayor pH en suelos CM (7.87) y T (7.86). La EC fue mayor en AM ( $446 \mu \text{Scm}^{-1}$ ) y T ( $419 \mu \text{Scm}^{-1}$ ). Por otro lado, AM mostró el mejor resultado en el contenido de SOM ( $21.80 \pm 1.10 \text{ gC kg}^{-1}$ ). **Implicación.** Por lo tanto, el AM en cultivos de maíz tiene el potencial de conservar o restaurar el stock de C en tierras áridas degradadas, aumentando la diversidad bacteriana, favoreciendo la salud del suelo. **Conclusión.** El manejo agroecológico de suelos de cultivos de maíz en el norte árido de México promueve una mayor diversidad bacteriana, lo que favorecerá la disponibilidad de nutrientes para el desarrollo futuro de plantas sanas.

**Palabras clave:** Diversidad bacteriana; Conductividad eléctrica; Microbioma; pH; 16S rRNA.

## INTRODUCTION

Modern agriculture faces new challenges as it integrates ecological and molecular approaches to achieve higher crop yields while minimizing environmental impacts. Farmers have generally improved their productivity by applying synthetic fertilizers and pesticides to crops, especially in arid and semi-arid areas; however, their uncontrolled use over the years has led to major environmental consequences, including air and water pollution as well as loss of soil fertility (Kosty *et al.*, 2020). In addition, the structure and function of soil microbial communities have been affected by irrational agricultural practices (Semenov, 2021; Shen *et al.*, 2021). Thus, there is a growing interest in the agroecological management of the agroecosystem, integrating the ecology of soil food systems, and the economic and social aspects of sustainable agriculture (Altieri, 2020; Francis *et al.*, 2003; Gliessman, 2013). Agroecology offers an integrated set of solutions that reconciles three central challenges facing agriculture today: (i) Feeding a growing population, (ii) Conserving natural resources, providing sustainable lives and livelihoods for farmers in the food system for agricultural workers, and (iii) For the people who consume their products (Gliessman, 2020). To improve production without the use of fertilizers of synthetic origin is necessary to focus on the beneficial intervention of microorganisms in the soil due to their potential to promote plant growth and reduce the use

and abuse of chemically synthesized contaminants (Moreno-Reséndez *et al.*, 2018). Moreover, agroecology tends to achieve system self-regulation by biodiversification in first place (Chavarria *et al.*, 2018). Furthermore, the key role of microorganisms in nutrient cycles and organic matter mineralization, as well as their interactions with plants could be used as indicators to evaluate the effect of different agricultural management systems on soil's quality (Burton *et al.*, 2010). Therefore, analyzing the microbiome is necessary to improve plant growth, which in turn will benefit sustainable food production.

For a long time, research on soil microbial composition was limited by isolation procedure in Petri plates. Moreover, in the mid-1980s, cultivable microbial colonies were estimated to constitute a small proportion (0.1–5%) of the total soil microbial diversity (Torsvik *et al.*, 1990; Torsvik and Ovreas, 2002). In addition, the high variability of the physicochemical properties of the soil (the content of organic carbon, nitrogen, pH, humidity, and porosity, among others) influences the diversity of its microbial communities (Semenov, 2021). According to Marchesi and Ravel (2015), new molecular technologies and bioinformatics have transformed how the soil microbial community is interpreted. Therefore, microbiome analysis is considered the predominant research approach in soil microbiology and provides a starting point for new research

directions and hypotheses (Nesme *et al.*, 2016). Knowing the dominant bacterial taxa of the soil could improve the ability to actively manage communities and promote their functional capacities to increase the production of plants of agricultural importance (Delgado-Baquerizo *et al.*, 2018). Due to this, the objective of the present study was to establish the bacterial communities' alteration by agroecological management in maize crops in arid northern Mexico comparing three scenarios *i*) Agroecological management (AM), *ii*) Conventional management (CM), and *iii*) Control (T, with no vegetation, that represents degraded soil), and considering changes in the physicochemical properties, as well as on carbon sequestration in a semi-arid region of Northern Mexico.

## MATERIALS AND METHODS

### Study area and sampling

The study was conducted in the Universidad Autónoma Agraria Antonio Narro, Unidad Regional Laguna, Torreón, Coahuila, Mexico, on December 2020. During summer the air temperature ranges from 22 to 35 °C, and in winter from 8 to 22 °C; the annual total rainfall average is 225 mm; the climate is mainly very dry semi-warm (BWH); there are sandy loam soils. Soil samples (texture sandy clay crumb) were collected at three contrasting sites: *i*) Agroecological management (AM-1 to AM-3; coordinates 25.553589N, -103.372848W; adding corn crop and weeds residues from the last year; no fertilizers were applied), *ii*) Conventional management (CM-4 to CM-6; 25.555654N, -103.372333W; adding NPK fertilizers (250 N, 70 P<sub>2</sub>O<sub>5</sub>, both in kg ha<sup>-1</sup>), artificial irrigation, mechanized land tilling systems, and machinery-assisted weed control measures), and *iii*) Control (T-7 to T-9; 25.565618N, -103.372189; without crops or plants to reflect the degradation degree of analyzed soils). Three random samples from each treatment were taken at a depth of 10 cm, using a California Type auger (SP06505 Model J), which was sterilized with alcohol 96° and fired for 1 min between samples. Likewise, a conventional auger was used to collect other three soil samples from each treatment (mixed to get a combined sample) to determine the physicochemical parameters.

### DNA extraction, amplification, and sequencing

An approximate portion of 100 mg was taken from the soil samples and placed in BashingBead™ lysis tubes; then 750 µl of Xpedition™ Zymo Research™ lysing buffer/stabilizer was added. Each tube was placed in a cell disruptor (TerraLyzer™) for 30 sec. DNA from the samples was extracted using the Zymo Research™ DNA Zymobiomics MiniPrep kit according to the manufacturer's instructions. The

amount of DNA per sample was measured in a Qubit® brand fluorometer. The amplification of the V3-V4 region of the 16S rRNA gene was performed using the primers suggested by Klindworth *et al.* (2013): S-D-Bact-0341-b-S-17, 5'-CCTACGGGNGGCWGCAG-3' and S-D-Bact-0785-a-A-21, 5'-GACTACHVGGGTATCTAATCC-3', which produces an amplicon of ~460 bp. The PCR was made following Illumina protocol for 16S metagenomics, as well as quantification, normalization (equimolarity), library pooling, and massive next-generation sequencing (MiSeq Illumina® 2 × 250 paired-end reads) (Illumina, 2017a).

### Bioinformatic analysis

The analysis of the sequences was carried out in an Oracle VM VirtualBox 5.1.14 virtual machine in MGLinux using the bioinformatics software Quantitative Insights into Microbial Ecology (QIIME) v.1.9.0 (Caporaso *et al.*, 2010). The process began by assembling the forward and reverse sequences using the PEAR program (Zhang *et al.*, 2014), with quality criteria Q30. Chimeras were eliminated with USEARCH (Edgar, 2010) and the selection of OTUs was performed with the UCLUST method (Edgar, 2010) at 97% similarity; a representative sequence was obtained for each OTU and the taxonomy was assigned taking as reference the updated 2020 EzBioCloud database (Yoon *et al.*, 2017). The absolute abundance of OTUs was obtained and the number of sequences was plotted by the number of taxa at the genus level to observe the coverage depth (asymptote trend curves); PAST Ver. 3.15 software was used (Hammer *et al.*, 2001). A simple random rarefaction process was carried out (Weiss *et al.*, 2017) to match the samples to the same number of sequences. Beta diversity was calculated using the Bray-Curtis Index (Beals, 1984); the obtained matrix was used to perform a PERMANOVA test ( $p < 0.05$ ) to observe significant difference in the microbiota among groups. Also, relative abundances for phylum and genera were obtained. The genera, whose relative abundance was greater than 0.01%, were represented in a heatmap; the hierarchical cluster method with Euclidean measurement was used for the dendrogram of the samples; this visualization was made using Morpheus software (<https://software.broadinstitute.org/GENE-E/>).

### Physicochemical properties of the soil

The pH and electrical conductivity (EC) were determined in a soil water suspension of 1:2 and 1:2.5 (w/v) ratio, respectively (Luján-Soto *et al.*, 2021). On the other hand, the soil organic matter (SMC, gC kg<sup>-1</sup>) was measured by Walkley and Black

(1934), and labile carbon (L) and non-labile (NL) carbon fractions due to the oxidation of C by  $\text{KMnO}_4$  (Blair *et al.*, 1995).

### Statistical analysis

First, the data normality and homogeneity of variance were examined in IBM SPSS Statistics program. After both assumptions were met, one-way ANOVA was applied to analyze differences among treatments. The taxa were enumerated to visualize the groups of samples using a principal component analysis (PCA) to observe associations between the ten main phyla and the physicochemical variables of the soils using RStudio (ver. 2021.09.2), and thus, determine the variables that have greater relationship to the phyla. The patterns in AM, CM, and T physicochemical properties were analyzed by multivariate redundancy. A redundancy analysis (RDA) was performed on RStudio (Di Felice *et al.*, 2012). Finally, a LEfSe analysis was made to statistically and biologically determine the key biomarkers, which contributed to the differences among groups. The selected clades were those less than 0.05 in the alpha value of the Kruskal-Wallis factorial test  $>4.0$  in the logarithmic LDA score. This analysis was performed on the website <http://huttenhower.sph.harvard.edu/lefse/>.

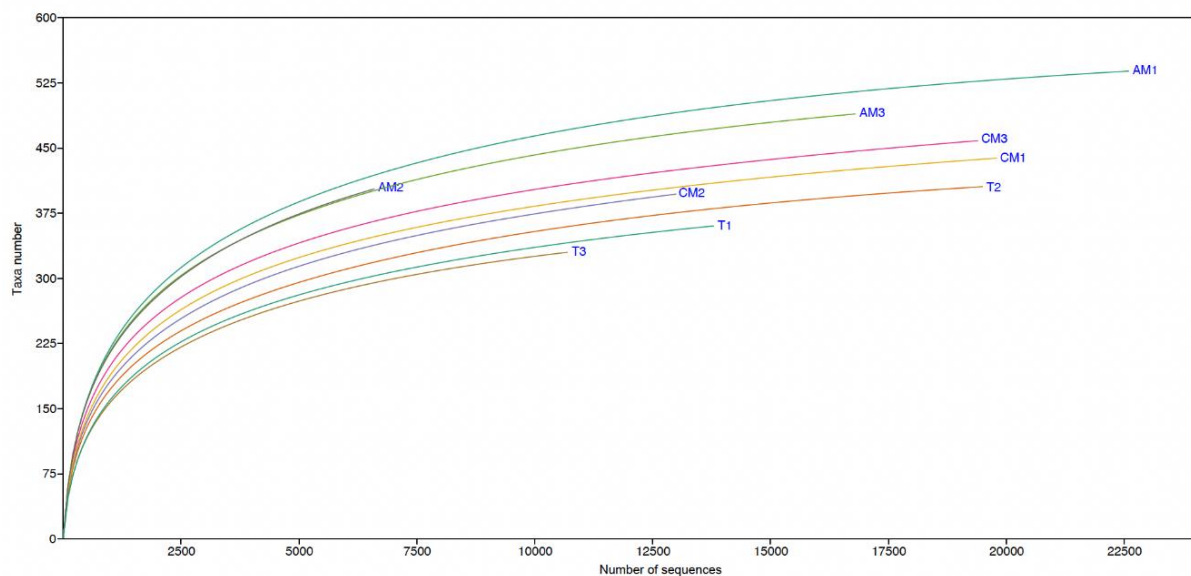
## RESULTS

### Analysis of the bacterial microbiota

The mean obtained from the total number of sequences in AM before performing the assembly was 135,976; for the CM was 123,261, and for T was 106,820. Averages of the assembled sequences were 50,133; 48,016, and 48,759, respectively. After the elimination of the singletons, the respective averages were 15,471; 17,549, and 14,799 (Table 1). An acceptable coverage depth was observed since the curves tended to the asymptote (Fig. 1).

In total, 20 phyla, 59 classes, 99 orders, 223 families, and 630 genera were recorded. For phyla, Actinobacteria ( $\bar{x} = 38\%$ ), Proteobacteria ( $\bar{x} = 29\%$ ), Chloroflexi ( $\bar{x} = 14\%$ ), Planctomycetes ( $\bar{x} = 11\%$ ), Acidobacteria ( $\bar{x} = 3\%$ ), and Saccharibacteria\_TM7 ( $\bar{x} = 0.5\%$ ) were the most abundant (Fig. 2). From 630 bacterial genera registered in the samples, *Tepidisphaera*, *Sphingomonas*, FJ479147\_g, GQ396871\_g, *Microvirga*, *Blastococcus*, *Geodermatophilus*, *Streptomyces*, among others, were the most abundant (Fig. 3).

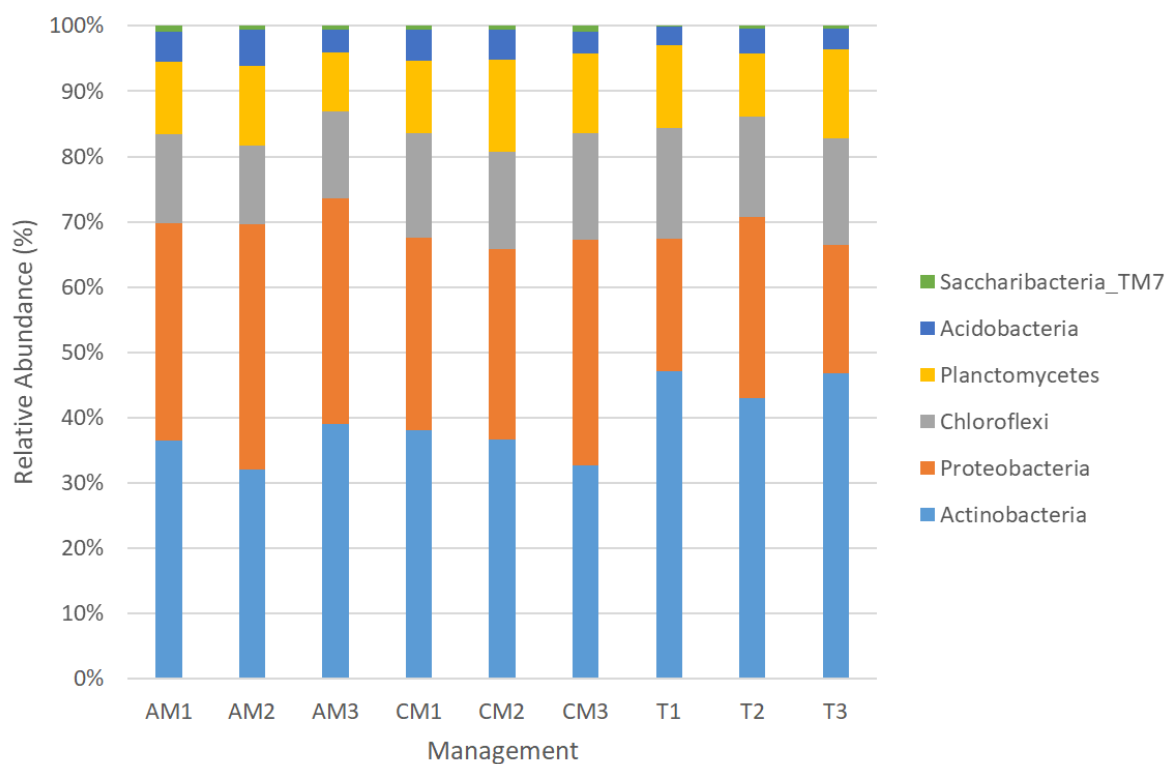
When performing the PERMANOVA test a significant difference was observed among groups ( $p = 0.004$ ). The AM, CM, and T groups were visualized using principal coordinate analysis (PCoA), showing the separation among them (Fig. 4).



**Figure 1.** Rarefaction curve sequences showing the microbiome cover depth (number of sequences vs. taxa number) from the Agroecological management (AM1-3), Conventional management (CM1-3), and Control (T1-3) soil samples.

**Table 1. Information of the 16S rRNA V3-V4 region sequences obtained from the Agroecological management (AM1-3), Conventional management (CM1-3), and Control (T1-3) soil samples. TS= Total sequences, AS= Assembled sequences, Q = Chimeras removed, QS= Quality sequences after chimeras removal, BS= Bacterial sequences after taxonomic assignment.**

Samples	TS	AS	Q	QS	BS
AM-1	177913	63047	561	62266	52971
AM-2	118629	38603	350	38121	29518
AM-3	111387	48749	612	47927	40677
Mean	<b>135976</b>	<b>50133</b>	<b>508</b>	<b>49438</b>	<b>41055</b>
CM-1	109509	46095	735	45188	39381
CM-2	128492	49646	570	48918	39552
CM-3	131782	48307	578	47550	40915
Mean	<b>123261</b>	<b>48016</b>	<b>628</b>	<b>47219</b>	<b>39949</b>
T-1	97340	44760	434	44182	36924
T-2	114981	52820	520	52120	44635
T-3	108139	48698	416	48125	37833
Mean	<b>106820</b>	<b>48759</b>	<b>457</b>	<b>48142</b>	<b>39797</b>

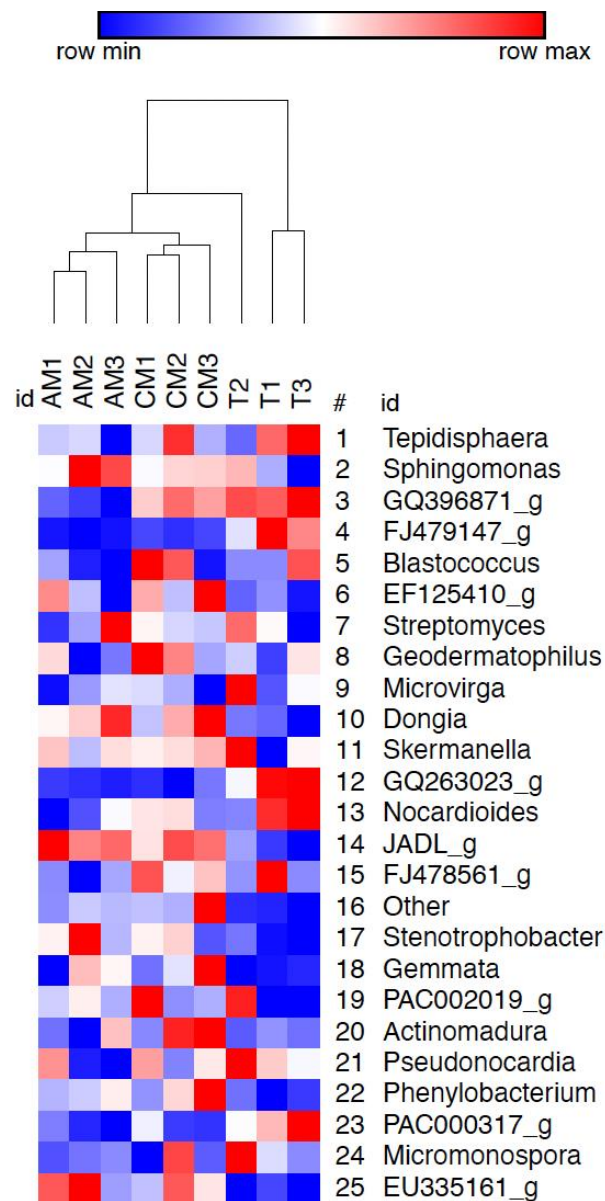


**Figure 2.** Relative abundance (%) of the main bacterial phyla obtained from the Agroecological management (AM1-3), Conventional management (CM1-3), and Control (T1-3) soil samples.

### Taxa biomarkers

A bar graph was made for a discriminant analysis of biomarkers (Fig. 5A), and a cladogram to represent the results obtained through the LEfSe analysis to make a comparison of high-dimensional classes with

a focus on taxa biomarkers (Fig. 5B). The discriminant analysis and cladogram showed an evident separation among the AM, CM, and T samples, in addition to identifying the predominant taxa by population.



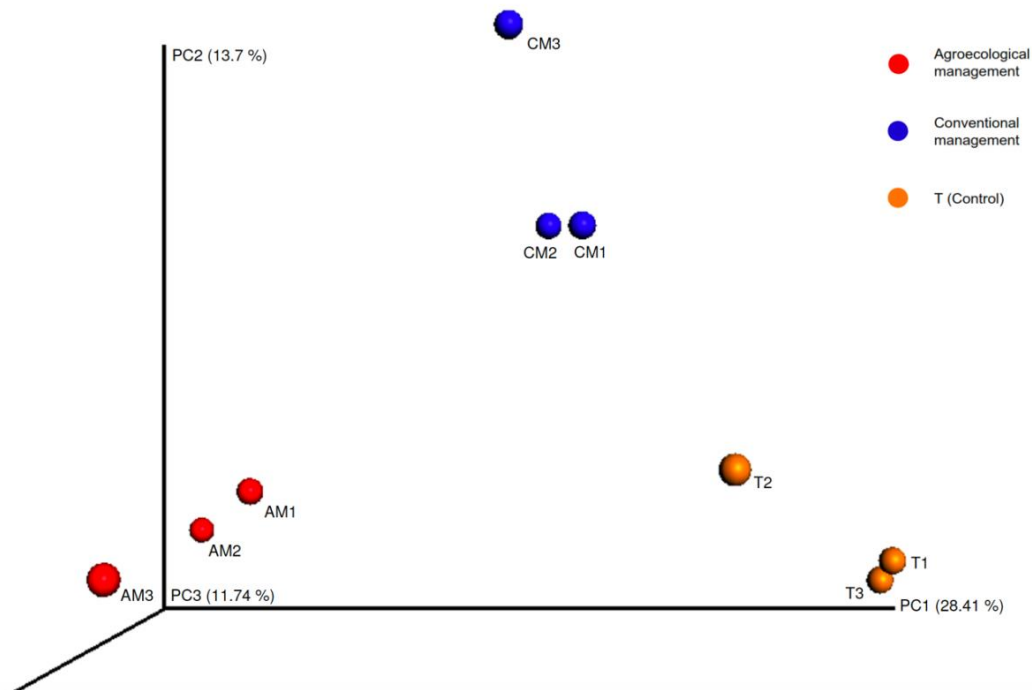
**Figure 3.** Heatmap based on the hierarchical clustering solution (complete clustering method) of the soil samples. Rows represent the 25 predominant bacterial phylum (average abundance >0.01%). Columns represent the soil samples (Agroecological management (AM1-3), Conventional management (CM1-3), and Control (T1-3)).

### Physicochemical properties of the soil

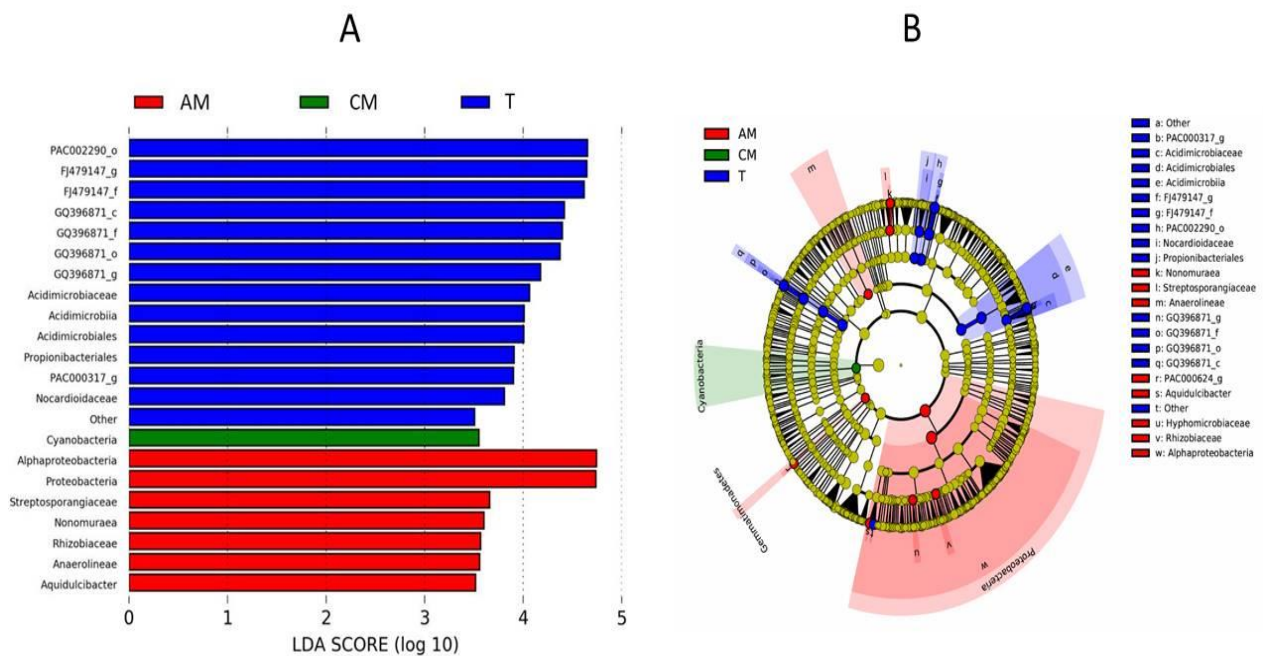
The results of the physicochemical characterization of the soils are shown in Table 2. The pH of soils was affected by the agroecological management (AM), showing the lowest mean ( $7.62 \pm 0.05$ ). Also, electrical conductivity showed significant difference among treatments, being higher for AM ( $446.0 \pm 25.3 \mu\text{Scm}^{-1}$ ), and T ( $419.3 \pm 14.86 \mu\text{Scm}^{-1}$ ).

Regarding SOM, significant differences were found among treatments, where its content was higher in the AM soil ( $21.80 \pm 1.10 \text{ gC kg}^{-1}$ ). The AM soil also displayed a higher TC than the CM soil, but not concerning to T (control). Within the labile carbon (L) and non-labile (NL) carbon fractions due to the oxidation of C by  $\text{KMnO}_4$ , both showed significant differences among treatments, AM soil showing the highest fraction of L ( $17.82 \pm 0.61 \text{ gC kg}^{-1}$ ), and T registering the highest fraction of carbon NL ( $5.94 \pm 0.43 \text{ gC kg}^{-1}$ ).





**Figure 4.** Principal coordinate analysis (PCoA) of the dissimilarity among the soil samples: PCoA plotted against the PC1 vs. PC2 vs. PC3 axes. The percentages indicate the relative contribution of the three principal coordinates (PC1-PC2-PC3).



**Figure 5.** LefSe analysis of bacterial microbiome from Agroecological management (AM), Conventional management (CM), and Control (T) soil samples. (A) Bar graph shows LDA scores which indicate the taxonomic key for differentiation among treatments. (B) The cladogram generated by LefSe indicates the main biomarkers among treatments. Each successive circle represents one phylogenetic level. Red-colored regions indicate taxa enriched in AM, green-colored region represents taxa enriched in CM, and blue-colored regions indicate taxa enriched in T.

**Table 2. Physicochemical parameters from Agroecological management (AM), Conventional management (CM), and Control (T) soil samples. Mean values  $\pm$  standard deviation. Superscript letters indicate significant difference among treatments (One-way ANOVA;  $p < 0.05$ ). EC - Electrical conductivity; SOM - Soil organic matter; TC - Total carbon; L- Labile carbon fraction; NL- Non labile carbon fraction.**

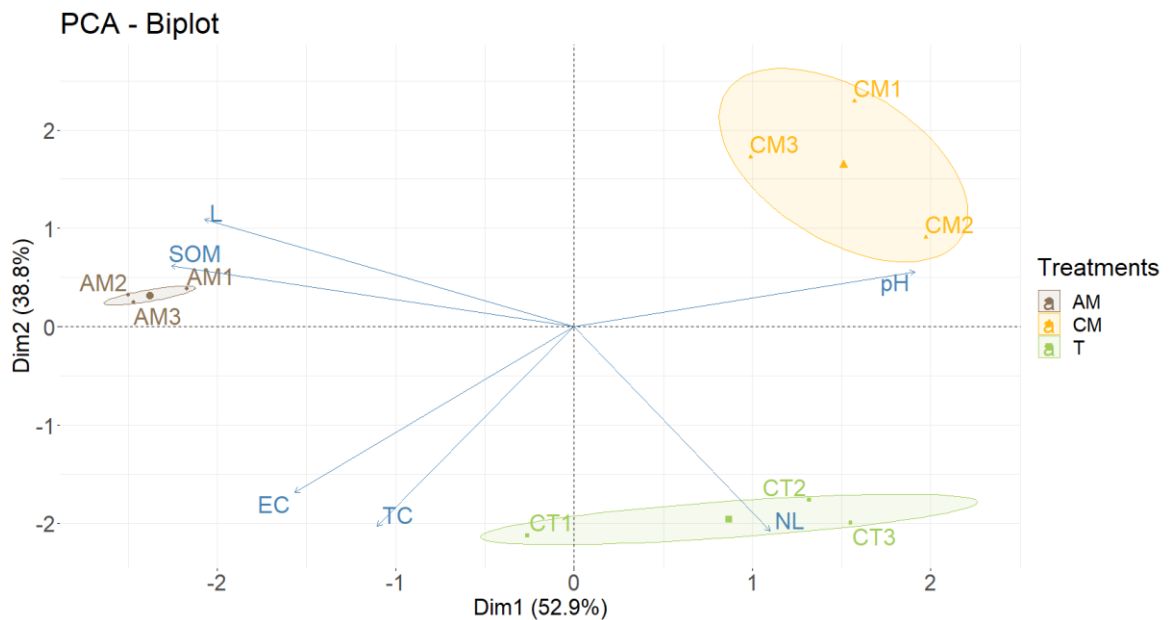
Variable	AM	CM	T	p
pH	7.62 $\pm$ 0.05b	7.87 $\pm$ 0.16a	7.86 $\pm$ 0.32a	0.010
EC, $\mu\text{Scm}^{-1}$	446.00 $\pm$ 25.35a	265.17 $\pm$ 38.04b	419.25 $\pm$ 14.86a	0.001
SOM, $\text{gC kg}^{-1}$	21.80 $\pm$ 1.10a	17.5 $\pm$ 1.50b	18.0 $\pm$ 0.60b	0.000
TC, $\text{gC kg}^{-1}$	19.30 $\pm$ 0.10b	17.1 $\pm$ 0.10c	19.8 $\pm$ 0.10a	0.026
L, $\text{gC kg}^{-1}$	17.82 $\pm$ 0.61a	15.2 $\pm$ 0.61b	13.86 $\pm$ 0.43c	0.000
NL, $\text{gC kg}^{-1}$	1.54 $\pm$ 0.22b	1.88 $\pm$ 0.61b	5.94 $\pm$ 0.43a	0.001

Principal component analysis (PCA) indicated differences between the analyzed soils with a decentralized distribution and an aggregate one (Fig. 6), explaining 92.7% of the variance with 2 components. From the results, it was possible to observe how the addition of organic amendments is related to a higher content of SOM and SOC, especially labile C. Similarly, CM influences a higher pH, while soils without vegetation seem to be related to non-labile C fractions (Table 1). Therefore, the environmental variables that were selected for their importance on the analyzed soils were SOM, SOC, L, NL, and pH.

#### Correlations between environmental variables and bacterial community

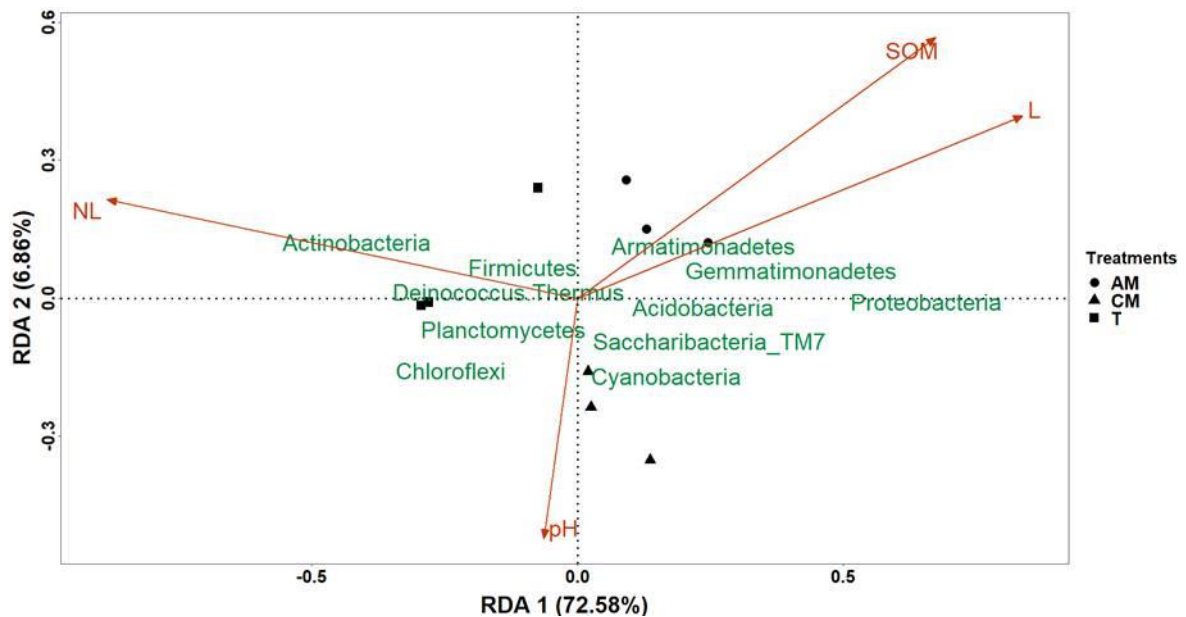
The canonical redundancy analysis of the samples is found in Figure 7. All the phyla found to the left of

the "Y" axis are related to the NL carbon and the T (control) soil (Actinobacteria, Firmicutes, *Deinococcus*). On the other hand, all the bacterial phyla found to the right will be related to the labile carbon fraction and the SOM (Armatimonadales, Gemmatimonadales, Proteobacteria (AM); Acidobacteria, Saccharibacteria\_TM7, and Cyanobacteria (CM)). It is highlighted that the control samples were located on the left side of the axis; while the CM and AM samples are on the right. In this way, all the bacterial phyla that are found up the "X" axis are more abundant in soils with a higher pH (Armatimonadales, Gemmatimonadales, and Proteobacteria), such as soil with CM; at the same time phyla below "X" axis will be in soils with higher EC, and higher concentration of TC and SOM, (*Deinococcus*, *Thermus*, *Planctomyces*, phylum Cyanobacteria, and *Chloroflexi*).



**Figure 6.** Principal component analysis (PCA) of soil properties under different managements. Vectors show the physicochemical soil properties which were related to each treatment. Vectors that pointed in the same direction are associated variables since samples closer to each other exhibited similarity. AM – Agroecological management; CM – Conventional management; T – Control; SOM – Soil organic matter; EC – Electrical conductivity; pH - Potential of hydrogen; TC – Total carbon; L – Labile carbon fraction; NL – No labile carbon fraction.





**Figure 7.** Redundancy analysis showing the effect of soil properties on the relative abundance of the most abundant bacterial taxa in soil samples under different treatments. AM – Agroecological management; CM – Conventional management; T – Control; SOM – Soil organic matter; pH - Potential of hydrogen; L – Labile carbon fraction; NL – No labile carbon fraction.

## DISCUSSION

The pH is one of the most important chemical properties of the soil and it is a good indicator of the balance among nutrients (Smita-Tale and Ingole, 2015) due to its significant effects on the concentration and absorption of solutes (Akpoveta *et al.*, 2010). In The Comarca Lagunera, calcisols (from the Latin calx, lime) are soils with a substantial accumulation of calcareous material, so their pH ranges from 7.2 to 8.3 (moderately alkaline), with the presence of carbonates (Yescas-Coronado *et al.*, 2018), such as the pH in the soils of the present study. The lowest pH was for the site with agroecological management (7.62), which also had the highest content of SOM and SOC with 1.27% and 2.19%, respectively. The decrease in pH can be attributed to SOM rise (Martínez *et al.*, 2008). The pH decreased by organic matter incorporation favors processes such as nitrification and mineralization, and a certain acidity generated by the various edaphic microbial groups. This coupled to the increase of CO<sub>2</sub> (due to the combination of atmospheric CO<sub>2</sub> and soil water that results in carbonic acid), which when dissociated, generates H<sup>+</sup> and causes the pH reduction (Carrasco, 1992; cited by Martínez *et al.*, 2008).

Soil organic matter increased under agroecological management (Table 2). Additionally, degraded soil (T) presented the lowest SOM content. The soil organic carbon (SOC) is directly associated with soil organic matter content (SOM), thus the addition of an

exogen organic amendment source normally increases its concentration. Once in soil, organic matter plays an important role in soil biological activity, due to it gives energetical resources to organisms. SOM also provides colloids with a high cation exchange capacity, which effect on physical properties is manifested by modifying the structure and distribution of the pore space of the soil. The amount of SOM and SOC not only depends on local environmental conditions but is strongly affected by its management (Martínez *et al.*, 2008; Yescas-Coronado *et al.*, 2018). On the other hand, electrical conductivity measures ions present in a solution and varies with depth (Dutta and Ram, 2012; Ingole, 2015). It is a determination that correlates with soil properties that affects its texture, the capacity of cation exchange, drainage conditions, organic matter level, salinity, and subsoil characteristics (Solanki and Chavda, 2012). If the electrical conductivity is less than 1000 (μS cm<sup>-1</sup>), it is considered a normal soil (Deshmukh, 2012), thus the results of this research correspond to this indicator (265–419 μS cm<sup>-1</sup>). The electrical conductivity of soils varies according to the amount of moisture retained by soil particles and is useful for monitoring the mineralization of its organic matter (De Neve *et al.*, 2000), which could indicate the reason for the differences in the EC between treatments. In that sense, the use of bovine manure seems to be a cause of a higher content of salts and increasing the EC in the AM, thus the type of manure should be considered.

Identification of labile SOC fractions let to understand the turnover of SOC and the enhancement of soil quality by management. The results showed an increase of LC under AM, where the incorporation of crop residues seems to increase this C fraction as other agroecological management were reported (Ma *et al.*, 2021; Xue *et al.*, 2018; Zhang *et al.*, 2020). The increase of labile  $\text{KmO}_4\text{-C}$  fraction could be related with a low C/N ratio in added amendments, favoring their decomposition (Mi *et al.*, 2016). However, other parameters such as soil microbiota could also affect the lability of SOC. The benefits from residue management in soil quality and crop yields have been reported by several authors (Piccoli *et al.*, 2020; Shafi *et al.*, 2007; Singh *et al.*, 2018), results that agree with this research. Nevertheless, their potential increases when are combined with other agroecological management such as legumes crop rotation, amendments incorporation or conservation agriculture (Singh *et al.*, 2018; Yescas-Coronado *et al.*, 2018).

Metasequencing has been used to study different soil environments (Alteio *et al.*, 2020; Cabrera-Rodríguez *et al.*, 2020; Clark *et al.*, 2021). Moreover, metagenomic analysis allows analyzing the composition and function of soil microbial communities (Navarrete *et al.*, 2015). There are two fundamental attributes of organisms that affect their distribution and species diversity: their niches and metabolism. The metabolic capacities of an organism must, at least in part, determine its niche, and its niche imposes restrictions on its distribution. Thus, niche distribution, mediated by metabolic requirements, constrains the size and composition of the subset of organisms with niches that match local conditions; thereby limiting local species richness ( $\alpha$  diversity) and change in species composition among sites ( $\beta$  diversity) (Okie *et al.*, 2015). In the present study, significant statistical differences were found for  $\beta$  diversity, as indicated by the PERMANOVA results ( $p < 0.004$ ), and the distribution of groups by principal component results (Fig. 6).

According with PCA, higher pH was associated with conventional management, as well as the genus *Cyanobacteria* (Figure 5). *Cyanobacteria* proliferation in soils is related with several physicochemical properties, as well as other issues determined by biodiversity in the ecosystem. This genus participates in different soil processes such as nitrogen fixation, soil genesis and conservation, SOM decomposition, soil phosphorus cycling, biocontrol, soil aggregation and aeration, among others (Alvarez *et al.*, 2021; Crouzet *et al.*, 2019). Moreover, *Cyanobacteria* could protect plants from adverse environmental conditions due to their physiological characteristics, including the high viscosity of their protoplasmic gel and sheaths with high molecular-

weight heteropolysaccharides and proteins (Bertocchi *et al.*, 1990; Gantar *et al.*, 1995; S. Singh, 2014; Zulpa *et al.*, 2008). In addition, their exopolysaccharides interact with soil particles to stabilize them (Sepehr *et al.*, 2019), and at the same time, they are a source of SOC for soil microorganisms also rising microbial activity (Crouzet *et al.*, 2019; Nisha *et al.*, 2018). Those mechanisms to resist extreme environmental conditions could be the reason why *Cyanobacteria* was a biomarker for conventional management in maize crops; Knapen *et al.*, (2007) reported that *Cyanobacteria* and algae increase cropped topsoil resistance to degradation.

The agroecological management was linked to higher SOC, SOM and L content, as the time that Proteobacteria and Alphaproteobacteria are their more representative biomarkers; *Aquidulcibacter* was the only biomarker genus. Alphaproteobacteria can conform to several environmental conditions, as well as they can interact with plants by symbiotic and non-symbiotically. Besides, this phylum is an important group to measure anthropogenic and environmental impact in a soil ecosystem functional diversity (Singh *et al.*, 2022). For example, Matsushita *et al.* (2015) reported more abundance and diversity of Alphaproteobacteria in organic apple Orchards than in conventional ones; also, Gazdag *et al.* (2018) had similar results in organic and conventional crop managements of maize, wheat, rye and sunflower production systems. Those results agree with the obtained data in the present study. On the other hand, Proteobacteria are copiotrophic bacteria which prefer living in nutrient-sufficient environmental, which could be the reason of their proliferation in the analyzed agroecosystem with manure addition. Mei *et al.* (2021) also reported a significant increase in Proteobacteria abundance in maize agroecosystems where manure was added to soils. Moreover, Zhang *et al.* (2019) found that the abundance of Proteobacteria is directly related with SOC and its labil fractions, such as our results. Likewise, *Aquidulcibacter* population was also increased by agroecological management. This genus was reported recently isolated from cyanobacterial aggregates in the eutrophic Lake Taihu in China as part of the family Caulobacteraceae (Cai *et al.*, 2017). *Aquidulcibacter* are Gram-negative aerobic bacteria, which growth at a pH range between 5.5 and 8.5 (optimum pH 7.0) and at 20-40°C (optimum 30°C). They are catalase and oxidase positive and are capable of nitrate reduction.

Control soil with no vegetation, was related with higher no labile fraction probably due to a higher content of soil inorganic C (SIC) with respect of the other sites, which represent an important carbon fraction in arid and semiarid areas (Gao *et al.*, 2018).

Barren spots areas are also an indicator of high salt content in soils (Reddy *et al.*, 2017). The content of soil carbon (SIC and SOC) varies with several environmental variables, including temperature, precipitation, nitrogen availability, among others (Shi *et al.*, 2012). Additionally, three unidentified phylotypes were the highest biomarkers in T samples: PAC002290\_o, FJ479147\_g and FJ479147\_f, all of them part of Actinobacteria phylum. The phylum Actinobacteria, considered one of the most widely distributed in soils, is recognized for its ability to degrade plant residues *in vitro*, encoding 16% of the total enzymes with activity on carbohydrates. According to Bao *et al.* (2021), their taxonomic and functional compositions were relatively stable during straw decomposition. In our study, it was the dominant phylum (38%), which demonstrates its importance in the degradation process of the incorporation of crop residues, especially in less fertile soils, since they have genes to fix nitrogen and produce antibiotics that favor them to compete and acquire carbon sources and protect against environmental disturbances (Swarnalakshmi *et al.*, 2016; van Bergeijk *et al.*, 2020; Bao *et al.*, 2021). Moreover, members of this phylum live under extreme conditions, due to this taxon size and diversity, its geographical expansion and ecological relevance (Bao *et al.*, 2021). FJ479147\_g, for example, was reported as one of the most abundant actinobacteria members in Atacama Desert in Chile, the most extreme non-polar biome on Earth (Idris *et al.*, 2017). In a similar way, Thomson *et al.* (2010) reported that Acidobacteria increased their abundance in bare soils, as occurs in this study. Thus, microorganisms exhibit an extraordinary phylogenetic and functional diversity and support biogeochemical cycles (Okie *et al.*, 2015), this explains the differences in biomarkers among sites (Fig. 4), with pH being a determining factor at a global level (Delgado-Baquerizo *et al.*, 2018; Lauber *et al.*, 2009; Zhou *et al.*, 2020).

## CONCLUSION

The agroecological management of the soil is the way to conserve and expand microbial diversity and favor the development of bacterial genera that efficiently degrade the organic matter that is incorporated, which will favor the health of the soil. Genomic technologies allow us to have an approach to understanding the importance of microbial groups in soils and their biotechnological implications for sustainable and resilient agricultural development in the medium and long term, especially of the main predominant genera *Tepisiphaera*, *Sphingomonas*, *Microvirga*, *Geodermatophilus* and *Blastococcus*, among others.

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**Author contribution.** J. Vásquez-Arroyo - Conceptualization, Investigation, Writing original draft., Z.L. Ramírez-Guajardo - Methodology, Data curation., E. Blanco-Contreras - Conceptualization, Resources., E. Nava-Reyna - Investigation, Formal analysis, Writing review & editing., G. Zapata-Sifuentes - Project administration., F. Vaca-Paniagua - Methodology, Resources., C.E. Díaz-Velásquez - Methodology, Resources., A. de la Cruz-Montoya - Methodology, Resources., L. M. Valenzuela-Núñez - Methodology, Resources., C. García-De la Peña - Supervision, Visualization, Writing review & editing.

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