

# BACTERIAL DIVERSITY IN SUGARCANE ACROSS DIFFERENT CULTIVATION PERIODS IN SOUTHERN TAMAULIPAS, MEXICO

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## ABSTRACT

This study analyzed the rhizosphere bacterial communities in sugarcane fields subjected to different durations of agronomic management: recent (5 years) and long-term (over 50 years). The objective was to evaluate the diversity of rhizosphere bacteria using 16S rRNA gene sequencing and assess their correlation with soil management practices. Results showed distinct bacterial compositions between recently and long-term managed fields. Significant associations were observed between bacterial species and soil conditions characterized by high electrical conductivity (EC), elevated concentrations of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Fe}^{2+}/\text{Fe}^{3+}$ , and  $\text{Mg}^{2+}$ , and low  $\text{CO}_3^{2-}$  levels. Dominant species included *Bacillus megaterium*, *Bacillus subtilis*, and *Bacillus simplex*, representing the phyla Actinobacteria, Proteobacteria, and Firmicutes, respectively. An analysis of the outlying mean index explained 76.2 % of the total variation, highlighting key soil factors. The first axis (44.7 %) correlated with EC and organic matter content (OM), while the second axis (31.5 %) was associated with extractable phosphorus (P) and potassium (K) levels. These findings underscore the influence of soil physicochemical properties-such as EC, OM, and nutrient availability-on rhizosphere bacterial diversity and composition. These variables modify the soil microenvironment, favoring certain bacterial groups over others and directly impacting soil fertility and agricultural productivity. This study provides insights into the interaction between rhizosphere bacteria and soil management practices. Understanding microbial dynamics in long-term managed systems paves the way for strategies that improve soil health and crop productivity in intensive agricultural systems.

**Additional keywords:** Agricultural soil, microbiota, rhizosphere

## RESUMEN

### Diversidad bacteriana en caña de azúcar en diferentes periodos de cultivo en el sur de Tamaulipas, México

Se estudiaron las comunidades bacterianas en la rizósfera de cultivos de caña de azúcar sometidos a diferentes duraciones de manejo agronómico: reciente (5 años) y a largo plazo (más de 50 años). El objetivo fue evaluar la diversidad de bacterias rizosféricas mediante la secuenciación del gen 16S rRNA y su correlación con las prácticas de manejo del suelo. Los resultados mostraron composiciones bacterianas distintas entre los campos con manejo reciente y prolongado. Se observaron asociaciones significativas entre las especies bacterianas y las condiciones del suelo, caracterizadas por alta conductividad eléctrica (CE), concentraciones elevadas de  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Fe}^{2+}/\text{Fe}^{3+}$  y  $\text{Mg}^{2+}$ , y bajos niveles de  $\text{CO}_3^{2-}$ . Las especies dominantes incluyeron *Bacillus megaterium*, *Bacillus subtilis* y *Bacillus simplex*, pertenecientes a los filos Actinobacteria, Proteobacteria y Firmicutes, respectivamente. El análisis del índice de marginalidad media explicó el 76,2 % de la variación total, destacando factores clave del suelo. El primer eje (44,7 %) se correlacionó con la CE y el contenido de materia orgánica (MO), mientras que el segundo eje (31,5 %) se relacionó con los niveles de P y  $\text{K}^+$  extraíbles, lo que subraya la influencia de las propiedades fisicoquímicas del suelo, tales como la CE, el contenido de MO y la disponibilidad de nutrientes, en la diversidad y composición bacteriana de la rizósfera. Estas variables modifican las condiciones del microambiente del suelo, favoreciendo la presencia de ciertos grupos bacterianos sobre otros, lo que impacta directamente en su fertilidad y productividad agrícola. Este estudio ofrece información sobre la interacción entre las bacterias rizosféricas y las prácticas de manejo del suelo. Comprender las dinámicas microbianas en sistemas de manejo prolongado permite implementar estrategias para mejorar la salud del suelo y la productividad del cultivo en sistemas agrícolas intensivos.

**Palabras clave adicionales:** Microbiota, rizósfera, suelo agrícola

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## INTRODUCTION

The study of bacterial populations in commercial agricultural plots across Mexico

remains limited, despite their critical role in sustainable agriculture. Understanding the physicochemical characteristics of soils in

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cultivated areas and the relationship between microbial community composition in the rhizosphere and cultivation duration is essential because it allows the development of targeted soil management strategies that improve crop productivity and maintain long-term soil health. Such insights can facilitate the development of sustainable land management practices by leveraging the benefits of native microbiota (Sanguin *et al.*, 2006; He *et al.*, 2023; Zhang *et al.*, 2022). Soil microorganisms significantly influence plant health, growth, and productivity, forming intricate interactions with host plants (Cruz *et al.*, 2023; Elbagory, 2023; Palansooriya *et al.*, 2023). Plants actively select microorganisms that promote their growth and reproduction, particularly in monoculture systems, where this selection often leads to a reduction in soil microbial diversity. This phenomenon reflects an adaptive strategy in plant-microbe interactions (Roesch *et al.*, 2007; Wang *et al.*, 2011; Bhattacharyya and Jha, 2012; Ciccazzo *et al.*, 2014).

Identifying soil properties linked to microbial community structure provides a basis for tailored management strategies to enhance crop productivity (Dolédéc *et al.*, 2000; Galperin, 2013; Nyenda *et al.*, 2023). Among these microbial communities, Plant Growth-Promoting Rhizobacteria (PGPR) or rhizobacteria stand out. These microorganisms colonize the rhizosphere, forming symbiotic relationships that benefit both plants and coexisting microbial populations (Sanguin *et al.*, 2006; Aguilar *et al.*, 2013; Ali *et al.*, 2015).

Sugarcane cultivation in production areas faces challenges such as declining productivity and the adverse effects of excessive chemical fertilizer use. These practices degrade the physicochemical and microbiological properties of soils, with cascading impacts on crop yields and economic outcomes. Excessive use of synthetic fertilizers leads to nutrient imbalances, soil acidification, and reduced organic matter content, which in turn diminish microbial diversity and soil fertility. Over time, this degradation lowers the soil's capacity to support healthy plant growth, resulting in decreased productivity and higher dependency on external inputs (Jongenburger *et al.*, 2010; Visi *et al.*, 2013; Adams *et al.*, 2015; House *et al.*, 2015). Alternative strategies, including reducing

chemical fertilizer dependence and increasing PGPR inoculation, have been proposed to mitigate these impacts. These approaches improve soil microbiota, enhance productivity, and lower production costs (Arunrat *et al.*, 2022; Ducousso *et al.*, 2022).

This study focuses on the diversity of soil bacteria associated with varying durations of sugarcane cultivation in Southern Tamaulipas, Mexico. It aims to investigate the relationship between bacterial diversity and soil management practices. Using 16S rRNA gene sequencing, the study seeks to analyze the distribution and diversity of rhizospheric bacteria, offering insights into their connection to sustainable soil management within sugarcane cultivation systems.

## MATERIALS AND METHODS

**Study area and sampling.** Six sugarcane plots cultivated with the CP 72-2086 variety (*Saccharum officinarum* L.) were selected for this study based on their cultivation history: three plots with less than five years of management, and three with over 50 years of continuous sugarcane cultivation. The plots are located near the city of Ocampo, Tamaulipas, Mexico (22°50' N, 99°22' W). The soil in these areas is classified as pelic vertisol, according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015).

In each plot, rhizosphere samples were collected from five randomly selected sugarcane plants within a circular area of 1-meter diameter centered in the plot, avoiding border effects by keeping an adequate distance from plot boundaries. Sampling was performed at five random points per plot. Samples were transported to the laboratory under controlled conditions and stored at -80 °C for subsequent analysis. A 1 kg subsample was air-dried at room temperature for 72 hours until reaching a constant weight, corresponding to a final moisture content of approximately 10%, before being subjected to physicochemical analysis conducted by the Soil Analysis Laboratory at the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) in Rio Bravo, Tamaulipas, Mexico.

**Physicochemical analysis.** The physicochemical properties analyzed included organic matter (%)

OM), extractable potassium (K), pH, electrical conductivity (EC), soil texture components (% sand, % clay, % silt), and nutrient contents (N, P, Ca, Mg, Na, K). Additional parameters such as carbonates ( $\text{CO}_3$ ), bicarbonates ( $\text{HCO}_3$ ), chlorides (Cl), sulfates ( $\text{SO}_4$ ), and trace elements (Cu, Zn, Mn, Fe) were also evaluated. These variables were selected for their relevance to sugarcane cultivation. Differences between plots with varying cultivation durations were analyzed using a Kruskal-Wallis ANOVA (STATISTICA v8.0, StatSoft, Inc.) (Ali *et al.*, 2015).

**Bacterial isolation and cultivation.** To isolate beneficial bacterial strains, 1 g of rhizosphere soil from each sampling point was processed in triplicate. Samples were serially diluted to  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$ , and 100  $\mu\text{L}$  of each dilution was spread onto sterile Petri dishes containing Miller's LB agar medium (10  $\text{g}\cdot\text{L}^{-1}$  casein peptone, 5  $\text{g}\cdot\text{L}^{-1}$  yeast extract, 10  $\text{g}\cdot\text{L}^{-1}$  NaCl, 15  $\text{g}\cdot\text{L}^{-1}$  agar; pH 7.0-7.2). Plates were incubated at 30 °C for 48 hours. Colony counts were performed, and the average colony number was calculated for each dilution, adjusted by the dilution factor.

**Selection and purification of bacterial strains.** Bacterial strains were selected based on distinct colony morphology. Selected colonies were subcultured on LB agar plates and incubated at 30 °C for 48 hours. This process was repeated until pure isolates were obtained. For preliminary identification, morphological characteristics were compared using Bergey's Manual of Systematic Bacteriology (Garrrity *et al.*, 2005).

**DNA extraction and sequence analysis.** Genomic DNA was extracted from pure cultures using the PrepMan® Ultra Sample Preparation Reagent (Applied Biosystems). The 16S rRNA gene was amplified using universal primers 27F and 1492R through PCR. The reaction conditions included an initial denaturation at 95 °C for 3 min., followed by 25 cycles of denaturation at 95 °C (30 s), annealing at 55 °C (30 s), and extension at 72 °C (30 s), with a final extension at 72 °C for 5 min. Amplicons were subjected to sequencing for bacterial identification. Sequence quality, editing, and assembly were performed using Sequence Scanner Software v2 (Applied Biosystems) and DNASTAR Lasergene v12.2. BLAST analysis against the NCBI database was performed using a sequence similarity threshold of 97 % for species-level identification.

**Statistical analyses.** Mean Marginality Analysis (Outlying Mean Index): this analysis, performed using an ADE-4 software, assessed the relationship between bacterial presence/absence and soil variables. A one-way PERMANOVA was conducted using Past 3.04 software to evaluate differences in bacterial presence across cultivation durations. A Kruskal-Wallis Test, was used to compare the soil physicochemical characteristics between plots, performed in STATISTICA v8.0. Finally, a Phylogenetic Analysis: the genetic relationships among bacterial isolates were analyzed using the Jukes-Cantor distance and the Neighbor-Joining method. A phylogenetic tree was constructed with bootstrap support (1000 replicates) using CLC Sequence Viewer v7.0.2 (QIAGEN).

## RESULTS AND DISCUSSION

The physicochemical characterization of the soil samples revealed significant variations linked to the duration of agronomic management in sugarcane plots. While parameters such as pH, electrical conductivity, and soil texture (sand, clay, and silt content) did not show notable changes, others, including organic matter (OM), extractable potassium ( $\text{K}^+$ ), nitrates ( $\text{NO}_3^-$ ), phosphorus (P), calcium ( $\text{Ca}^{2+}$ ), sulfates ( $\text{SO}_4^{2-}$ ), copper ( $\text{Cu}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), manganese ( $\text{Mn}^{2+}$ ), and iron ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ), exhibited significant differences (Table 1). These findings reflect the cumulative impact of agronomic practices on soil fertility and nutrient availability over time.

**Soil physicochemical properties.** Both soil management conditions maintained an alkaline pH (~8), indicative of salinity and alkalinity issues commonly reported in sugarcane monoculture systems. This pH stability, alongside consistent soil texture parameters, suggests resilience to agronomic modifications over time. However, nutrient profiles exhibited distinct differences. Soils under long-term management (>50 years) demonstrated higher concentrations of calcium, magnesium, and zinc. These elevated nutrient levels, combined with increased organic matter content, likely enhanced microbial activity and diversity, contributing to improved soil fertility. Interestingly, sugarcane plots with extended agronomic management (50 years) reported higher fruit yields (100  $\text{tons}\cdot\text{ha}^{-1}$ ) compared to plots with

recent management (5 years), which achieved 80 tons·ha<sup>-1</sup>. Nevertheless, the average sugarcane yield in Tamaulipas (44 tons·ha<sup>-1</sup>) remains significantly below the national peak yield, reported in Morelos (121 tons·ha<sup>-1</sup>), the leading sugarcane-producing state in Mexico. It is worth

noting that elevated concentrations of chlorides (Cl<sup>-</sup>) and sulfates (SO<sub>4</sub><sup>2-</sup>) were detected in long-term managed plots, indicating the cumulative effects of prolonged chemical fertilizer use, which may contribute to soil salinity and long-term productivity challenges.

**Table 1.** Result of the physicochemical analysis of soil samples from plots cultivated with sugarcane (variety CP 72-2086) collected in the municipality of Ocampo, Tamaulipas, Mexico.

Soil parameters*	Agricultural plots					
	1	2	3	4	5	6
	Management duration					
	< 5 years	< 5 years	< 5 years	> 50 years	> 50 years	> 50 years
OM (%)	8.6	4.6	8.6	9.2	4.1	3.4
Extractable K <sup>+</sup> (ppm)	976	420	100	147	265	132
pH	8.9	8.1	8.0	8.0	7.9	8.0
EC (dS/m)	1.6	1.2	1.6	1.9	1.0	0.8
Sand (%)	51.8	55.8	37.8	47.8	38.2	31.8
Clay (%)	34.2	26.2	46.2	32.2	44.2	50.2
Silt (%)	14.0	18.0	16.0	20.0	17.6	18.0
Textural Class	Scl*	Scl	Clay	Cl*	Clay	Clay
N-NO <sub>3</sub> <sup>3</sup> (ppm)	66.7	49.8	61.6	55.1	24.4	9.6
P(ppm)	83.7	15.5	9.1	10.5	1.8	13.6
Ca <sup>2+</sup> (me/l)	6.8	5.9	7.7	8.3	5.2	4.1
Mg <sup>2+</sup> (me/l)	4.6	3.8	4.5	5.0	4.3	3.7
Na <sup>+</sup> (me/l)	0.1	0.1	0.1	0.1	0.1	0.1
K <sup>+</sup> (me/l)	0.3	0.1	0.6	0.7	0.2	0.1
CO <sub>3</sub> <sup>2-</sup>	2.7	3.3	2.2	1.6	2.2	4.3
HCO <sub>3</sub> <sup>-</sup>	16.3	24.9	17.9	46.7	14.1	19.5
Cl <sup>-</sup>	6.0	6.6	7.6	9.4	9.0	5.8
SO <sub>4</sub> <sup>2-</sup>	7.5	4.2	2.8	7.2	1.9	2.4
Cu <sup>2+</sup>	2.4	2.6	3.1	1.8	1.0	1.6
Zn <sup>2+</sup>	42.3	34.7	54.7	27.8	33.4	22.1
Mn <sup>2+</sup>	51.6	57.7	264.0	46.1	48.7	40.8
Fe <sup>2+</sup> /Fe <sup>3+</sup>	2.3	2.7	11.7	9.7	0.9	1.1

\*OM= Organic Matter content expressed as a percentage; K<sup>+</sup>= Potassium; pH= Hydrogen ion potential (measure of acidity/alkalinity); EC= Electrical Conductivity(measure in deciSiemens per meter);N-NO<sub>3</sub><sup>3</sup>= Nitrate; P= Phosphorus; Ca<sup>2+</sup>= Calcium; Mg<sup>2+</sup>= Magnesium; Na<sup>+</sup>= Sodium; CO<sub>3</sub><sup>2-</sup>= Carbonates; HCO<sub>3</sub><sup>-</sup>= Bicarbonates; Cl<sup>-</sup>= Chlorides; SO<sub>4</sub><sup>2-</sup>= Sulfates; Cu<sup>2+</sup>= Copper; Zn<sup>2+</sup>= Zinc; Mn<sup>2+</sup>= Manganese; Fe<sup>2+</sup>/Fe<sup>3+</sup>= Iron; ppm= Parts Per Million; me/L= Milliequivalents per Liter; Scl= Sandy clay loam; \*Cl= Clay loam.

Organic matter content was notably higher in soils with long-term management, aligning with studies that emphasize the role of organic inputs from crop residues in enhancing soil quality (Cabrera and Zuaznábar, 2010; Wakgari *et al.*, 2018). Furthermore, the elevated levels of calcium and magnesium likely contributed to soil structure stabilization, facilitating microbial colonization and nutrient cycling processes. Essential soil

nutrients such as phosphorus and potassium also significantly influence the composition and abundance of bacterial communities (Cumpa *et al.*, 2021). Conversely, reduced concentrations of these essential elements' phosphorus, potassium, and nitrogen can activate the presence of bacteria from the order *Rhizobiales*. This order includes species known for forming symbiotic relationships with plant roots and playing a critical role in

nutrient cycling (Rathinasabapathi *et al.*, 2018; Yaghoubi *et al.*, 2021).

**Microbial composition and diversity.** The bacterial communities associated with the sugarcane rhizosphere exhibited significant differences between recently and long-term managed systems. A total of 38 bacterial species were identified, classified into 11 genera across three phyla: Firmicutes, Proteobacteria, and Actinobacteria. The phylum Firmicutes dominated, comprising 26 species, followed by Proteobacteria with 8 species, and Actinobacteria with 4 species.

The distribution of bacterial species varied according to soil management duration. Twelve species were exclusive to recently managed plots, while 14 were found exclusively in long-term managed plots. The remaining 12 species were shared between both systems (Table 2 and Figure 2). This variation highlights the impact of soil physicochemical properties and agricultural

practices on bacterial community composition, a phenomenon previously documented by Ciccazzo *et al.* (2014).

Particularly noteworthy is the prominent presence of *Bacillus* species in the sugarcane rhizosphere across both study plots. This observation aligns with prior research on sugarcane rhizospheres (Pirhadi *et al.*, 2018), which reported a strong dominance of the *Bacillus* genus. *Bacillus* species are well-documented for their roles in promoting plant growth, enhancing nutrient availability, and improving resistance to biotic and abiotic stressors.

These findings underline the significant influence of agricultural management duration on the structure and composition of rhizosphere bacterial communities. Furthermore, the prevalence of *Bacillus* spp. in both systems suggest its adaptive advantage and potential utility in biotechnological applications to enhance sugarcane productivity and sustainability.

**Table 2.** Presence of identified bacterial species from the rhizosphere of sugarcane (variety CP 72-2086) considering the variable agricultural management time in the municipality of Ocampo, Tamaulipas, Mexico.

Agronomic management time		
Less than 5 years	More than 50 years	Both
<i>Arthrobacter siccitolerans</i>	<i>Acinetobacter</i> sp.	<i>Arthrobacter globiformis</i>
<i>Arthrobacter</i> sp.	<i>Bacillus barbaricus</i>	<i>Bacillus megaterium</i>
<i>Bacillus aryabhattai</i>	<i>Bacillus cereus</i>	<i>Bacillus pumilus</i>
<i>Bacillus methylotrophicus</i>	<i>Bacillus circulans</i>	<i>Bacillus simplex</i>
<i>Bacillus nealsonii</i>	<i>Bacillusidriensis</i>	<i>Bacillus</i> sp.
<i>Bacillus safensis</i>	<i>Bacillus licheniformis</i>	<i>Bacillus subtilis</i>
<i>Bacillus subterraneus</i>	<i>Bacillus mycoides</i>	<i>Bacillus thuringiensis</i>
<i>Brevundimonas aurantiaca</i>	<i>Bacillus niacini</i>	<i>Fictibacillus barbaricus</i>
<i>Ensifer adhaerens</i>	<i>Bacillus soli</i>	<i>Pseudomonas koreensis</i>
<i>Paenibacillus barcinonensis</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas</i> sp.
<i>Paenibacillusillinoisensis</i>	<i>Pseudomonas mediterranea</i>	<i>Staphylococcus</i> sp.
<i>Pseudomonas putida</i>	<i>Staphylococcus haemolyticus</i>	<i>Terribacillus saccharophilus</i>
	<i>Streptomyces venezuelae</i>	
	<i>Virgibacillus</i> sp.	

Statistical analyses confirmed significant differences in bacterial communities between the two soil management systems. A PERMANOVA

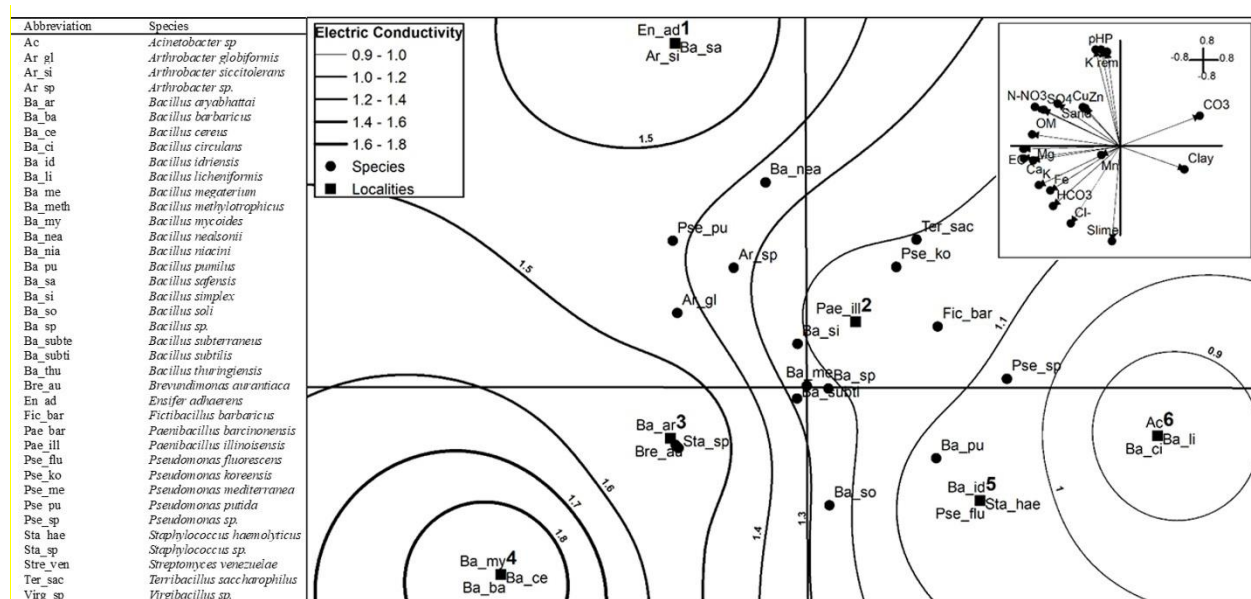
test demonstrated distinct microbial compositions ( $F= 4.232$ ;  $P= 0.0003$ ), with Marginal Mean Analysis accounting for 76.2 % of the observed

variance. Key drivers included electrical conductivity and organic matter along the first axis (44.7 %), and phosphorus and potassium along the second axis (31.5 %) (Figure 1). These results emphasize the intricate relationships between soil physicochemical properties and microbial community structures.

Specific bacterial species were strongly associated with particular soil characteristics, such as elevated electrical conductivity and key elements like calcium, potassium, iron, and magnesium. These correlations suggest that the bacterial community composition in the rhizosphere is shaped by both the availability of essential nutrients and broader soil chemical properties.

Notably, several bacterial species were highly representative in sugarcane plots, with their presence or absence significantly associated with specific soil conditions ( $p \leq 0.0001$ ). These included *Bacillus barbaricus*, *B. cereus*, *B. megaterium*, *B. mycoides*, *Pseudomonas mediterranea*, *Streptomyces venezuelae*, and *Virgibacillus sp.*

Understanding these associations provides critical insights into microbial ecology within sugarcane cultivation systems. These findings may inform agricultural management strategies aimed at enhancing soil fertility, optimizing microbial interactions, and improving crop productivity under varying management practices.



**Figure 1.** Bivariate dispersion of the centroid of the bacterial species and factor structure of the edaphic variables obtained from the Mean Marginality Analysis in soils cultivated with sugarcane, collected in the municipality of Ocampo, Tamaulipas, Mexico.

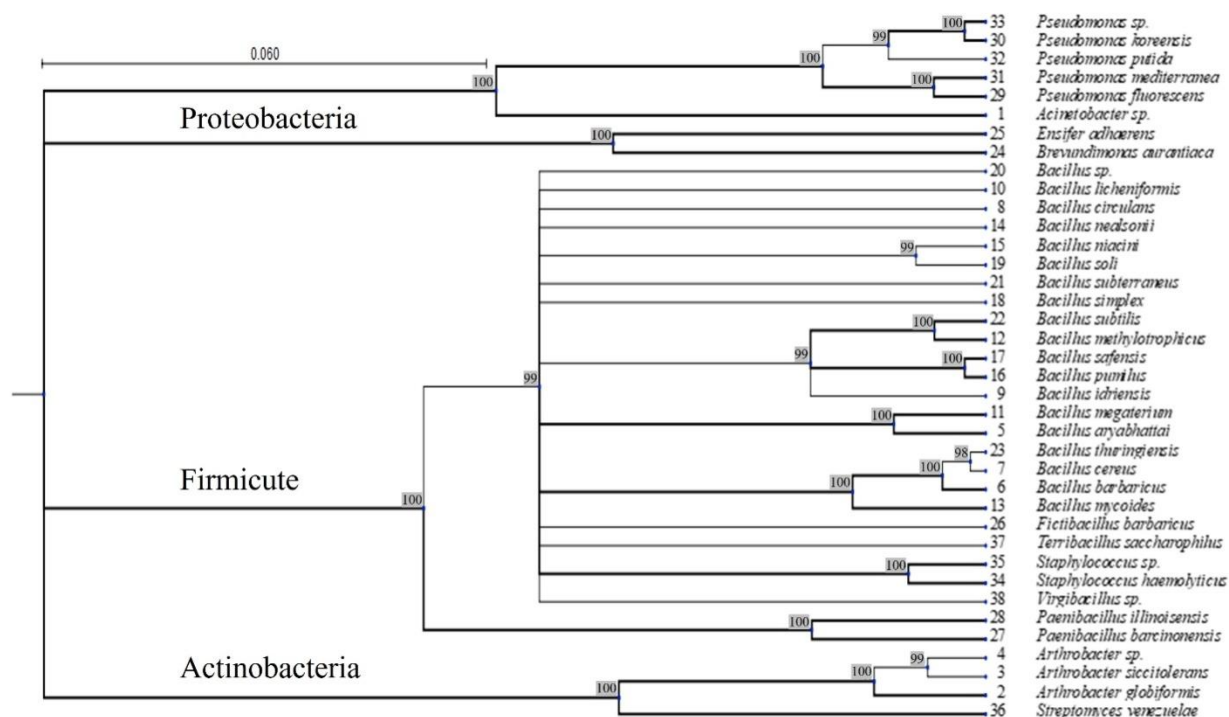
**Cluster analysis of bacterial communities.** Cluster analysis using the Jukes-Cantor distance and neighbor-joining algorithm identified three distinct bacterial groups based on their distribution across management systems (Figure 2). Two groups (34 species) were dominated by members of the genus *Bacillus* and *Pseudomonas*, with a relatively even distribution between management soil. A third group (four species) included the genera *Arthrobacter* and *Streptomyces* highlighting broader ecological functions within the microbiome.

Notably, species such as *Bacillus barbaricus*, *B. cereus*, *B. megaterium*, *B. mycoides*, *Pseudomonas mediterranea*, *Streptomyces venezuelae*, and *Virgibacillus sp.* were significantly associated with soils rich in calcium, potassium, iron, and magnesium. These associations suggest that long-term management conditions favor bacteria with specific nutrient requirements, potentially contributing to sustained soil fertility and crop productivity

**Functional role of microbial communities.** The presence of beneficial bacterial species in

both management systems highlights their functional roles in nutrient cycling, plant growth promotion, and stress mitigation. Members of the genus *Bacillus* are well-known for their plant growth-promoting abilities, including phosphorus solubilization, nitrogen fixation, and production of

phytohormones (Kumar and Chandra, 2020). Similarly, *Pseudomonas mediterranea* and *Streptomyces venezuelae* are associated with biocontrol properties, offering resistance against soil-borne pathogens (Quecine *et al.*, 2012; Ciccazzo *et al.*, 2014).



**Figure 2.** Phylogenetic relationship of bacteria associated with the rhizosphere of sugarcane cultivation (variety CP 72-2086) in the municipality of Ocampo, Tamaulipas, Mexico.

Interestingly, the dominance of Firmicutes in both systems aligns with their adaptive strategies under stress conditions, such as salinity and alkalinity, which are characteristic of the studied soils. Their ability to form endospores enables them to thrive in fluctuating environmental conditions, providing a stable microbial foundation for plant-microbe interactions.

**Agronomic implications and potential for sustainable practices.** The productivity analysis revealed that long-term managed plots achieved average yields of  $\sim 100$  tons $\cdot$ ha $^{-1}$ , outperforming recently managed plots ( $\sim 80$  tons $\cdot$ ha $^{-1}$ ). This observation challenges the conventional notion that prolonged monoculture depletes soil fertility. Instead, it suggests that microbial communities in long-term managed soils contribute significantly to maintaining productivity by enhancing nutrient availability and plant resilience.

The role of organic matter in sustaining these microbial communities cannot be overstated. As observed, higher organic matter content in long-term managed soils provided a conducive environment for diverse microbial populations. This is consistent with findings by Pisa *et al.* (2011) and Juma *et al.* (2018), who emphasized the importance of organic inputs in promoting soil health and microbial diversity.

The results highlight the potential for leveraging microbial communities to enhance sugarcane productivity sustainably. The presence of functionally diverse bacteria across both management systems underscores the importance of maintaining soil health through balanced nutrient management and organic inputs.

Future strategies could focus on integrating microbial inoculants, such as *Bacillus* and *Pseudomonas* species, into agronomic practices to

promote plant health and resilience. Additionally, monitoring shifts in microbial communities in response to environmental changes can provide insights into soil quality and productivity trends.

The findings align with previous research demonstrating the central role of soil microbiota in crop productivity and sustainability. Studies by Pirhadi *et al.* (2018) and Rosa *et al.* (2020) have highlighted similar microbial patterns in sugarcane rhizospheres, reinforcing the idea that long-term management systems can sustain productive microbial communities. However, the observed variations in bacterial composition and function in this study emphasize the need for localized approaches to soil management, tailored to specific agroecological conditions.

**Limitations and future directions.** While this study provides valuable insights into the interplay between soil properties and microbial communities, several limitations warrant consideration. First, the reliance on 16S rRNA sequencing, although effective for bacterial identification, may overlook functional attributes at the genomic level. The obtained sequences had an average length of approximately 1,400 base pairs, covering the nearly full-length 16S rRNA gene. Future studies could incorporate metagenomics to gain a deeper understanding of microbial functionality.

Second, the study is focused on bacterial communities, excluding other important microbial groups such as fungi and archaea. Including these groups in future research could provide a more comprehensive view of soil microbiota and their contributions to sugarcane productivity.

Finally, the study's observational design limits causal inferences about the relationship between soil properties, microbial communities, and crop yields. Experimental interventions, such as controlled inoculations or nutrient amendments, could help clarify these interactions.

## CONCLUSIONS

The study demonstrated significant differences in the composition and structure of bacterial communities in the sugarcane rhizosphere under recent ( $\leq 5$  years) and long-term ( $>50$  years) agricultural management. These differences were closely linked to variations in soil physicochemical characteristics, such as organic

matter, phosphorus, potassium, and micronutrient availability.

The findings highlight the influence of agronomic practices and soil conditions on microbial ecology, emphasizing the importance of nutrient management to maintain soil health and optimize crop productivity. The adaptive responses of bacterial species, particularly those within the *Bacillus* genus, underscore their role in nutrient cycling and potential contributions to sugarcane resilience under diverse management conditions.

Understanding the interplay between microbial communities and soil characteristics provides valuable insights for developing sustainable agricultural strategies aimed at enhancing sugarcane yield and long-term soil fertility.

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