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Soil Core Bacteria and Main Edaphic Functions in Pecan Nut Trees (*Carya illinoinensis*) Rhizosphere in an Arid Agroecosystem

Núcleo Bacteriano del Suelo y sus Principales Funciones Edáficas en la Rizósfera de Árboles de Nuez Pecanera (*Carya illinoinensis*) en un Agroecosistema Árido

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SUMMARY

Microorganisms are fundamental for preserving plant health and productivity, thus knowledge of their composition and metabolism in a particular agroecosystem could be used as a tool to sustainably improve or maintain crop performance. Therefore, this study aimed to identify the bacterial core and its functional characteristics in the rhizosphere of *Carya illinoinensis*. This study was carried out in the arid north of Mexico (Viesca, Coahuila) in 2022 in an orchard of 20-25-year-old young trees, with a nut production per tree of 20-22 kg. Sampling was carried out at five points in the orchard: the four corners and the center. Metagenomic analyses of the 16S rRNA gene were performed to determine the taxonomic composition of the microbial species. In addition, functional predictions were made using Phylogenetic Community Research by Reconstruction of Unobserved States (PICRUSt) to estimate gene abundance within metabolic pathways. The ASVs of the bacterial population of the studied orchard were classified and identified into 60 phyla, 130 classes, 392 orders, 40 families, 40 genera, and 1766 species. Proteobacteria, Acidobacteriota, and Actinobacteriota were the most abundant phyla. At the genus level, Pararheinheimera ($\bar{X} = 5.5\%$) and two unknown genera of the Thermoanaerobaculia class ($\bar{X} = 3.2\%$ and $\bar{X} = 2.7\%$, respectively) were the main taxa. In addition, glucose oxidation, phospholipase, and L-rhamnose production were the main metabolic pathways associated with the bacterial community, which confer plant growth-promoting properties and crop resilience under biotic and abiotic stress conditions. Thus, knowledge of the local bacteriome is a tool to propose the appropriate agronomic management to exploit the potential of key taxa to promote plant growth or isolate specific strains for the generation of biofertilizers.

Index words: *arid zones, metagenomics, plant growth-promoting bacteria.*

RESUMEN

Los microorganismos del suelo son fundamentales para preservar la salud y productividad de las plantas, por lo que conocer su composición y metabolismo en un agroecosistema particular puede ser utilizado como herramienta para mantener o mejorar el rendimiento de los cultivos sosteniblemente. Por lo anterior, este estudio tuvo como objetivo identificar el núcleo bacteriano y sus características funcionales en la rizosfera de *Carya illinoinensis*. Este estudio se realizó en el norte árido de México (Viesca, Coahuila) en 2022 en una huerta de árboles jóvenes de 20-25 años de edad, con una producción de nuez por árbol de 20-22 kg. El muestreo se realizó en cinco puntos del huerto: las cuatro esquinas y el centro. Se hicieron análisis metagenómicos del gen 16S rRNA para determinar la composición taxonómica de las especies microbianas. Además, se realizaron predicciones funcionales utilizando la Investigación Filogenética de Comunidades por Reconstrucción de Estados. No Observados (PICRUSt) para estimar la abundancia de genes dentro de las rutas metabólicas. Las ASV de la población bacteriana del huerto estudiada se clasificaron e identificaron en 60 filos, 130 clases, 392 órdenes, 40 familias, 40 géneros y 1766 especies. Proteobacteria, Acidobacteriota y Actinobacteriota fueron los filos más abundantes. A nivel de género, Pararheinheimera ($\overline{X}$ = 5,5%) y dos géneros desconocidos de la clase Thermoanaerobaculia ($\overline{X}$ = 3.2% y $\overline{X}$ = 2.7%, respectivamente) fueron los principales taxones. Además, la oxidación de la glucosa, la producción de fosfolipasas y la producción de L-ramnosa fueron las principales vías metabólicas asociadas a la comunidad bacteriana, lo que le confiere propiedades promotoras del crecimiento vegetal y resiliencia del cultivo bajo condiciones de estrés biótico y abiótico. Así, el conocimiento del bacterioma local es una herramienta para plantear el manejo agronómico adecuado para explotar el potencial de los taxones clave para promover el crecimiento vegetal o aislar cepas específicas para la generación de biofertilizantes.

Palabras clave: *zonas áridas, metagenómica, bacterias promotoras del crecimiento vegetal*.

INTRODUCTION

Pecan nut (*Carya illinoinensis* (Wangeh.) K. Koch) is the most important deciduous fruit tree in the southern United States and northern Mexico. Moreover, Mexico is the second pecan nut producer worldwide, with a planted area of 84 429 ha, with an average yield of 1.91 Mg ha^{-1} (González, Ortega, Morales, Palacios, and Lucero, 2022; SIAP, 2022), concentrated mostly in the arid northern Mexico, where the state of Coahuila is an important walnut-producing state with a production of 18 328 Mg ha^{-1} from a harvest of 16 768 ha of pecan nut (SIAP, 2022). Climate change represents an ecological and productivity concern for nut crops (Bernard, Lheureux, and Dirlewanger, 2017), where there is a special interest in decreasing chemical inputs and developing novel strategies to manage crops to resist current and future biotic and abiotic stress conditions adapted to local conditions without affecting yields. It is known that the environment highly influences interactions between plants and their microbiome and is fundamental for plant health, production, and resilience to stressed conditions (Singh, Liu, and Trivedi, 2020; Trivedi, Mattupalli, Eversole, and Leach, 2021; Trivedi, Batista, Bazany, and Singh, 2022), making these microorganisms an important strategy to improve yield, decrease production cost, and increase the sustainability of crops.

Microorganisms represent the largest biotic group in soil (Hartmann and Six, 2023). Moreover, microbiomes play a key role in agroecosystems, ranging from soil fertility to potential tolerance to soil stress. Thus, the microbiome performs key functions such as soil structure, aggregation, water flow regulation, and nutrient utilization (Kennedy, 1999; Hartmann and Six, 2023). However, little is known about the microbiota harbored in agroecosystems of forest interest, which represent areas of opportunity for the use and conservation of biotic resources (Sánchez-Ledesma, Garibay, Guevara, Ávila, and Arreola, 2022), in order to prevent degradation and loss of soil biodiversity, mainly in arid zones. It is estimated that there are between one million and one billion microorganisms per gram of agricultural soil (Kopittke, Menzies, Wang, McKenna, and Lombi, 2019), making soils the most complex and diverse ecosystems in the world. Furthermore, since microbial communities are fundamental for preserving plant health and productivity, knowledge of their composition and metabolism in a particular agroecosystem could be used as a tool to improve or maintain plant performance. In this context, specific microbial taxa such as *Rheinheimera* and *Thermoanaerobaculi*a have attracted attention due to their ecological and biotechnological roles. *Rheinheimera* participates in the bioremediation of contaminated waters; in addition, its use as a probiotic and its potential as a source of bioactive compounds with antimicrobial and antioxidant properties are being investigated (Sisinthy, Chakraborty, Adicherla, and Gundlapally, 2017; Wang *et al*., 2022). Moreover, *Thermoanaerobaculia* have the potential to metabolize nitrate but not to fix molecular N, ammonia, or urea, and could utilize carbohydrates, including chitin, cellulose, lactose, and simple sugars such as sucrose and glucose (Flieder *et al*., 2021; White III *et al*., 2023).

On the other hand, advances in molecular techniques now enable the detection of more than 98% of soil bacterial diversity (Green, Bohannan, and Whitaker, 2008; Segata *et al*., 2013; Zarraonaindia, Smith, and Gilbert, 2013), providing a comprehensive understanding of microbial consortia that influence plant growth and agricultural productivity. However, knowledge regarding the microbiota associated with pecan (*Carya illinoinensis*) remains limited. We hypothesize that the rhizosphere of pecan trees under high-yield conditions harbors a core bacterial community with specific taxonomic and functional traits that enhance plant growth and resilience under arid environments. Therefore, this study aimed to characterize the bacterial composition and predominant metabolic pathways in the rhizosphere of a highly productive pecan orchard in Coahuila, Mexico, using next-generation sequencing approaches. The findings are expected to expand the current understanding of microbial communities in arid agroecosystems and to provide a foundation for designing agronomic strategies and developing biofertilizers.

MATERIALS AND METHODS

Study Area

The present study was conducted in an orchard of *Carya illinoinensis* located in the municipality of Viesca, Coahuila, Mexico (Figure 1), which has an elevation of 1100 m of altitude, an average temperature of 25 °C, and an annual rainfall of 100-400 mm, with a very dry semi-warm climate during most of the year (INEGI, 2024). The orchard has Wichita and Western varieties, in a 12 × 12 m real frame plantation system, with a subsurface drip irrigation system and a clay loam-textured soil, a field capacity of 0.35 m^3 m^{-3}, and a permanent wilting point of 0.19 m^3 m^{-3} (Sánchez-Ledesma *et al*., 2022). The soils had a pH of 8.18, an electrical conductivity of 421 87, and an organic matter content of 34.6 g kg^{-1}. The orchard was managed conventionally according to the technological package for pecan nut production (INIFAP, 2012).

Soil Sampling

A total area of 25 ha was sampled, comprising young trees of 20-25 years, with nut production per tree ranging between 20-22 kg. X-shaped sampling was carried out and used in agriculture for its high precision, where the five sites comprising the four corners and the center of the orchard were sampled. Rizospheric soil samples for each site were deposited in BashingBead™ lysis buffer with 750 µL Xpedition™ Zymo Research™ Lyse/Stabilizer buffer. Samples were then shaken in a cell disruptor (TerraLyzer™) and frozen until DNA was extracted.

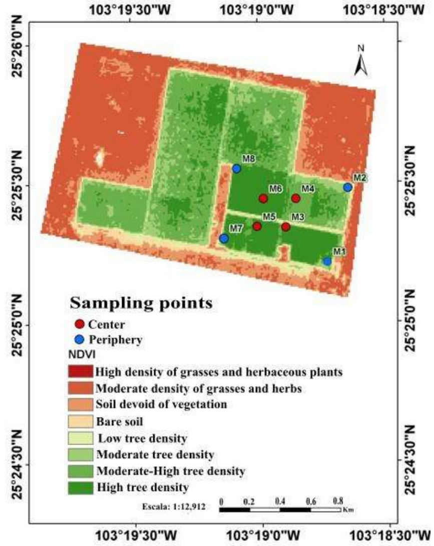

Figure 1. Location of the study site, "Tierra Blanca" orchard, located in the municipality of Viesca, Coahuila, Mexico.

DNA Extraction

DNA extraction was performed using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research Corp., Irvine, CA, USA), following the manufacturer's protocol. The concentration and quality of DNA were measured using a Qubit® 2.0 (Invitrogen, Carlsbad, CA, USA).

16S rRNA Amplicon Sequencing

The samples were processed by Novogene Corporation Inc. (Davis, CA), where V3–V4 16S rRNA amplicons were obtained using 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) primers. PCR reactions were carried out with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 2 µM of forward and reverse primers, and approximately 10 ng of template DNA. Thermal cycling consisted of an initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The same volume of 1X loading buffer (containing SYBR Green) and PCR products were mixed and electrophoresed on a 2% agarose gel for detection. PCR products were then pooled in equimolar proportions and purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA), following the manufacturer's recommendations, and index codes were added. Library quality was assessed on a Qubit® 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Finally, the libraries were sequenced on an Illumina NovaSeq platform to generate 250 bp paired-end reads.

Bioinformatic and Statistical Analysis

Taxonomic composition. Sequence analysis was performed using QIIME2 (Bolyen *et al*., 2019). Subsequently, we used the Divisive Amplicon Denoising Algorithm 2 (DADA2) to denoise the sequence, which included removing low-quality sequences, filtering noisy, chimeric, and singleton sequences, and correcting errors in the edge sequences (Callahan *et al*., 2016; Callahan, McMurdie, and Holmes, 2017). Then, the output files from DADA2 were obtained, including a feature table (raw Amplicon Sequence Variants (ASVs) table) and representative ASVs sequences. The QIIME2 feature-classifier plugin was then used to align the ASV sequences to the Greengenes2 database (McDonald *et al*., 2022) to assign taxonomy. Relative abundance was obtained at all taxonomic levels, and plots were developed using RStudio and Morpheus software (Broad Institute).

Predicted metabolic pathways. The metabolic pathways of the soil bacterial communities were predicted using the Picrust2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) pipeline (Douglas *et al*., 2020). The gene function annotation and classification analysis were performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) and MetaCyc library to obtain the metabolic pathways.

Data Availability

The files used in this study were deposited in the NCBI Sequence Read Archive (SRA) database (Accession number: SUB14450874).

RESULTS AND DISCUSSION

The mean number of sequences assembled in the pecan orchard was 109 493.16, with a mean quality of the bacterial sequences in all the samples of 103 973.33. The abundance of ASVs in the tree soil population was 70 895 (Table 1).

Taxonomic Composition of the Microbiome

The ASVs of the bacterial population of the studied orchard were classified and identified into 60 phyla, 130 classes, 392 orders, 40 families, 40 genera, and 1766 species. From the total bacteria, 677 are non-cultivable species, 1031 are unknown, and 58 have taxonomic names, which were confirmed by means of a BLAST in NCBI, using the representative sequences. Sequences whose percentage of identity was 97% and with an E value of 0.0 were considered as valid species (Table 2).

Table 1. Process for obtaining non-chimeric sequences (clean sequences) from DADA2 in soil samples from pecan nut orchards.

ID	Total, readings	Filtered	% of filter	Readings denoised	Merged reading	% of merged reading	Non-chimeric	% of non-chimeric
M01	151 536	108 578	71.6	103 998	74 566	49.21	59 702	39.4
M02	148 536	130 272	87.7	120 911	74 091	49.88	57 036	38.4
M03	148 804	109 008	73.2	105 407	86 589	58.19	75 759	50.9
M04	64 207	54 482	84.8	52 412	17 587	27.39	15 581	24.2
M05	161 078	119 245	74.0	115 047	94 522	58.68	83 274	51.7
M06	151 345	135 374	89.4	126 065	78 015	51.55	49 812	32.9
Mean	137 584.33	109 493.16	80.1	103 973.33	70 895	49.15	56 860.6	39.5

The most abundant phylum in the total sequence was Proteobacteria ($\bar{X}$ = 39.17%), followed by Acidobacteriota ($\bar{X}$=24.46%) and Actinobacteriota ($\bar{X}$ = 4.41%) (Figure 2). Proteobacteria have been reported as a predominant phylum in several plant soil microbiomes (Liu *et al*., 2018; Xi *et al*., 2023), including pecan nut trees (Cabrera-Rodriguez *et al*., 2020; Rodriguez-Ramos, Scott, Marty, Kaiser, and Hale, 2022; Shi *et al*., 2023). Bacteria from this phylum are copiotrophic, which grow in carbon-rich soils for fast growth and high metabolism (Kuzyakov and Razavi, 2019). Acidobacteria can also respond to labile carbon sources, which facilitates their rapid growth and adaptation to various types of plant rhizospheres, and helps to degrade cellulose and lignin (i.e., carbon cycling) (Salas-Leiva *et al*., 2021). In addition, several species of Actinobacteria are plant symbionts linked to metabolic processes related to nitrogen fixation and release of P from organic sources (Garaycochea, Romero, Beyhaut, Neal, and Altier, 2020; Salas-Leiva *et al*., 2021; Sellstedt, and Richau, 2013). The undoubted role of Proteobacteria, Acidobacteria, and Actinobacteria as adjacent natural ecosystem species in predicting bacterial diversity is important in *C. illinoinensis* trees for biodiversity management and conservation in agroecosystems and as plant growth promoters (Palma-Cano *et al*., 2021; Peng *et al*., 2024).

Table 2. Bacteria identified at the species level in pecan nut (*Carya illinoinensis*) orchard, in Viesca, Coahuila, Mexico.

Species	Species	Species	Species
Accumulibacter aalborgensis	*Bacteroides fragilis*	*Pseudomonas kunmingensis*	*Rubellimicrobium aerolatum*
Acidaminococcus fermentans	*Brevundimonas aveniformis*	*Pseudorhodoplanes sinuspersici*	*Rubellimicrobium rubrum*
Acinetobacter baumannii	*Brevundimonas kwangchunensis*	*Pseudoxanthomonas daejeonensis*	*Ruminococcus bicirculans*
Acinetobacter johnsonii	*Campylobacter portucalensis*	*Pseudoxanthomonas kaohsiungensis*	*Sphingomicrobium sediminicola*
Adlercreutzia hattorii	*Cetobacterium somerae*	*Pseudoxanthomonas Mexicana*	*Sphingosinicella ginsenosidimutans*
Aestuariivirga litoralis	*Cloacibacterium normanense*	*Ralstonia pickettii*	*Sutterella parvirubra*
Agathobacter rectalis	*Clostridium perfringens*	*Ramlibacter montanisoli*	*Suttonella indologenes*
Aliibacillus thermotolerans	*Collinsella tanakaei*	*Reyranella aquatilis*	*Tahibacter aquaticus*
Allisonella histaminiformans	*Filifactor alocis*	*Reyranella massiliensis*	*Terribacillus halophilus*
Arenimonas daejeonensis	*Flavimobilis soli*	*Reyranella soli*	*Terrimicrobium sacchariphilum*
Arenimonas oryziterrae	*Flavobacterium ustbae*	*Rhabdothermincola sediminis*	*Thalassobacillus pellis*
Asticcacaulis benevestitus	*Metarhizobium album*	*Rheinheimera faecalis*	*Traorella massiliensis*
Azohydromonas australica	*Pseudalkalibacillus decolorationis*	*Rhizorhapis suberifaciens*	*Xenophilus arseniciresistens*
Azotobacter chroococcum	*Pseudobdellovibrio exovorus*	*Rhodocytophaga rosea*	
Bacteroides fluxus	*Pseudokineococcus lusitanus*	*Roseburia inulinivorans*	

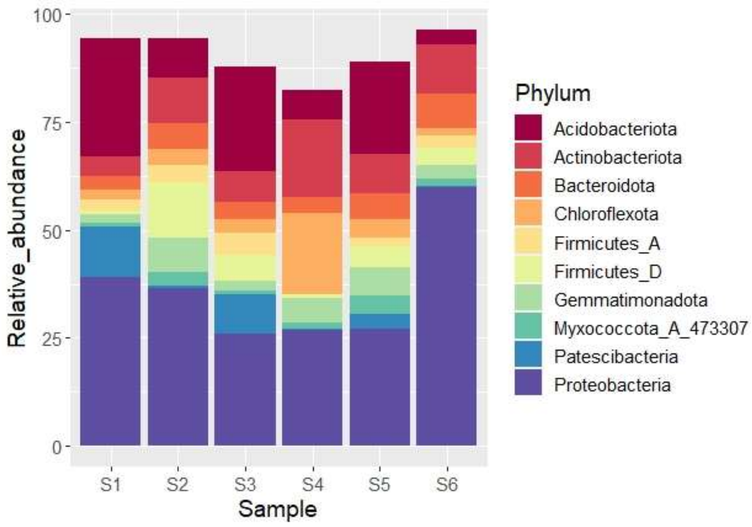

Figure 2. Relative abundance of healthy and productive soil bacterial communities in pecan nut *Carya illinoinensis* orchards at the phylum level. Each bar represents the top 10 bacterial taxa.

At the class level, the most dominant were Gammaproteobacteria ($\overline{X}$ = 21.0%), Alphaproteobacteria ($\overline{X}$ =14.9%), and Vicinamibacteria ($\overline{X}$ =7.3%), whereas at order level, Enterobacterales ($\overline{X}$ = 8.4%), Vicinamibacterales ($\overline{X}$ = 6.8%), and Sphingomonadales ($\overline{X}$ = 4.0%) were the most abundant taxa. Overall, at family level, Alteromonadaceae ($\overline{X}$ = 5.6%), Sphingomonadaceae ($\overline{X}$ = 4.0%), and Thermoanaerobaculia; UBA5704 ($\overline{X}$ = 3.3%) were the most representative taxa, as long as at genus level, they were Pararheinheimera ($\overline{X}$ = 5.5%), and two unknown genus of Thermoanaerobaculia class ($\overline{X}$ = 3.2% and $\overline{X}$ =2.7%, respectively) (Figure 3).

Thermoanaerobaculia is an important taxon capable of nitrogen fixation and thrives at temperatures above 45 °C (Ivanova, Zhelezova, Chernov, and Dedysh, 2020). This class has also been described in the rhizosphere of a few plants, such as switchgrass and *Robinia pseudoacacia* (Li, Fan, Yang, Yang, and Shangguan, 2023; White III *et al*., 2023). In addition, it is involved in the metabolism of L-rhamnose (glucosidases), participating in the catalysis and hydrolysis of rhamnosides (Romero-Calvo, 2018[1]), and has been reported as an important organism in sulfur cycling in marine sediments (Flieder *et al*., 2021). On the other hand, bacteria from the genus *Pararheinheimera* are denitrifying and photosynthetic, with potential roles as bioremediation agents in saline soils due to the production of L-lysine oxidase, an oxidoreductase enzyme with antimicrobial activity that may also influence plant–pathogen interactions (Dang *et al*., 2021; Iskandar *et al*., 2021; Sardinha-Francisco *et al*., 2023). This genus has also been reported in the rhizosphere of peanut and wheat (Hirpara, Hinsu, and Kothari, 2024; Sardinha-Francisco *et al*., 2023). The life beneath the soil is crucial for resource availability and for understanding the impact of environmental and anthropogenic factors. Soil microbiota, together with management practices, contribute significantly to sustainable agriculture (Gupta *et al*., 2022; Ray, Lakshmanan, Labbé, and Craven, 2020; Solomon, Janda, and Molnár, 2023). Consequently, associations of specific plant species with bacterial taxa are primarily linked to their active roles in promoting plant growth (Berg, Grube, Schloter, and Smalla, 2014). The agriculturally important microorganisms identified in this study are expected to play vital roles in crop health and productivity by contributing to integrated nutrient management and providing disease and pest control, thereby reducing reliance on agrochemicals without compromising yields (Bano, Wu, and Zhang, 2021; Nwachukwu and Babalota, 2021; Solomon *et al*., 2023). Moreover, studies on agroecosystem habitats have shown that various biotic and abiotic edaphic factors strongly influence bacterial diversity and structure, suggesting that they may act as ecological drivers of the species richness found in this study (Peng *et al*., 2024). Recent studies have advanced the characterization of the *Carya illinoinensis* microbiome across different locations, consistently revealing the presence of a conserved bacterial core. Shi *et al*. (2025) reported significant β-diversity differences between aboveground and belowground habitats, indicating niche-driven community assembly. Similarly, Cervantes, Heerema, and Randall (2022) identified a core microbiome in New Mexico orchards that included families such as

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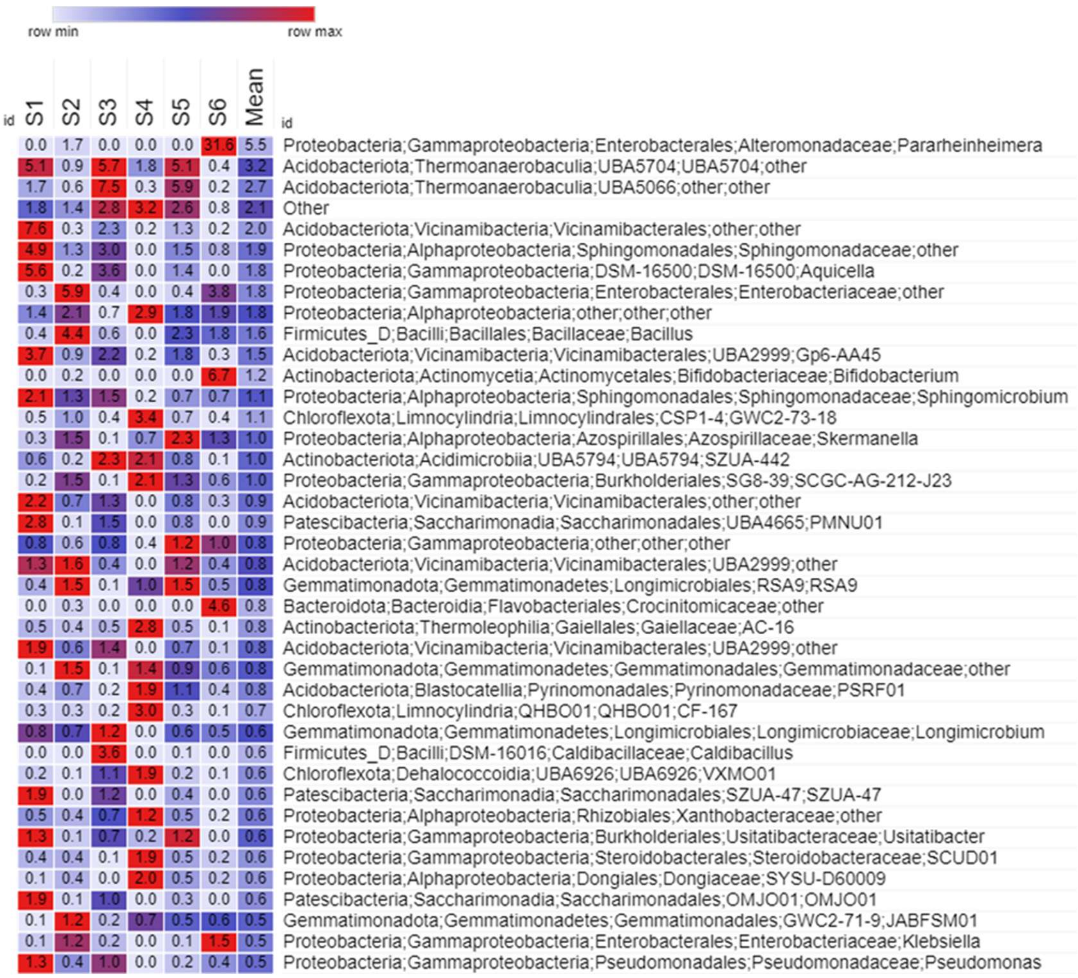
[1] Romero-Calvo, P. (2018). *Identificación y caracterización bioquímica de una nueva ramnosidasa de Lactobacillus plantarum WCFS1*. Tesis para obtener el grado de Maestria en Ciencias. Universidad Complutense de Madrid. Disponible en: http://hdl.handle.net/10261/172510

Rhizobiaceae, Chitinophagaceae, Burkholderiaceae, Sphingomonadaceae, and Pseudomonadaceae, with Sphingomonadaceae emerging as a central taxon; these findings were later corroborated by Ren, Zhang, Tondre, Wang, and Xu (2024). Tang, Liu, Bao, Chu, and Peng (2022) further demonstrated that soil properties–particularly pH, phosphorus, moisture, and nitrogen—strongly shape community composition, while Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi consistently dominate at the phylum level. In addition, Cervantes *et al*. (2022) reported organ-specific microbiomes in seedlings, with Chitinophagaceae enriched in roots and Rhizobiaceae and Moraxellaceae predominating in stems and leaves. Collectively, these studies support the existence of a conserved bacterial core in pecan rhizospheres, providing a relevant framework to contextualize our findings of abundant *Pararheinheimera* and Thermoanaerobaculia taxa.

Genome Prediction Analysis by PICRUSt

According to the PICRUSt analysis, the main metabolic pathways associated with the bacterial communities in the soil pecan nut samples were aerobic respiration I (cytochrome c), L-isoleucine biosynthesis II, DHGLUCONATE-PYR-CAT-PWY (glucose oxidation), LIPASYN-PWY (phospholipase production), and PWY-6713 (L-rhamnose production).

From the prediction of metabolic functions with the KEEG database, it was found that aerobic respiration I (cytochrome c) is one of the core functional traits of microbial communities. This metabolic pathway, apart from being present in the mitochondrial matrix, is also involved in ATP production (Sousa, D'Imprima, and Vonck, 2018). Another pathway inferred in the studied pecan orchard soils is the biosynthesis of L-isoleucine II, which is related to increased fruit production and facilitates proper nutrient uptake (Karl *et al*., 2020). In addition, it improves flowering size and fruit quality (Ghouili *et al*., 2022).



S1	S2	S3	S4	S5	S6	Mean	id
0.0	1.7	0.0	0.0	0.0	31.6	5.5	Proteobacteria;Gammaproteobacteria;Enterobacterales;Alteromonadaceae;Pararheinheimera
5.1	0.9	5.7	1.8	5.1	0.4	3.2	Acidobacteriota;Thermoanaerobaculia;UBA5704;UBA5704;other
1.7	0.6	7.5	0.3	5.9	0.2	2.7	Acidobacteriota;Thermoanaerobaculia;UBA5066;other;other
1.8	1.4	2.8	3.2	2.6	0.8	2.1	Other
7.6	0.3	2.3	0.2	1.3	0.2	2.0	Acidobacteriota;Vicinamibacteria;Vicinamibacterales;other;other
4.9	1.3	3.0	0.0	1.5	0.8	1.9	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;other
5.6	0.2	3.6	0.0	1.4	0.0	1.8	Proteobacteria;Gammaproteobacteria;DSM-16500;DSM-16500;Aquicella
0.3	5.9	0.4	0.0	0.4	3.8	1.8	Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;other
1.4	2.1	0.7	2.9	1.8	1.9	1.8	Proteobacteria;Alphaproteobacteria;other;other;other
0.4	4.4	0.6	0.0	2.3	1.8	1.6	Firmicutes_D;Bacilli;Bacillales;Bacillaceae;Bacillus
3.7	0.9	2.2	0.2	1.8	0.3	1.5	Acidobacteriota;Vicinamibacteria;Vicinamibacterales;UBA2999;Gp6-AA45
0.0	0.2	0.0	0.0	0.0	6.7	1.2	Actinobacteriota;Actinomycetia;Actinomycetales;Bifidobacteriaceae;Bifidobacterium
2.1	1.3	1.5	0.2	0.7	0.7	1.1	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomicrobium
0.5	1.0	0.4	3.4	0.7	0.4	1.1	Chloroflexota;Limnocylindria;Limnocylindrales;CSP1-4;GWC2-73-18
0.3	1.5	0.1	0.7	2.3	1.3	1.0	Proteobacteria;Alphaproteobacteria;Azospirillales;Azospirillaceae;Skermanella
0.6	0.2	2.3	2.1	0.8	0.1	1.0	Actinobacteriota;Acidimicrobiia;UBA5794;UBA5794;SZUA-442
0.2	1.5	0.1	2.1	1.3	0.6	1.0	Proteobacteria;Gammaproteobacteria;Burkholderiales;SG8-39;SCGC-AG-212-J23
2.2	0.7	1.3	0.0	0.8	0.3	0.9	Acidobacteriota;Vicinamibacteria;Vicinamibacterales;other;other
2.8	0.1	1.5	0.0	0.8	0.0	0.9	Patescibacteria;Saccharimonadia;Saccharimonadales;UBA4665;PMNU01
0.8	0.6	0.8	0.4	1.2	1.0	0.8	Proteobacteria;Gammaproteobacteria;other;other;other
1.3	1.6	0.4	0.0	1.2	0.4	0.8	Acidobacteriota;Vicinamibacteria;Vicinamibacterales;UBA2999;other
0.4	1.5	0.1	1.0	1.5	0.5	0.8	Gemmatimonadota;Gemmatimonadetes;Longimicrobiales;RSA9;RSA9
0.0	0.3	0.0	0.0	0.0	4.6	0.8	Bacteroidota;Bacteroidia;Flavobacteriales;Crocinitomicaceae;other
0.5	0.4	0.5	2.8	0.5	0.1	0.8	Actinobacteriota;Thermoleophilia;Gaiellales;Gaiellaceae;AC-16
1.9	0.6	1.4	0.0	0.7	0.1	0.8	Acidobacteriota;Vicinamibacteria;Vicinamibacterales;UBA2999;other
0.1	1.5	0.1	1.4	0.9	0.6	0.8	Gemmatimonadota;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;other
0.4	0.7	0.2	1.9	1.1	0.4	0.8	Acidobacteriota;Blastocatellia;Pyrinomonadales;Pyrinomonadaceae;PSRF01
0.3	0.3	0.2	3.0	0.3	0.1	0.7	Chloroflexota;Limnocylindria;QHBO01;QHBO01;CF-167
0.8	0.7	1.2	0.0	0.6	0.5	0.6	Gemmatimonadota;Gemmatimonadetes;Longimicrobiales;Longimicrobiaceae;Longimicrobium
0.0	0.0	3.6	0.0	0.1	0.0	0.6	Firmicutes_D;Bacilli;DSM-16016;Caldibacillaceae;Caldibacillus
0.2	0.1	1.1	1.9	0.2	0.1	0.6	Chloroflexota;Dehalococcoidia;UBA6926;UBA6926;VXMO01
1.9	0.0	1.2	0.0	0.4	0.0	0.6	Patescibacteria;Saccharimonadia;Saccharimonadales;SZUA-47;SZUA-47
0.5	0.4	0.7	1.2	0.5	0.2	0.6	Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;other
1.3	0.1	0.7	0.2	1.2	0.0	0.6	Proteobacteria;Gammaproteobacteria;Burkholderiales;Usitatibacteraceae;Usitatibacter
0.4	0.4	0.1	1.9	0.5	0.2	0.6	Proteobacteria;Gammaproteobacteria;Steroidobacterales;Steroidobacteraceae;SCUD01
0.1	0.4	0.0	2.0	0.5	0.2	0.6	Proteobacteria;Alphaproteobacteria;Dongiales;Dongiaceae;SYSU-D60009
1.9	0.1	1.0	0.0	0.3	0.0	0.6	Patescibacteria;Saccharimonadia;Saccharimonadales;OMJO01;OMJO01
0.1	1.2	0.2	0.7	0.5	0.6	0.5	Gemmatimonadota;Gemmatimonadetes;Gemmatimonadales;GWC2-71-9;JABFSM01
0.1	1.2	0.2	0.0	0.1	1.5	0.5	Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Klebsiella
1.3	0.4	1.0	0.0	0.2	0.4	0.5	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas

Figure 3. Heatmap diagram of microbiota composition at the genus level for pecan nut orchard soil. The top 40 genera shared by all samples are shown.

Another three main metabolic pathways were identified in this study: DHGLUCONATE-PYR-CAT-PWY (glucose oxidation), LIPASYN-PWY (phospholipase production), and PWY-6713 (L-rhamnose production). For glucose oxidation, bacterial communities assist plant roots with glucose-oxygen reactions, and it is easily metabolized in soils with little aeration, helping not only the growth of crops but also the aerobic microorganisms that inhabit them (Borer, Kleyer, and Or, 2022; Kim, Fravel, and Papavizas, 1988; Ross, 1968). Furthermore, the phospholipase pathway is ubiquitous in all organisms, and plants respond to auxin as a growth stimulator and help protect against pathogens (Fang, Jiang, Ding, Li, and Xie, 2023). Therefore, the presence of this pathway in agricultural soils maintains maximum yields in production and phosphorus enrichment in the soil (Kameyama *et al*., 1996; Kuroshima and Hayano, 1988; Shakeel-ur-Rehman, Fleming, Farkye, and Fox, 2003; Yang *et al*., 2024). In contrast, the L-rhamnose metabolic pathway is produced by plants and microorganisms present in nature (Jiang, Dillon, Silva, Gomez, and Grotewold 2021; Linhardt, Bakhit, Daniels, Mayerl, and Pickenhagen, 1989). Some bacteria possess a degradation pathway that involves non-phosphorylated intermediates and structural polysaccharides that can be used as an additive to improve soil aggregation, thereby increasing the amount of temporary cementing agents and carbon stock. Thus, this pathway is important in agroecosystems as an indicator of soil quality and influences further degradation of organic matter (Muñoz-Bojorges *et al*., 2015).

CONCLUSIONS

This study characterized the rhizosphere bacterial community of *Carya illinoinensis* in highly productive orchards under arid conditions, identifying the dominant taxa and their predicted metabolic pathways. The results confirm the presence of a core microbiota with functional traits potentially linked to plant growth promotion and resilience to environmental stress. These findings provide a robust baseline for understanding the microbial ecology of pecan agroecosystems and highlight the importance of leveraging native microbial communities to enhance soil health and crop productivity. While descriptive, this work establishes the groundwork for future applied research focused on isolating key bacterial strains, developing biofertilizers, and designing sustainable agronomic strategies tailored to arid environments.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS’ CONTRIBUTIONS

Conceptualization: C.G.P. and J.A.S.L.; Methodology: E.N.R. and J.A.S.L.; Software: I.P.T. and P.J.L.; Validation: E.N.R., C.G.P., and J.A.S.L.; Formal analysis: J.A.S.L., C.G.P., and E.N.R.; Investigation: J.A.S.L. and I.P.T.; Resources: J.A.S.L.; Data curation: C.G.P. and J.A.S.L.; Writing – original draft preparation: J.A.S.L., C.G.P., and E.N.R.; Writing – review and editing: C.G.P. and E.N.R.; Visualization: J.A.S.L., E.N.R., and C.G.P.; Supervision: E.N.R.; Project administration: J.A.S.L. and E.N.R.; Funding acquisition: J.A.S.L., C.G.P., and E.N.R.

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