TERRA LATINOAMERICANA

Soil Physicochemical Parameters Determine the Structure of Bacterial Communities in Cacao (*Theobroma cacao* L.) Agroecosystems: Evidence from Metabarcoding

Los Parámetros Fisicoquímicos del Suelo Determinan la Estructura de la Comunidad Bacteriana en Agroecosistemas de Cacao (*Theobroma cacao* L.): Evidencia a partir de Metabarcoding

Ronald Oswaldo Villamar-Torres[1], Edgar Efraín Milán-Chela[2], Gregorio Humberto Vásconez-Montufar[1], Danilo Yánez-Cajo[1‡], Seyed Mehdi Jazayeri[3], and Ousseini Issaka Salia[4]

[1] Universidad Técnica Estatal de Quevedo, Campus La María, Facultad de Ciencias Pecuarias y Biológicas, Carrera Agropecuaria. Vía Quevedo-El Empalme km 7, Campus "La María". 120501 Quevedo, Los Ríos, Ecuador; (R.O.V.T), (G.H.V.M.), (D.Y.C.).
[‡] Corresponding autor: dyanezc@uteq.edu.ec
[2] Universidad Técnica Estatal de Quevedo, Campus Central, Facultad de Posgrados, Maestría en Biotecnología Agropecuaria. Av. Quito km 11.5, vía a Santo Domingo de los Tsáchilas. 120501 Quevedo, Los Ríos, Ecuador; (E.E.M.Ch.).
[3] Universidad de Aviñón, Unidad Propia de Investigación Innovadora. 301 Rue Baruch de Spinoza. 84140 Aviñón, Francia; (S.M.J.).
[4] Washington State University. 305 Clark Hall, PO Box 646353, Pullman, WA 99164-6353, USA; (O.I.S).

SUMMARY

This study evaluated the effect of three cacao (*Theobroma cacao* L.) production systems monoculture (T1), diversified (T2), and agroforestry (T3) on soil physicochemical properties and bacterial community structure. Soil samples were collected using a systematic sampling design and analyzed for physical and chemical indicators. Bacterial communities were characterized through high-throughput sequencing of the 16S rRNA gene (V3–V4 region), followed by bioinformatic processing and multivariate statistical analyses. The diversified system (T2) exhibited significantly improved soil conditions, including higher pH, organic matter, and nutrient availability (N, P, K, Ca, Mg), compared to monoculture (T1), while T3 showed intermediate values. These edaphic differences were associated with changes in microbial diversity and composition. Alpha diversity indices (Observed, Chao1, Shannon, Simpson) were significantly higher in T2 and lowest in T1 ($p < 0.05$). Beta diversity analyses (PCoA based on Aitchison and Bray–Curtis distances) revealed clear separation among treatments, indicating distinct community structures. At the taxonomic level, differences were observed not only at the family level but also at the genus level. Monoculture soils were relatively enriched in genera affiliated with *Acidobacteria* and *Bradyrhizobium*, whereas diversified systems showed higher relative abundance of genera such as *Rhizobium, Burkholderia*, and *Streptomyces*, commonly reported in association with nutrient cycling processes. Agroforestry systems presented intermediate compositions, including genera such as *Sphingomonas* and *Pseudomonas*. Multivariate analyses (db-RDA) indicated that soil pH, organic matter, and nutrient availability were significant drivers of bacterial community structure. These results provide evidence that cacao production systems are associated with shifts in soil properties and microbial assemblages. Overall, diversification was linked to improved soil conditions and greater microbial diversity, suggesting its potential role in promoting more stable soil environments in tropical agroecosystems.

Index words: *agricultural sustainability, bioinformatics, high-throughput sequencing, microbial diversity.*

RESUMEN

Este estudio evaluó el efecto de tres sistemas de producción de cacao (*Theobroma cacao* L.) monocultivo (T1), diversificado (T2) y agroforestal (T3) sobre las propiedades fisicoquímicas del suelo y la estructura de la comunidad bacteriana.

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Las muestras de suelo se recolectaron mediante un diseño sistemático y se analizaron indicadores físicos y químicos. La comunidad bacteriana se caracterizó mediante secuenciación de alto rendimiento del gen 16S rRNA (región V3–V4), seguida de análisis bioinformático y estadístico multivariante. El sistema diversificado (T2) presentó condiciones edáficas significativamente mejores, incluyendo mayor pH, contenido de materia orgánica y disponibilidad de nutrientes (N, P, K, Ca y Mg), en comparación con el monocultivo (T1), mientras que el sistema agroforestal (T3) mostró valores intermedios. Estas diferencias se asociaron con cambios en la diversidad y composición microbiana. Los índices de diversidad alfa (Observed, Chao1, Shannon y Simpson) fueron significativamente mayores en T2 y menores en T1 ($p < 0.05$). Los análisis de diversidad beta (PCoA con distancias de Aitchison y Bray–Curtis) evidenciaron una clara separación entre tratamientos. A nivel taxonómico, se observaron diferencias tanto a nivel de familia como de género. El monocultivo presentó mayor abundancia relativa de géneros asociados a *Acidobacteria* y *Bradyrhizobium*, mientras que el sistema diversificado mostró mayor representación de géneros como *Rhizobium*, *Burkholderia* y *Streptomyces*, comúnmente vinculados con procesos de ciclado de nutrientes. El sistema agroforestal presentó una composición intermedia, incluyendo géneros como *Sphingomonas* y *Pseudomonas*. El análisis multivariante (db-RDA) indicó que el pH, la materia orgánica y la disponibilidad de nutrientes fueron factores determinantes en la estructuración de la comunidad bacteriana. En conjunto, los resultados evidencian que los sistemas de manejo del cacao están asociados con cambios en las propiedades del suelo y en el microbioma, y sugieren que la diversificación puede contribuir a mejorar las condiciones edáficas y la diversidad microbiana en agroecosistemas tropicales.

Palabras clave: *sostenibilidad agrícola, bioinformática, secuenciación de alto rendimiento, diversidad microbiana*.

INTRODUCTION

The cacao chain is strategic for tropical economies and for Ecuador due to its fine aroma cocoa, whose demand sustains productive linkages and significant foreign exchange on a global scale (e.g., recent production and trade outlook). However, intensification under monoculture schemes has caused soil degradation and pressures on soil biodiversity, with consequences on productivity, stability, and resilience of the agroecosystem. Evidence indicates that management practices aimed at improving physicochemical properties such as crop rotation, organic amendments, and reduced agrochemical use create more favorable soil environments and preserve microbial biodiversity, which is key for soil structure and functionality (Bertola, Ferrarini, and Visioli, 2021).

In parallel, frameworks of microbial biotechnology and the use of beneficial microorganisms (biofertilizers, biocontrol agents) have emerged as promising alternatives to enhance crop performance while reducing dependence on synthetic inputs (Sawant, Park, Sim, Kim, and Choi, 2025). From an ecological perspective, the bacterial microbiome plays a central role in biogeochemical cycling processes (C, N, P), phosphorus solubilization, biological nitrogen fixation, phytohormone production, and pathogen suppression, which are critical determinants of plant growth-promoting rhizobacteria (PGPR) activity (Santoyo, Urtis, Loeza, Orozco, and Glick 2021). Moreover, environmental factors such as pH, organic matter, moisture, and nutrient availability strongly influence microbial diversity and community composition, with direct implications for soil multifunctionality (Qiu *et al*., 2021; Salazar-García, Lagunes, Cámara y Arévalo, 2023).

In cacao production systems, management practices are expected to shape soil conditions and, consequently, microbial assemblages. Diversified and agroforestry systems, characterized by higher structural complexity, increased litter inputs, and moderated microclimatic conditions, may promote more stable soil environments and support more diverse and functionally robust microbial communities compared to monoculture systems (Fahad *et al*., 2022). Despite this theoretical framework, empirical evidence linking production system, soil properties, and bacterial community structure in cocoa agroecosystems remains limited and fragmented, particularly under tropical conditions. Recent advances in high-throughput sequencing technologies have significantly improved the capacity to characterize soil microbial communities with greater taxonomic and ecological resolution (Satam *et al*., 2023). These developments have opened new opportunities to better understand the interactions between soil management, physicochemical properties, and microbiome dynamics. However, there is still a lack of integrative studies that simultaneously evaluate soil quality indicators and microbial community patterns across contrasting cacao production systems, limiting the development of evidence-based management strategies.

Therefore, the objective of this study was to evaluate the effect of different cacao production systems (monoculture, diversified, and agroforestry) on soil physicochemical properties and bacterial community structure, and to identify the relationships between soil conditions and microbiome composition. This approach aims to contribute to a better understanding of how management practices influence soil functionality and to provide scientific evidence to support the transition toward more sustainable and resilient cocoa production systems.

MATERIALS AND METHODS

Study Location and Sample Collection

The field phase was carried out in the San Gabriel community, Mocache canton, Los Ríos province, Ecuador (coordinates: 1° 01′ 43″ S, 79° 27′ 49″ W). In decimal notation: –1.0286°, –79.4636°. The site corresponds to a cocoa-growing area on the Ecuadorian Coast and was the sampling point for comparing the three evaluated production systems (Figure 1).

Using sterilized tools, soil cores of 2.5 cm in diameter and 10 cm in depth were collected directly adjacent to the plant roots. Once collected, the samples were placed in sterile tubes and immediately sealed to prevent cross contamination and degradation. The samples were transported in a cooler with dry ice, maintaining the cold chain to the Soil and Water Laboratory of the Technical State University of Quevedo (UTEQ), "La María" Campus, located at km 5 on the Quevedo – El Empalme road. The physical variables were analyzed in the same laboratory, and the chemical variables were prepared for analysis at the Soil Laboratory of the National Institute of Agricultural Research (INIAP, Ecuador). Regarding the remaining portion of the samples, these were stored at −80 °C, later stabilized, and prepared for DNA sequencing, which was carried out in the city of Quito by the company providing the service of Biosequence Company.

Treatments, Experiment Management, and Physicochemical Indicators

Samples of soil from the three cacao production systems were compared. The contrast focused on the physicochemical properties of the soil and the structure of the bacterial microbiome. Table 1 below shows the areas considered for each treatment, as well as the organization of the analysis for each evaluation axis (soil and microbiome).

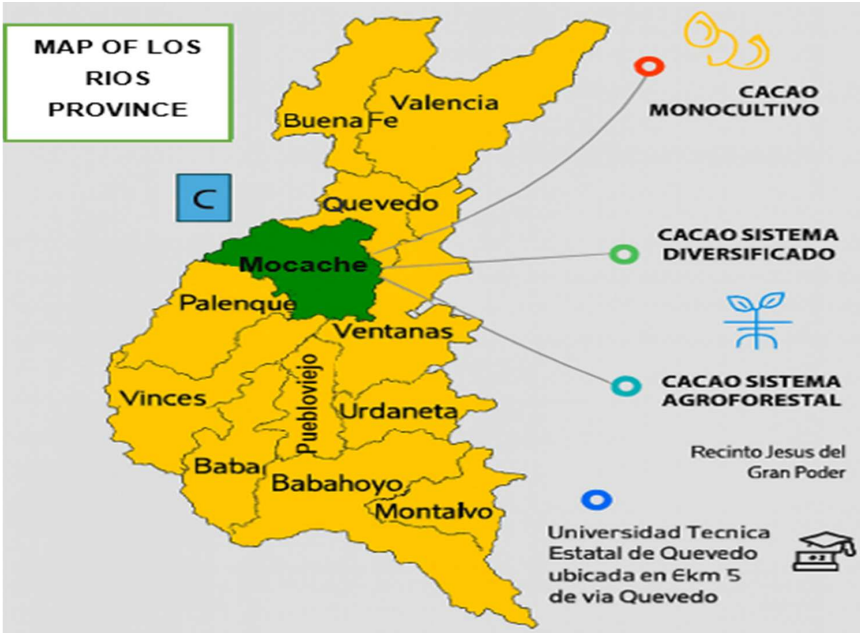

Figure 1. Location of the study area in Los Ríos province, Ecuador. The three evaluated cacao production systems are shown: monoculture (T1), diversified (T2), and agroforestry (T3).

Table 1. Description of the three cacao production systems (monoculture, diversified, and agroforestry), indicating the management applied and the area used in the study.

Treatments	Description	Area (ha)	Samples
T1: Cacao monoculture	Productive units are composed exclusively of cacao (*Theobroma cacao*) aged 8 years. Clonal material CCN-51 grafted onto EET-400 rootstock. No associated species present.	5.0	15
T2: Cacao diversified system	Cacao as the dominant species, integrated into a multistrata vegetation arrangement with the presence of plantain (*Musa* sp.), teak (*Tectona grandis*), and fruit trees such as orange (*Citrus sinensis*) and rambutan (*Nephelium lappaceum*). These species form complementary strata that increases the structural and functional complexity of the system.	3.5	15
T3: Cacao agroforestry system	Cacao as the main crop established under a dominant timber tree canopy, composed mainly of teak (*Tectona grandis*). The upper stratum provides permanent shade, microclimate modulation, and greater biomass input.	2.3	15

Sampling Strategy and Sample Preparation

Sampling was carried out following a zigzag pattern using a systematic design with 8 m intervals, avoiding plot edges and disturbed areas adjacent to cocoa plants. The sampling path was established within the central zone of each plot corresponding to the different treatments. Soil samples were collected from the topsoil layer (0–10 cm). For each treatment, 15 individual samples were collected, each weighing approximately 0.5 kg, and were used for physicochemical analyses. From this set, three samples per treatment (100 g each) were randomly selected to obtain representative material for metabarcoding analyses. All the samples were properly labeled. The fraction intended for physicochemical analysis was air-dried and sieved to 2 mm, whereas the fraction designated for molecular analyses was stored at −80 °C, subsequently stabilized, and sent to the laboratory for DNA extraction and sequencing, being transported under cold-chain conditions until receipt.

Physical Indicators

Bulk density (BD) was determined in undisturbed soil, based on the calculation of total porosity and particle density. Texture (sand, silt, and clay): textural fractions were determined using the Bouyoucos hydrometer method, with verification by wet sieving at 0.053 mm in required cases. Moisture and water-holding capacity were estimated by gravimetric quantification in undisturbed samples.

Chemical Indicators

Soil pH was determined both in the field (preliminary reading) and in the laboratory, using reference readings with duplicates and blank controls. Organic matter (OM) was quantified using the Walkley and Black method, following the standardized procedure established by INIAP (2014). Total nitrogen (N) was determined by the Kjeldahl method, while available phosphorus (P) was analyzed through extraction with Bray II. Exchangeable basespotassium (K), calcium (Ca), and magnesium (Mg)were determined by extraction with 1 N ammonium acetate (pH 7) and measured by atomic absorption spectrophotometry. Analyses were performed in duplicate, including blank controls and verification of analytical precision (CV ≤ 10%), ensuring the quality and reproducibility of the results.

DNA Extraction and Library Preparation

From the total soil containing the microrganism, DNA was extracted following the laboratory's operational protocol. Quality was verified by electrophoresis (integrity) and fluorometric quantification (concentration). Bacterial characterization was performed on the 16S rRNA gene (V3–V4 regions) using primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). Libraries were prepared through a two-step PCR process (dual indexing), followed by magnetic bead cleanup to remove residues and equimolar normalization prior to pooling. Procedural laboratory controls were included according to standard practice.

High-Throughput Sequencing

Once the libraries were qualified, they were sequenced using the Illumina sequencing-by-synthesis method with paired-end reads: 250 × 2, delivering BCL/FASTQ files and a quality metrics report. The demultiplexed FASTQ files constituted the input for the bioinformatic analysis. Traceability, run date, and quality acceptance criteria at the library level were documented.

Bioinformatic and Multivariate Statistical Analysis of the Soil Microbiome

The bioinformatic processing of the sequences was performed using a standardized pipeline in QIIME2 version 2023.5 (Bolyen, 2019). Raw reads in FASTQ format were subjected to quality control, filtering, dereplication, and chimera removal, followed by taxonomic assignment against a curated database (SILVA 138). Sequences were grouped into amplicon sequence variants (ASVs), and a normalized abundance table was constructed and treated as compositional data through centered log-ratio (CLR) transformation.

Alpha diversity was estimated using the Observed, Chao1, Shannon, and Simpson 1–D indices, while beta diversity was analyzed through Principal Coordinates Analysis (PCoA) employing Aitchison and Bray–Curtis distances. Differences in community structure among treatments were evaluated using permutational multivariate analysis of variance (PERMANOVA) on distance matrices. Canonical ordination (db-RDA) was applied to explore the relationship between edaphic variables (pH, organic matter, and nutrients) and bacterial composition, using permutation tests to determine significance ($p < 0.05$). Finally, the internal coherence of the treatments was examined through hierarchical clustering (Ward.D2) based on CLR coordinates, visualizing the similarity among microbial communities under the different cocoa production systems.

RESULTS AND DISCUSSION

Chemical Indicators of Soil Quality

Figure 2 shows that the diversified system (T2) consistently exhibits higher contents of N and P, as well as higher pH and organic matter (OM), compared to the monoculture system (T1), while the agroforestry system (T3) generally presents intermediate values. The Kruskal Wallis test followed by Dunn's post-hoc comparisons indicates significant differences among treatments ($p < 0.05$), confirming that these patterns are statistically supported. From an edaphic perspective, the observed differences may be associated with the greater input of organic residues (e.g., litter and fine roots) and improved microclimatic conditions in T2, which can enhance organic matter accumulation and contribute to pH buffering. These conditions are known to influence nutrient availability, particularly for N and P, as moderate pH levels can reduce P fixation by Al and Fe (Blume *et al*., 2016). For K, a pattern of T3 ≈ T2 > T1 is observed, suggesting a similar availability between diversified and agroforestry systems. This may be related to the contribution of woody species in T3, which can enhance nutrient recycling through litterfall. In contrast, Ca and Mg tend to be higher in T2, which could indicate greater base saturation and improved cation exchange conditions (Tzilivakis, Warner, Green, and Lewis, 2015). Overall, the chemical profile observed in T2 characterized by moderate pH, higher organic matter, and greater nutrient availability may create favorable conditions for more active soil biological processes. Previous studies have shown that such environments can promote microbial activity and nutrient cycling, including nitrification and sulfur transformations, while reducing the dominance of acidophilic and oligotrophic microorganisms typically associated with nutrient-poor soils (Li *et al*., 2025; Dalmaso, Ferreira, and Vermelho 2015).

Alpha Diversity: Observed/Chao1, Shannon, Simpson 1–D

Figure 3 shows a consistent pattern across the four alpha diversity metrics: the diversified system (T2) exhibits the highest values for richness (Observed families and Chao1), diversity (Shannon), and evenness (Simpson 1–D), whereas the monoculture system (T1) presents the lowest values, and the agroforestry system (T3) shows intermediate values. These results indicate significant differences among treatments, with T2 supporting higher richness and a more even distribution of bacterial communities compared to T1. From an ecological perspective, these patterns may be associated with differences in soil conditions among systems. The higher values observed in T2 could be related to increased resource availability and environmental heterogeneity, which are known to favor the coexistence of diverse microbial groups. In contrast, the lower diversity observed in T1 may reflect more homogeneous conditions and a greater dominance of fewer taxa (Massaccesi *et al*., 2015; Matthews and Whittaker, 2015).

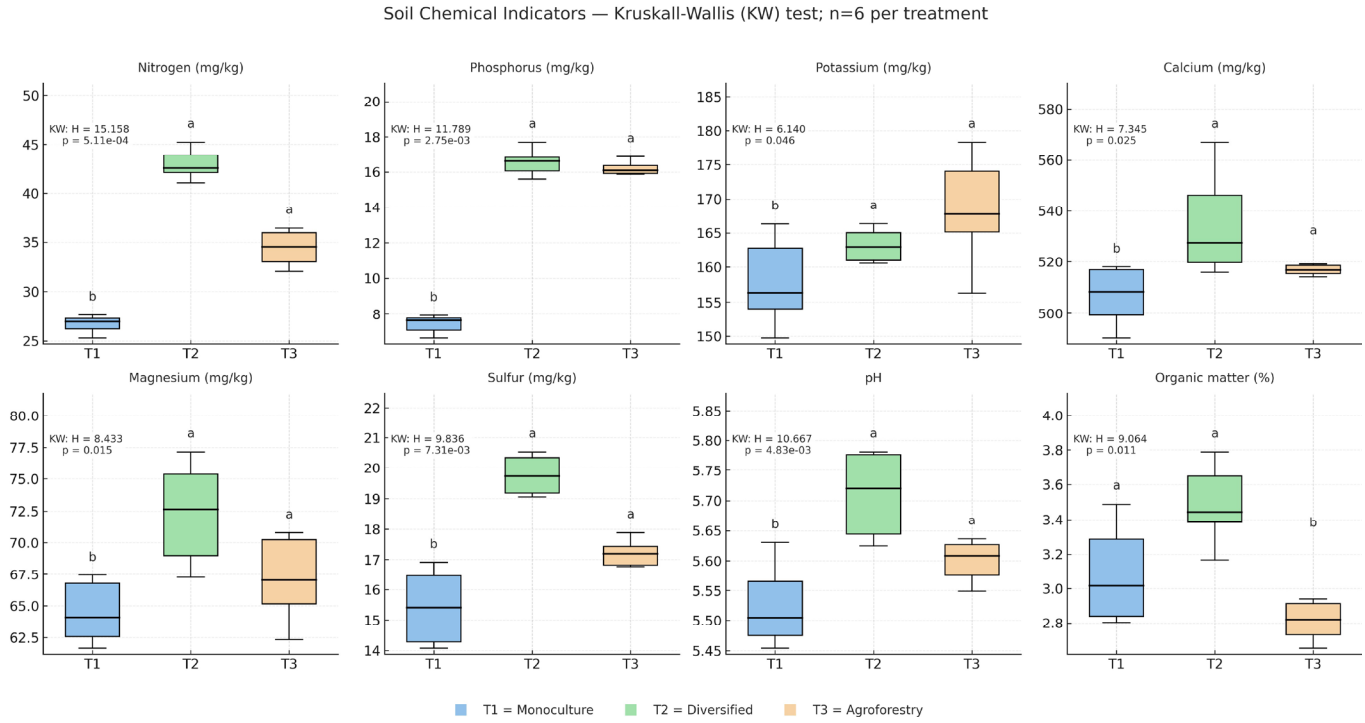

Figure 2. Chemical indicators of soil quality under three cacao production systems in Los Ríos province, Ecuador: monoculture (T1), diversified (T2), and agroforestry (T3). Mean values ± standard deviation of pH, organic matter (OM), total nitrogen (N), available phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) are presented. Different letters above the boxes indicate significant differences among treatments (Kruskal–Walli's test and Dunn post-hoc comparison, $p < 0.05$).

The intermediate values observed in T3 suggest that agroforestry systems share some of the conditions that promote diversity, although to a lesser extent than fully diversified systems. This may be influenced by factors such as vegetation structure, residue inputs, and microclimatic variability (Achat, Fortin, Landmann, Ringeval, and Augusto, 2015; Dobrowski *et al*., 2015). The use of multiple diversity metrics provides complementary information: Observed families and Chao1 emphasize richness (with Chao1 accounting for potential under sampling), Shannon incorporates both richness and evenness, and Simpson (1–D) reflects dominance patterns. The consistency across these indices strengthens the robustness of the observed trends. Overall, the results suggest that diversification of cocoa production systems is associated with higher bacterial alpha diversity. Previous studies have reported similar patterns, linking improved soil conditions and management practices with increased microbial diversity and activity in tropical agroecosystems (Stefan, Hartmann, Engbersen, Six, and Schöb, 2021; Faure *et al*., 2015; Wemheuer *et al*., 2020; Stenuit and Agathos, 2015; Țopa, Căpșună, Calistru, and Ailincăi, 2025; Munroe, Soto, Virginio Filho, Fulthorpe, and Isaac, 2015).

PCoA – Aitchison (CLR-Euclidean) to Analyze or Visualize Differences Among Microbial Communities

The Aitchison ordination in Figure 4 (based on log-ratio transformations) shows a clear separation by treatment: T2 replicates cluster on the right side, T1 shifts to the opposite end, and T3 occupies intermediate positions, with partial overlap toward T2. Because Aitchison distance operates within a compositional framework, this separation reflects differences in the relative abundance structure of taxa rather than absolute changes in total community size, highlighting shifts in the proportional dominance of bacterial groups across systems. The percentages explained by the first two axes (25.6% and 24.4%) indicate that a substantial fraction of the compositional variation is captured, supporting the robustness of the observed pattern. From an ecological perspective, this segregation can be interpreted in relation to the edaphic gradient identified among treatments. Increases in pH, organic matter, and moisture availability observed in T2 are known to reduce environmental filtering and promote the establishment of copiotrophic and metabolically flexible taxa, which respond positively to higher substrate

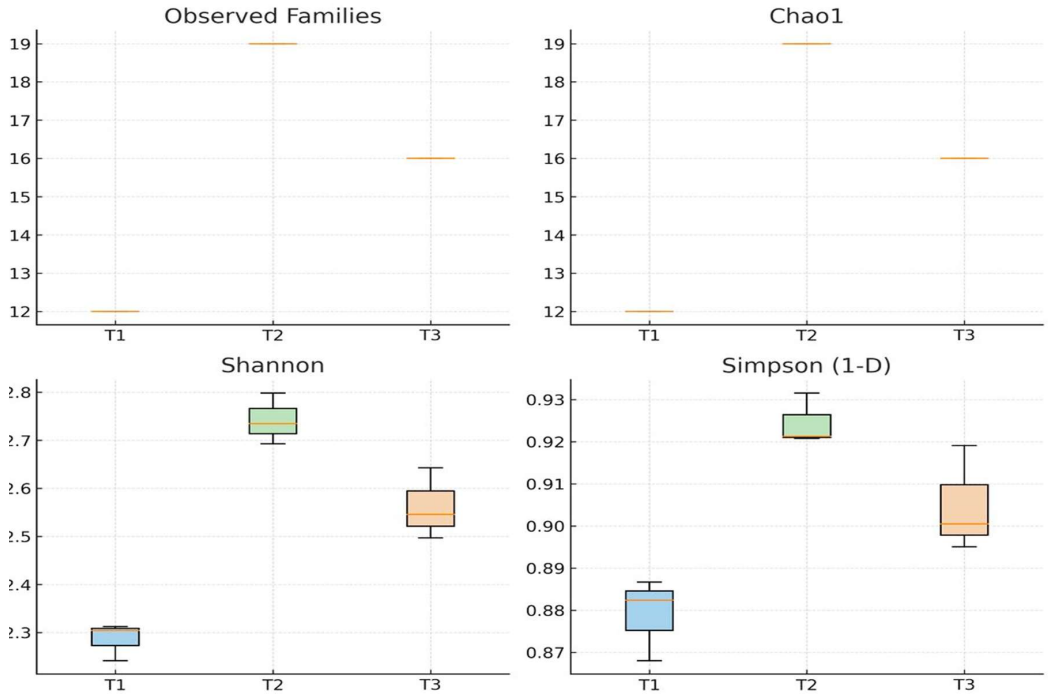

Figure 3. Alpha diversity indices (Observed families, Chao1, Shannon, Simpson [1-D]) of soil microbial communities under three cacao production systems: monoculture (T1), diversified (T2), and agroforestry (T3). Higher richness (Observed, Chao1) and diversity (Shannon, Simpson) were observed in T2 and T3 compared to T1, indicating that diversified and agroforestry systems promote more diverse and stable microbial communities in cacao soils.

availability and more neutral pH conditions (Gong *et al*., 2015; Wang *et al*., 2015). In contrast, more acidic and resource-limited soils, such as those observed in T1, tend to select for oligotrophic and acidophilic taxa adapted to low nutrient availability and slower growth rates, a pattern widely documented in soil microbial ecology (Fierer, Bradford, and Jackson, 2007; Lauber, Hamady, Knight, and Fierer, 2009). The intermediate positioning of T3 suggests a transitional microbial structure, likely reflecting partial improvements in soil conditions due to litter inputs and microclimatic buffering associated with woody vegetation. However, the magnitude and consistency of these changes appear lower than in T2, which may explain the greater dispersion observed in T3. Such variability is consistent with previous reports indicating that agroforestry systems can exhibit heterogeneous microbial responses depending on species composition, canopy structure, and management intensity (Edelstein *et al*., 2025; Wemheuer *et al*., 2020). Overall, the separation observed in beta diversity supports the interpretation that management-driven changes in soil physicochemical properties act as key environmental filters structuring microbial communities. Rather than being random, these compositional shifts are consistent with established ecological frameworks linking soil pH, organic matter, and resource availability to microbial community assembly processes, including niche differentiation and environmental selection (Fierer *et al*., 2007; Delgado-Baquerizo *et al*., 2018).

Diversification Standardizes Niches and Copiotrophic Assemblages: PCoA–Bray–Curtis (PC1 29.0%, PC2 21.7%) and Concordance with Aitchison in Bacterial Communities of Cocoa Soils

The PCoA with Bray–Curtis in Figure 5 (which strongly weights abundant taxa) reproduces the ordination pattern by treatment: T2 forms a compact cluster toward the positive side of PC1, T1 is located on the opposite side, and T3 lies between them with moderate variation. The fact that Bray–Curtis and Aitchison show a similar structure is convergent evidence: treatment changes not only alter relative proportions (Aitchison) but also the identity and dominance of the most common taxa (Bray–Curtis). The axes explain x = 29.0% and = 21.7% of the variation, sufficient to visualize consistent community-scale patterns. From a functional standpoint, the lower variability in T2 indicates that the diversified system tends to standardize soil conditions (pH, organic matter, and moisture), favoring a stable set of copiotrophic bacteria that could influence nutrient cycles indicative of

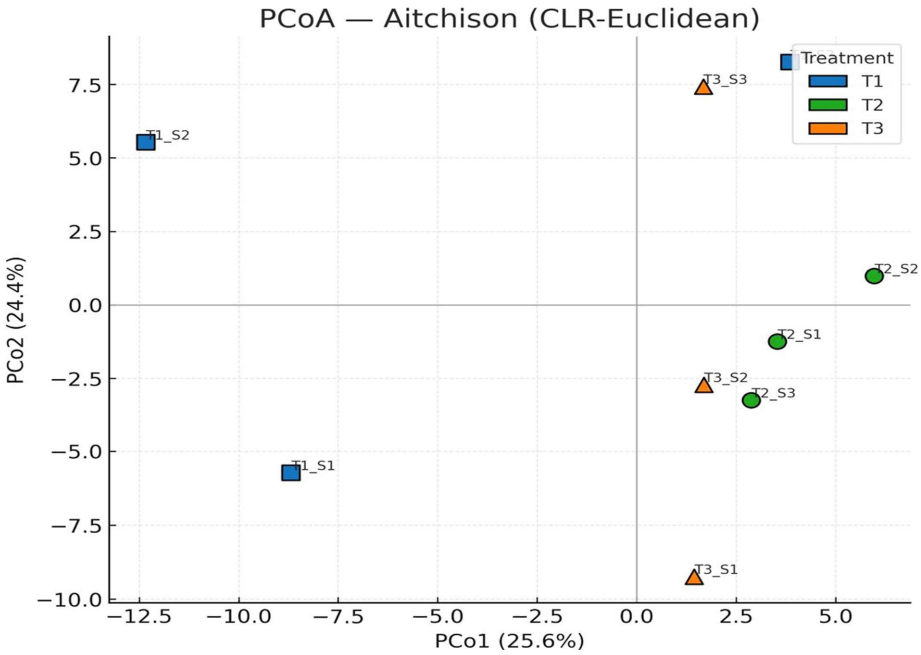

Figure 4. Principal Coordinates Analysis (PCoA) based on Aitchison (CLR-Euclidean) distances showing the ordination of soil microbial communities under three cacao production systems: monoculture (T1), diversified (T2), and agroforestry (T3). The clear separation of clusters indicates distinct microbial community compositions among treatments, with diversified and agroforestry systems (T2 and T3) differing markedly from monoculture (T1).

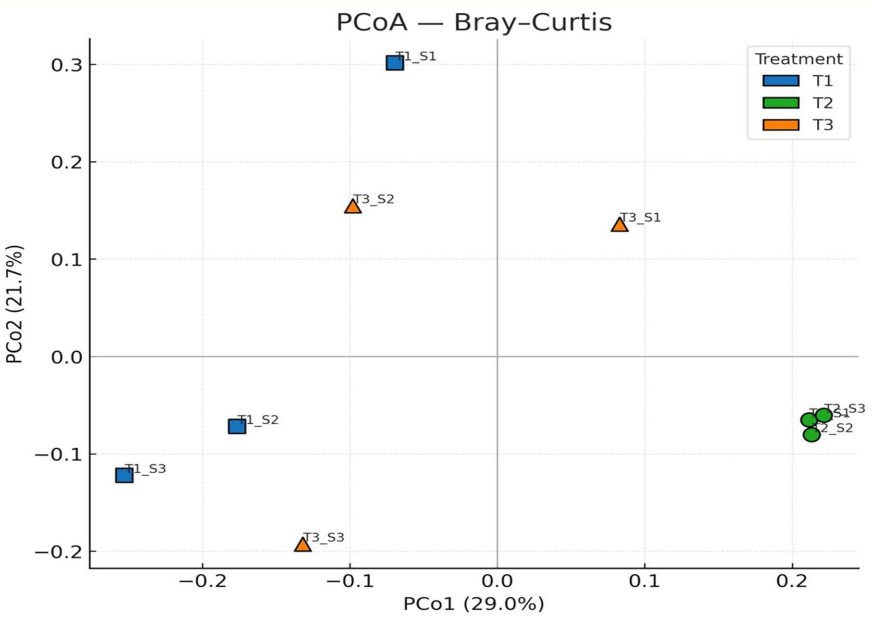

Figure 5. Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarities showing the similarity and dissimilarity of soil microbial communities among cacao production systems: monoculture (T1), diversified (T2), and agroforestry (T3). The ordination reveals clear compositional divergence, with T2 and T3 forming distinct clusters relative to T1. Bray–Curtis emphasizes differences in species abundance, indicating that management intensity strongly shapes microbial community composition.

optimal conditions (such as nitrogen and sulfur) (Lynch and Neufeld, 2015; Ortego, Noguerales, Gugger, and Sork, 2015). In contrast, T1 presents a different composition, characteristic of more acidic soil's poor in substrates, where oligotrophic bacteria predominate (Penny *et al*., 2015). T3 combines traits of both, which is expected given the heterogeneity of agroforestry arrangements. In summary, the PCoA Bray–Curtis confirms that diversification reconfigures the dominant community, consistently reinforcing the soil–microbiome relationship observed in the physicochemical indicators (Zeng *et al*., 2025).

Relative Abundance of the 20 Most Representative Families to Simplify or Display Patterns of Microbial Communities

Figure 6 illustrates differences in bacterial community composition among the three production systems (T1 = monoculture, T2 = diversified, and T3 = agroforestry). Each treatment shows a distinct microbial profile, suggesting that soil management is associated with variations in community structure. In T1 (monoculture), families such as *Bradyrhizobiaceae* and Acidobacteriaceae Subgroup 1 are relatively more abundant, which are commonly reported in soils under more restrictive conditions, including lower organic matter inputs and higher environmental stress (Caro-Quintero and Konstantinidis, 2015). In contrast, T2 (diversified) exhibits a more balanced distribution of taxa, with a higher relative representation of families such as *Rhizobiaceae, Burkholderiaceae*, and *Streptomycetaceae*. These groups have been frequently associated with nutrient cycling and soil biological activity in previous studies (Lehman *et al*., 2015; Souza, Ambrosini, and Passaglia, 2015). The agroforestry system (T3) shows an intermediate composition, with the presence of families such as *Sphingomonadaceae* and *Pseudomonadaceae,* which have been reported in environments with diverse organic inputs and complex substrates (Gianfreda, 2015). These patterns suggest that differences in vegetation structure and residue inputs among systems may contribute to shaping microbial assemblages. Overall, the observed differences in taxonomic composition indicate that diversified systems tend to support more heterogeneous bacterial communities compared to monoculture systems, although these patterns should be interpreted cautiously given the descriptive nature of the analysis. Functional interpretations based on taxonomic composition should be considered indicative rather than conclusive. While previous studies have linked similar bacterial groups to processes such as nitrogen cycling, organic matter decomposition, and pathogen suppression, these functions were not directly measured in this study. Nonetheless, metagenomic research in cocoa systems has reported higher functional diversity and activity under diversified and agroforestry management compared to monoculture systems (Nahon *et al*., 2024; Vaudour, Costantini, Jones, and Mocali, 2015).

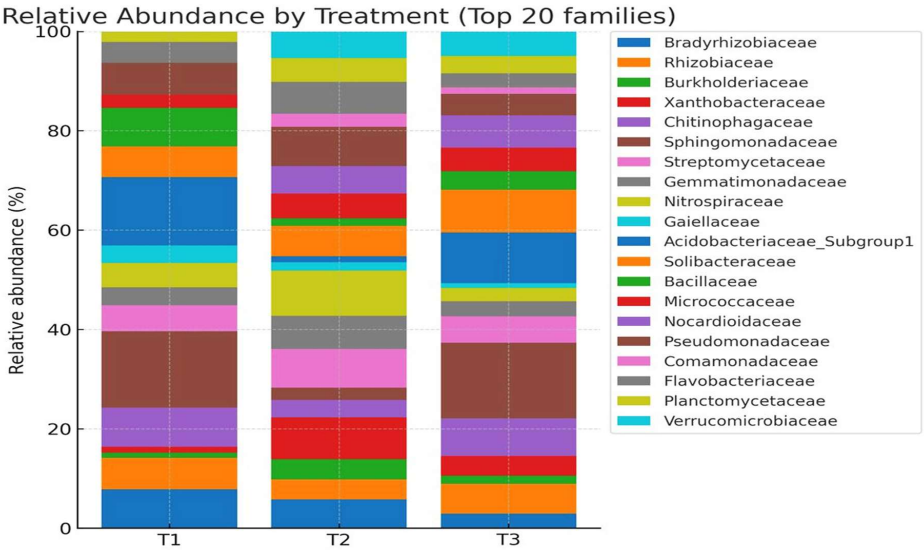

Figure 6. Relative abundance of the top 20 bacterial families in soils under three cacao production systems: monoculture (T1), diversified (T2), and agroforestry (T3). Distinct taxonomic compositions were observed among treatments, with higher representation of Bradyrhizobiaceae, Rhizobiaceae, and Burkholderiaceae in T2 and T3, suggesting that diversified and agroforestry systems favor beneficial microbial groups associated with soil fertility and nutrient cycling.

db-RDA – Bacterial Families and Edaphic Variables

In Figure 7, the canonical ordination shows that the variation of bacterial communities is strongly explained by the physicochemical indicators of the soil. On axis RDA1 (29.7%), the gradients of pH, phosphorus, sulfur, and organic matter mainly separate the diversified system (T2), suggesting that soil fertility and chemical stability are key drivers in the structuring of communities. On the other hand, axis RDA2 (21.6%) reflects the influence of bulk density and porosity, factors that particularly distinguish the monoculture (T1), where soil compaction and lower aeration are observed. Families with affinity toward T2, such as *Rhizobiaceae* and *Burkholderiaceae*, are associated with soils with higher nutrient and moisture availability, confirming the hypothesis that agricultural diversification enhances an edaphic environment favorable for beneficial bacteria. In contrast, in T1, families adapted to more restrictive conditions predominate, linked to physical and chemical limitations. In summary, the db-RDA reinforces the idea that microbial gradients are not random but closely respond to the edaphic conditions generated by each management system (Cline and Zak, 2015). This provides clear evidence of the interaction between agricultural management, soil quality, and bacterial community structure.

The close relationship between soil physicochemical indicators and the structuring of the bacterial microbiome, demonstrated in the db-RDA analysis, confirms that soil quality is a determining factor in the configuration of microbial communities (Algora *et al*., 2015). These findings provide strong evidence that agricultural diversification is an effective strategy to improve the sustainability and resilience of cocoa production systems, consistent with previous reports highlighting the contribution of agroforestry systems to soil health improvement and maintenance (Fahad *et al*., 2022). The db-RDA analysis showed that physicochemical variables, particularly pH, organic matter, and available nutrients, are strong determinants in the structuring of bacterial communities, with especially evident differences observed in the diversified system. This correlation confirms that agricultural management modifies the soil in a way that directly affects the microbiome, a finding that aligns with the G×E×M (Genotype × Environment × Management) framework used to understand the root microbiota of cocoa and its ecosystem services (Sousa *et al*., 2024).

Hierarchical clustering (Aitchison-Ward) and internal treatment coherence (c = 0.845)

In Figure 8, the hierarchical clustering based on Aitchison distance (CLR transformation with Euclidean metric) and Ward linkage shows high coherence among replicates (cophenetic coefficient = 0.845) and an early separation of the monoculture (T1) from the other systems, while the diversified (T2) and agroforestry (T3) systems cluster

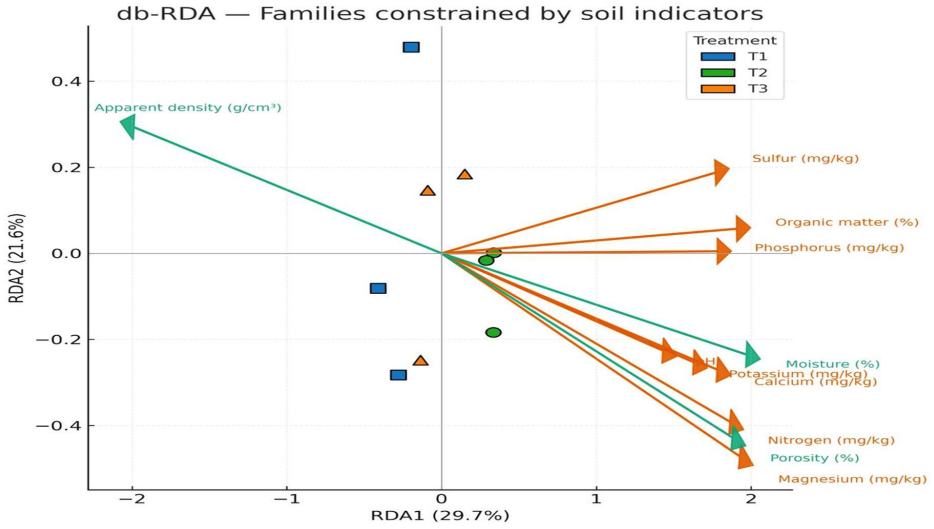

Figure 7. Distance-based redundancy analysis (db-RDA) showing the relationship between soil bacterial community composition and soil physicochemical indicators under three cacao production systems: monoculture (T1), diversified (T2), and agroforestry (T3). Vectors indicate environmental variables significantly correlated with community structure. Apparent density negatively correlated with microbial distribution, while sulfur, organic matter, and phosphorus strongly influenced community shifts in diversified and agroforestry systems.

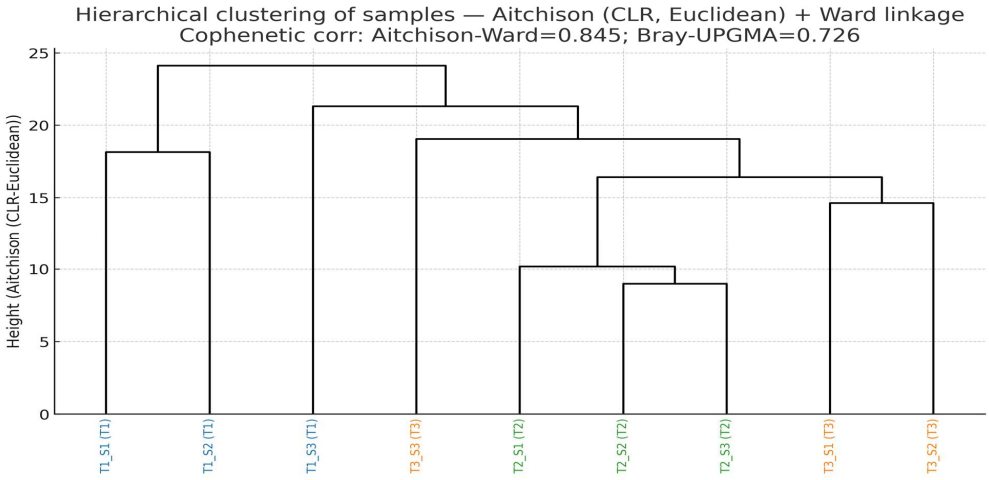

Figure 8. Hierarchical clustering of soil microbiome samples under three cacao production systems: monoculture (T1), diversified (T2), and agroforestry (T3). The dendrogram was generated using Aitchison (CLR–Euclidean) distance and Ward's linkage method. The clustering pattern indicates closer similarity between diversified and agroforestry systems, while monoculture forms a distinct branch, reflecting compositional divergence driven by management practices.

more closely together. This pattern indicates that system management significantly modulates the composition of the soil microbiome and is consistent with the compositional nature of the data and the suitability of the CLR+Ward approach for preserving distance structure (Sobrinho *et al*., 2015), in addition to aligning with recent evidence attributing greater microbial diversity and soil stability to diversification and agroforestry compared with monoculture (Deguine *et al*., 2015).

CONCLUSION

The evaluated cocoa production systems influence the edaphic properties and the structure of soil bacterial microbiomes. The diversified cocoa system was associated with more favorable physicochemical conditions compared to monoculture, while the agroforestry system showed intermediate behavior. These differences in soil quality define patterns in microbial diversity and composition, evidencing a close relationship between the edaphic environment and the organization of bacterial communities. In particular, the diversified system favored greater richness and evenness. In contrast, monoculture was associated with more restricted communities, typical of environments with greater physicochemical limitations. The multivariate analyses confirmed that edaphic gradients (pH, organic matter, and nutrient availability) explain a significant fraction of microbial variation. Overall, the evidence supports that diversification of cocoa cultivation acts on the soil microbiome, promoting more functional microbial profiles. This reinforces the relevance of agroecological practices in the management of cocoa systems to strengthen the sustainability of the agroecosystem.

ETHICAL 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, Formal analysis, Validation, Project administration, Methodology, and Funding acquisition: R.O.V.T., E.E.M.Ch., G.H.V.M., and D.Y.C.; Investigation: R.O.V.T., E.E.M.Ch., S.M.J., and O.I.S.; Methodology: R.O.V.T. and E.E.M.Ch.; Writing – original draft preparation: R.O.V.T., S.M.J., and O.I.S.; Writing – review and editing: R.O.V.T., D.Y.C., S.M.J., and O.I.S.

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